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Two Eyes, One Vision: Evaluating the Etuaptmumk/Two-Eyed Seeing Framework in Bridging Scientific and Indigenous Knowledge for Climate Solutions

Nicole Li

Abstract: Canada's colonial legacy has long marginalized Indigenous peoples and their knowledge in academia and policy-making. To bridge this gap, Mi'kmaw Elder Albert Marshall introduced the Etuaptmumk/Two-Eyed Seeing (E/TES) framework, aiming to optimize Indigenous knowledge (IK) and Western science. While E/TES is applied in research on fishery management and healthcare, its role in climate research remains underexplored in a cross-cultural context. Using a three-round Delphi study, this study identifies challenges in applying E/TES and strategies for fostering culturally respectful research environments. Findings reveal that common obstacles include the superficial inclusion of IK, the power imbalance between researchers, and difficulties reconciling opposing worldviews. To address these challenges, experts recommend abiding by the research protocols of Indigenous communities, establishing advisory bodies, and providing institutional support for Indigenous-led research. By scrutinizing the operational model of E/TES climate research, this study hopes to guide more inclusive, sustainable, and culturally appropriate research practices and environmental solutions.

Keywords: Etuaptmumk/Two-Eyed Seeing, Indigenous knowledge, Delphi study, cross-cultural research, climate research

1. Introduction

Climate change is reshaping coastlines, ecosystems, and entire ways of life—yet its impacts are only expected to intensify. Recent studies predict that the number of people at risk from sea-level rise will grow five-fold by the end of this century (Schechter et al., 2023). In particular, Indigenous communities are disproportionately affected by these devastating consequences given their deep, intergenerational relationship with the land. However, their profound cultural, spiritual, and practical experiences with the Earth equip them with holistic place-based environmental insights that are missing in scientific methods (Berry & Schnitter, 2022). Despite its significance, Indigenous knowledge (IK) largely remains undervalued in Canadian environmental politics due to the country's colonial past (Comberti et al., 2019; Datta et al., 2024; Deranger et al., 2021). To amplify Indigenous voices, academia has begun increasingly exploring how IK informs climate adaptation strategies, including land stewardship, seasonal ecological monitoring, and community-led conservation programs (Petzold et al., 2020; Vijay Kumar, 2019).

One major approach is the Etuaptmumk/Two-Eyed Seeing (E/TES) framework, which empowers Indigenous perspectives in environmental research (Bartlett et al., 2012; Deloria, 1999). However, Roher et al. (2024) note that much of the existing E/TES research oversimplifies its guiding principles and lacks a detailed description of its application. To address these gaps, this study will draw upon expert opinions, including those who have participated in E/TES climate research, to investigate the following question: How does adherence to the four E/TES principles affect the operation model of E/TES climate research projects in Canada? Based on the literature, it is hypothesized that E/TES research following the four guiding principles is more likely to yield culturally appropriate environmental solutions, as corroborated by Datta et al. (2024) and Whitney et al. (2020). Conversely, research that treats IK as supplementary knowledge may be less effective due to the risks of misinterpretation (Latulippe & Klenk, 2019). By comparing the experiences and insights of participants, this study will identify recurring challenges (i.e. delegitimization of IK and unequal power dynamics) and practices that contribute to a meaningful E/TES application.

2. Literature Review

The E/TES framework demonstrates high potential for fostering inclusive and effective environmental solutions (Datta et al., 2024; Latulippe & Klenk, 2019; Whitney et al., 2020). This literature review will explore the underlying factors behind IK's exclusion from Canadian environmental policy and research, detail the development of integration frameworks, and identify the gap this study aims to address.

2.1 The Exclusion of Indigenous Knowledge

While IK offers rich perspectives on climate adaptation, systemic barriers in Canada hinder its inclusion in research and policymaking. According to the report *Decolonizing Climate Policy in Canada*, "[Indigenous Peoples'] knowledge and approaches to climate change are systematically excluded from the creation and implementation of climate policies" (Deranger et al., 2021, p. 9). This epistemological marginalization is rooted in Canada's colonial legacy that dismisses IK as superstitious, undermining its credibility (Comberti et al., 2019; Mach et al., 2020). As a result, Western science has been consistently prioritized as the most trustworthy source in Canadian climate research (Comberti et al., 2019). Corroborating this claim, Teena Starlight (2024), a Tsuut'ina First Nation professor at Mount Royal University, and her colleagues interviewed Elders of the Blackfoot First Nation in Western Canada. Their findings, similarly, reveal that the delegitimization of IK is a direct consequence of Canada's colonial history.

The historical and systemic marginalization of IK is further compounded by the West's fixation on scientific knowledge, which prioritizes analytical and reductionist methods (Reid et al., 2022). This emphasis on scientific knowledge originated during the Scientific Revolution in the 17th-century, which promoted the ideas of experimentation and empirical observations (Mazzocchi, 2006; Brooks, 2020). Conversely, IK focuses on experiential intergenerational observations passed down through storytelling and ceremonies (Reid et al., 2022; Whitney et al., 2020). Due to these fundamental differences, Latulippe and Klenk (2019) contend that IK is often viewed as "supplementary knowledge," solely analyzed for its consistency with science or to fill scientific research gaps. Notably, this results in confirmation and selection biases that, when combined with pre-existing cultural and linguistic differences, prevent policymakers and researchers from understanding the nuances of IK (Latulippe & Klenk, 2019; Reid et al., 2022).

2.2 Etuaptmumk/Two-Eyed Seeing

Although less technical than scientific knowledge, IK offers profound insights into climate patterns and ecosystem dynamics through place-based wisdom passed down through generations (Datta et al., 2024; Filho et al., 2022; Whitney et al., 2020). The practice of planting the Three Sisters (i.e., corn, squash, and beans) exemplifies Indigenous agricultural knowledge, where each crop facilitates the growth of another, thereby optimizing harvest yields (Agriculture and Agri-Food Canada, 2021). Acknowledging the value of IK, Darlene Sanderson (2015), a professor of Cree descent at Thompson Rivers University, and her colleagues advocate for its inclusion in policymaking. They believe that it can expand researchers' understanding of climate change beyond the scope of Western science, a position supported by Datta et al. (2024) and Latulippe and Klenk (2019).

Indigenous scholars have developed multiple approaches to bridge the gap between Indigenous and Western knowledge systems (Smith et al., 2023). One of the earliest frameworks, Two Ways of Knowing, was designed by Indigenous activist Vine Deloria Jr. (1999) to differentiate between the teachings

of IK and Western science. In 2004, Mi'kmaw Elders Albert and Murdena Marshall, along with Tier 1 Canada Research Chair Cheryl Bartlett, extended Deloria's (1999) ideas by introducing the concept of Etuaptmumk/Two-Eyed Seeing, illustrated in Figure 1 (Peltier, 2018). In Marshall's words, "[E/TES] refers to learning to see from one eye with the strengths of Indigenous ways of knowing and from the other eye with the strengths of Western Ways of Knowing and to using both of these eyes together" (p. 335), symbolized by the tabs of the puzzle in Figure 1 (Cape Breton University, 2007).

Rather than solely distinguishing between knowledge systems, Bartlett et al., (2012) envisioned E/TES to reduce the clash between them, enabling researchers to optimize the value of all information. However, as Smith et al. (2013) emphasize, the purpose of E/TES is to empower IK and support Indigenous self-determination. Hence, this framework should be centred on IK, followed by the integration of scientific knowledge (p. 122). This order is critical to decolonizing Western research, allowing IK to challenge preconceived notions (MacRitchie, 2018).

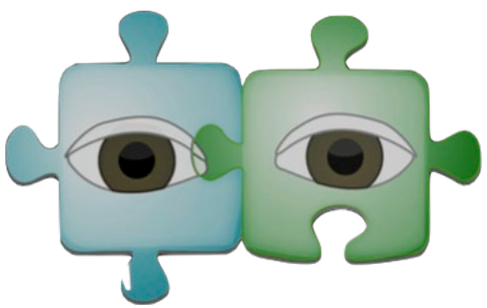

Figure 1
Visual representation of the Etuaptmumk/Two-Eyed Seeing framework (Cape Breton University, 2007).

2.3 Applications of Etuaptmumk/Two-Eyed Seeing

E/TES has been effectively applied across various areas of research, particularly in the fields of coastal management and healthcare. For instance, Denny and Fanning (2016) utilized this framework to re-examine salmon conservation in Nova Scotia. Similarly, Marsh et al. (2016) combined Indigenous and Western health practices to enhance treatments for substance use. These examples highlight E/TES's strength to adapt to different research objectives and contexts (Roher et al., 2024).

Regardless of variations in its application, Bartlett (2017) emphasizes that all E/TES-based research should abide by four key principles. These include 1) "i'l'oqaptmu'k" (co-learning): collaborative learning from all knowledge systems, 2) knowledge scrutinization: evaluating the strengths and weaknesses of each system, 3) knowledge validation: ensuring the participation of professional scientists and IK scholars, and 4) knowledge gardening: fostering opportunities for knowledge exchange (p. 37-64). However, after reviewing 83 health studies from 2004 to 2023 that employed E/TES, Roher et al. (2024) discovered that not all studies adhered to these principles. The team identified two main shortcomings: 1) an insufficient description of how E/TES was specifically applied during the research, and 2) an oversimplification of the framework's four guiding principles. This finding highlights a pervasive flaw in E/TES application, demonstrating the importance of adhering to the principles to ensure the authenticity of this framework.

2.4 Gap Analysis

Despite the growing recognition of E/TES in the scholarship, few studies apply this framework in climate research or assess its application process, indicating an empirical and knowledge gap. To address this limitation, this study gathered insights from professionals with relevant expertise regarding their experiences and understandings of E/TES research. To collect data, a Delphi study was conducted with Indigenous and non-Indigenous participants who hold expertise in environmental fields and/or have participated in E/TES climate research initiated by the government and/or independent organizations. This approach also addresses a methodological gap as it is rare to facilitate asynchronous discussions between professionals from fundamentally different knowledge systems.

3. Methodology

To address the identified research gaps, this chapter outlines the study's design and the data collection process utilized to investigate the hypothesis, including anticipated challenges and ethical concerns.

3.1 Design and Approach

3.1.1 The Delphi Method

This study employed the Delphi method, a qualitative research design that aims to obtain a reliable consensus among a panel of experts (Landeta, 2006; Taghipoorreyneh, 2023). According to its creators, Olaf Helmer and Norman Dalkey of RAND Corporation (1963), this is achieved through "a series of intensive questionnaires interspersed with controlled opinion feedback" (p. 458). The Delphi method is defined by four key characteristics: 1) iterative data collection featuring at least two survey rounds, 2) anonymity of participant responses, 3) controlled feedback, and 4) statistical response that generates a consensus on a disputed topic (Nasa et al., 2021; Shang, 2023). However, later applications of Delphi have eliminated the requirement for a full consensus, searching instead for a reliable group opinion supported by the majority of expert panelists (Landeta, 2006). Khodyakov (2023) argues that Delphi studies generate accurate prognoses because a group perspective is more reliable and objective than the viewpoint of one expert. As such, the Delphi method is a valuable tool for forecasting and policymaking (Bataller-Grau et al., 2019; Green et al., 2007). Additionally, this approach eliminates the psychological biases—such as the halo effect[1], groupthink[2], and bandwagon effect[3]—and intra-panelist animosity that is common in face-to-face group discussions (Bataller-Grau et al., 2019; Nasa et al., 2021).

3.1.2 Justification and Applicability

Delphi studies have been widely utilized in social science research (Landeta, 2006), with approximately 20,000 mentions in peer-reviewed journals (Khodyakov, 2023). According to Jandl et al. (2009), a Delphi study is particularly useful when dissecting complex social challenges where well-established knowledge is scarce. Additionally, qualitative methods are ideal for examining topics involving underrepresented populations (Creswell & Creswell, 2018; Morse, 1991). As this study investigated the application of E/TES in climate research, an underexplored area involving Indigenous scholars—a historically marginalized group—a qualitative Delphi design was better suited than a quantitative approach.

3.2 Data Collection

3.2.1 Sampling and Recruitment

Purposive sampling was employed to select an expert panel, choosing participants "that [were] most likely to yield appropriate and useful information" (Kelly, 2010, p. 317). The sampling population consisted of Indigenous and non-Indigenous scholars with experience in E/TES climate research, expertise in the environmental field, and/or background in conducting research with Indigenous communities. This study recruited a total of five participants, a number that served as an appropriate sample size while allowing a thorough exploration of each participant's opinions. Participants were identified through credible institutions, such as colleges, universities, and renowned climate organizations. Their participation was confirmed through email.

3.2.2 Survey Design

As Fish and Busby (2005) observed, a three-round Delphi study was sufficient to reach a reliable expert opinion. Studies exceeding three rounds showed

1 Cognitive bias in which one trait of an individual is used to make an overall judgement of that person

2 A phenomenon in which a group of individuals reach a consensus without critical reasoning

3 Phenomenon where people adopt certain behaviors and beliefs because other people are doing the same

minimal changes in perspectives and led to increased survey fatigue among respondents (Beiderbeck et al., 2021; Mahajan et al., 1975). Following this recommendation, participants in this study completed four questionnaires: one focusing on their professional background and the remaining three dedicated to the Delphi rounds.

3.2.3 Contextual Survey

The first questionnaire asked participants to detail their affiliated organization, years of experience in their field, and the number of environmental E/TES research studies they participated in. Participants also provided a professional description, only disclosing information of their choosing. This helped establish their qualifications as an expert and gauged the viewpoints they brought to the discussion.

3.2.4 Three-Round Delphi Survey

In the first round, participants answered open-ended questions about each of the four E/TES principles. They shared their understanding of the framework and described any challenges they encountered or foresaw in the research process. The second round extended upon these responses, including follow-up questions that explored emerging themes and subthemes. In particular, participants were asked to propose strategies to overcome the identified challenges. In the third round, they detailed the extent to which they agreed with the strategy by rating it on a 7-point Likert-scale and justified their decision. To mitigate the bandwagon effect, participants were not informed of other panelists' ranking of each solution (Barrett & Heale, 2020). Simultaneously, the controlled feedback in rounds two and three did not disclose identifiable information about the experts. Furthermore, Barrios et al. (2021) highlighted that the ability of panelists to change their perspective is critical to generating a reliable opinion. Accordingly, participants were allowed to revise or clarify their viewpoints after each round, as shown in Figure 2.

3.2.5 Synthesis

Through three iterative rounds, the challenges associated with upholding each of the four guiding principles were assessed. In addition, participants were allowed to vote on proposed solutions, allowing them to be ranked from most to least important.

3.3 Ethics

To ensure confidentiality, participants were referred to by pseudonyms, with a brief description of their affiliated organization and professional experience to establish their credibility. These descriptions were authored by the panelists, allowing them to choose the information they wished to disclose. All responses remained confidential and were stored in a password-protected file, only accessible to the researcher and her supervisor. All data will be deleted one year after the completion of this study. Additionally, the questionnaires invited participants to reflect on personal or professional conflicts. To respect their comfort level, none of the questions were mandatory, and participants could skip anything they preferred not to answer. This research was approved by the school's Internal Ethics Review Board.

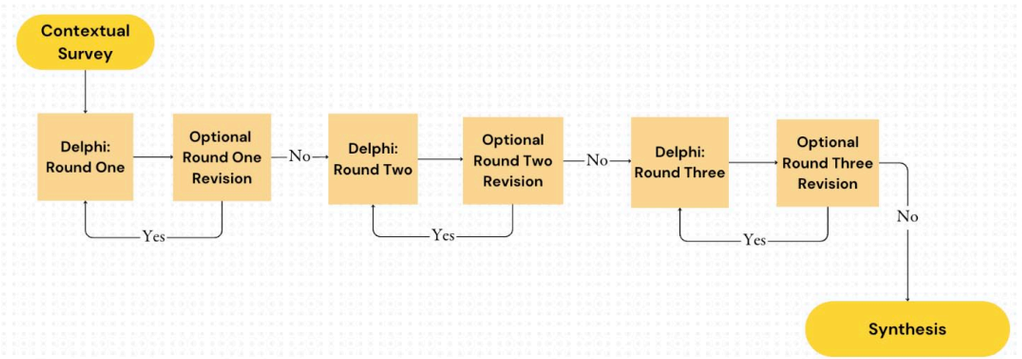

Figure 2
Flowchart showcasing the three-round Delphi process.

Participant Pseudonym	Professional Description	Number of Years Working in Field
Riley	Environmental Law and Policy Consultant	20
Parker	President and CEO of BG Gold (Nunavut-focused exploration company)	5
Skyler	Doctoral Student in Geography & Environment at Western University	3
Jesse	Professor Emeritus in Environmental Studies	55
Harper	Professor in the Departments of Human Geography and Physical and Environmental Sciences, University of Toronto Scarborough	7

Table 1
Table displaying the self-authored professional description of all five expert panelists.

4. Findings

The Delphi study gathered five expert panelists with diverse backgrounds, research experiences, and education levels (Table 1), forming a forum with multiple perspectives. Notably, several panelists provided personal insights from prior experiences leading an E/TES application and/or conducting research with Indigenous communities. For instance, Riley co-led the first E/TES research initiated by the Canadian government to study Boreal Woodland Caribous in 2017, while Harper conducted research with Anishinaabe peoples in the Great Lakes. As Khodyakov (2023) mentions, this range of expertise equips participants to engage in profound discussions that generate complex analyses and robust prognoses.

Over three weeks, participants responded to a contextual survey and three rounds of questionnaires featuring open-ended and Likert-scale type questions (Figure 3). The questions in the first round assessed participants' understanding of E/TES principles developed by Cheryl Bartlett (2017). The second and third questionnaires extended upon panelists' insights from previous rounds, refining the discussions based on new and recurring ideas. To avoid jeopardizing panelist anonymity, individual opinions were rephrased into generalized statements when incorporated into subsequent rounds. This chapter outlines the findings regarding the four E/TES principles (Figure 4), and subthemes that emerged throughout the three-round Delphi study.

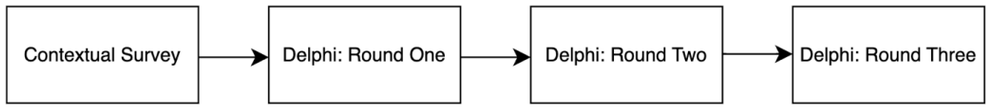

Figure 3
Simplified flowchart showcasing the data collection process.

4.1. Understanding of the E/TES Framework

All participants demonstrated a shared understanding of the E/TES framework, highlighting its purpose in weaving together IK and Western science to develop the most effective climate solutions. Responses underscore the idea of utilizing the framework for "the benefit of distinct but connected communities" (Harper), including Indigenous and non-Indigenous peoples and "current and future generations" (Parker). Specifically, Parker believes that adopting this framework enables researchers to acquire a holistic understanding of the environment and better address complex challenges.

However, disagreements emerged regarding the operational model of E/TES research. Conventionally, Indigenous and non-Indigenous researchers would conduct the research collaboratively, interchanging knowledge throughout the process. However, to meaningfully advance reconciliation, Riley contended that each knowledge stream should conduct research independently with adequate funding. Subsequently, the "decision-maker should receive the two conclusions simultaneously and be tasked with appropriately integrating [them]." Jesse supported this approach, emphasizing that each party should recognize their priorities and collectively reach a middle ground, noting that "the goal is not sameness." While the degree of

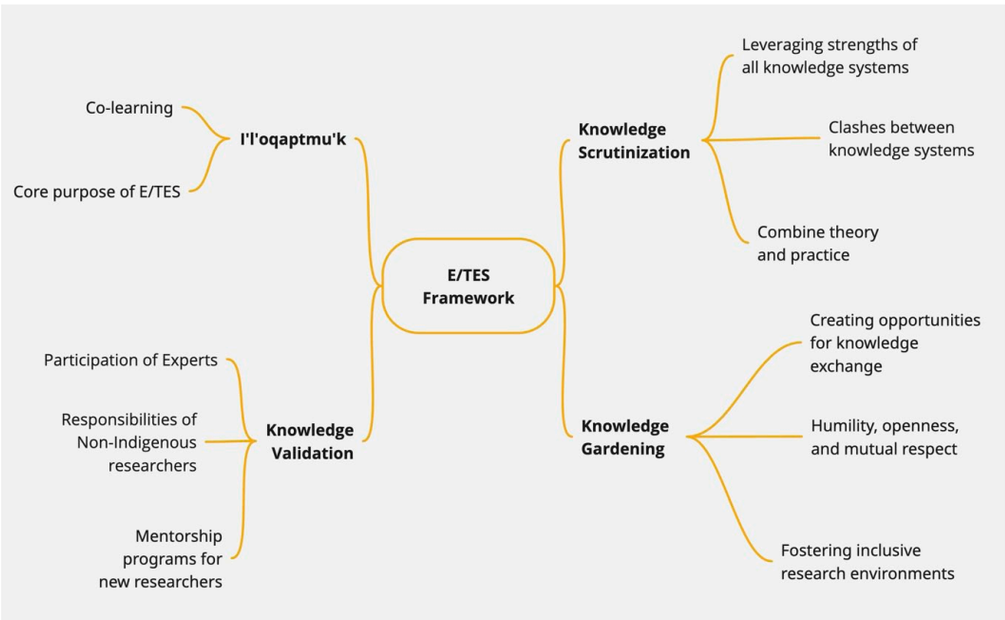

Figure 4
Mind map summary of key themes under the four E/TES principles.

agreement to this idea varied (see Table 2), most panelists possessed an open mind. For example, Harper, who disagreed, nevertheless recommended that "the decision-maker should be made aware of the respective strengths of the knowledge system to assist in evaluating the significance of the conclusions."

Participant Pseudonym	Level of Agreement
Riley	Strongly Agree
Parker	Agree
Skyler	Disagree
Jesse	Somewhat Agree
Harper	Somewhat Disagree

Table 2
Levels of agreement to the statement: In an E/TES research, Indigenous and non-Indigenous scholars should first conduct separate, independent research using their respective knowledge systems. Afterwards, researchers will seek to appropriately integrate the two conclusions rather than weaving together the two knowledge systems from the start.

4.2. E/TES Principle One: I'l'oqaptmu'k

Responses highlight that i'l'oqaptmu'k—collaborative learning—is central to the purpose of E/TES. Parker, who worked with Indigenous communities in Nunavut, described E/TES as a "strong guiding principle" for cross-cultural collaboration. Particularly, survey responses proposed three techniques to facilitate co-learning: 1) establishing advisory bodies (i.e., academic ethics boards or community advisory boards), 2) forming Special Operating Committees to oversee cross-cultural engagement, and 3) following the protocols of the Indigenous communities involved. When asked to rank these strategies, all five panelists unanimously placed the third option first, with the Special Operating Committees viewed as slightly more effective than advisory bodies.

Recommended Solutions	Number of First Ranks	Number of Second Ranks	Number of Third Ranks
Implementing Advisory Bodies	0	3	2
Utilizing Special Operating Committees	0	2	3
Following Indigenous Protocols	5	0	0

Table 3
Participant rankings of recommended solutions to foster collaborative learning.

In her justification, Harper emphasized that "following [Indigenous] protocols is far above the others. It is an expression of respect for Indigenous self-determination, laws, and rights. It embodies meaningful partnership and can have the most meaningful, far-reaching, holistic, and sustainable impacts."

4.3. E/TES Principle Two: Knowledge Scrutinization

The second principle, knowledge scrutinization, entails critically evaluating the strengths and limitations of both IK and Western science, as well as addressing conflicts that arise between the two systems. All panelists agreed that leveraging the strengths of both knowledge systems is crucial for addressing climate challenges, with Parker emphasizing the need to combine theoretical discussions with practical implementations. However, disagreements remain regarding which knowledge source should take precedence during knowledge clashes. Two strategies were proposed to resolve this challenge: 1) clearly outlining the conflict and allowing the end user to decide which knowledge is more appropriate, and 2) prioritizing IK, with non-Indigenous researchers first reflecting on their biases or potential misinterpretation of IK.

Participant Pseudonym	Level of Agreement
Riley	Strongly Disagree
Parker	Somewhat Agree
Skyler	Disagree
Jesse	Neither Agree nor Disagree
Harper	Somewhat Agree

Table 4
Levels of agreement to the statement:

When Indigenous knowledge clashes with Western science, Indigenous knowledge be prioritized instead of leaving the end user of the information to decide.

Responses varied when participants ranked the two solutions, as depicted in Table 4. Riley, who was not in favor of the default prioritization of IK, argued that "the pursuit of truth suggests that each knowledge system should [have] its opportunity to present and explain its unique conclusions." This would allow the decision-makers to truly understand and appreciate different perspectives, making more context-sensitive decisions. Conversely, Harper contended that IK "almost always leads to more holistic, long-term, sustainable, and transformative solutions than Western approaches to ecological problems." Panelists who shared this view echoed Smith's (2013) argument, detailing that the goal of E/TES is to honor IK as it has been systemically marginalized. Nevertheless, there was consensus that IK should be prioritized in research about traditional ecological territories (Table 5), underscoring the importance of respecting Indigenous sovereignty and traditional lands in research.

Participant Pseudonym	Level of Agreement
Riley	Somewhat Agree
Parker	Agree
Skyler	Strongly Agree
Jesse	Agree
Harper	Neither Agree nor Disagree

Table 5

Agreement levels to the statement: When conducting research relating to traditional ecological territories, Indigenous knowledge should be prioritized over science.

4.4. E/TES Principle Three: Knowledge Validation

The involvement of both Indigenous and non-Indigenous scholars is imperative to facilitate a reliable exchange of knowledge throughout the research process. To strengthen collaboration, Skyler suggests that non-Indigenous scholars unfamiliar with E/TES should participate in mentorship programs or cultural training to ensure that they engage in the research with appropriate awareness. Without such preparation, the risk of Western researchers dominating the process increases, treating IK "as ornamental, optional, and not as 'rigorous' as science" (Skyler). Given that IK embodies an "entirely [distinct] worldview" (Riley), these practices help ensure that the nuances of IK—which Reid et al. (2022) highlighted as a critical component in E/TES research—are fully understood. In particular, Jesse posits that IK offers an opportunity for non-Indigenous researchers to "re-set [their] values" and learn to cultivate a harmonious relationship with nature by shifting away from the Western perspective rooted in resource extraction and exploitation.

4.5. E/TES Principle Four: Knowledge Gardening

All panelists emphasized that opportunities for knowledge exchange largely depend on researchers' attitudes and willingness to "make space for Indigenous knowledge" (Jesse). Core characteristics like openness, humility and a genuine willingness to comprehend the teachings of a different culture were identified as critical traits for E/TES researchers, especially those who are non-Indigenous (Skyler, Jesse, and Harper). This mutual respect helps prevent IK from being treated as supplementary or peripheral knowledge, where it is commonly integrated artificially or only analyzed for consistency with Western science in the status quo (Latulippe & Klenk, 2019).

While opportunities for knowledge exchange differ across projects, most panelists highlighted that an open mind fosters the most effective exchange of information. To foster an inclusive environment for knowledge change, participants suggested three different approaches: 1) host regular in-person meetings to build trusting relationships; 2) implement research training across undergraduate, graduate, and professional levels; and 3) practice through test cases to help researchers familiarize themselves with the operation model of E/TES research.

Recommended Solutions	Number of First Ranks	Number of Second Ranks	Number of Third Ranks
In-Person Meetings	3	1	1
E/TES Research Training	2	1	2
Practice in Test Cases	0	3	2

Table 6

Participant rankings of recommended solutions to foster knowledge change.

While there was no full consensus (Table 6), in-person meetings were most highly valued, with three participants identifying them as their first choice. The rank is followed by researching training and practice in test cases. When justifying their views, four of the five panelists argued that cultivating personal relationships within the research group fosters a more comfortable space and facilitates a smoother research process. As Skyler noted, "Meeting in person is… great. [It] builds personal and trusting relationships that can be leveraged in many unique ways."

5. Discussion

Together, the four principles offer a holistic overview of the intent and application of the E/TES framework. By scrutinizing emerging themes, this chapter effectively addresses the empirical and knowledge gaps, thereby identifying strategies that contribute to an effective E/TES implementation, common challenges to applying the framework appropriately, and approaches to resolving clashes between knowledge systems. It also discusses the implications of the findings in Chapter 4 and detail the limitations of this study.

5.1. Research Context and Interdependency Between E/TES Principles

As demonstrated in the previous chapter, the four guiding principles are deeply interconnected and collectively essential to E/TES research. For instance, co-learning cannot occur without opportunities for knowledge exchange. These principles also highlight that operational methods should be flexible, as they vary depending on the research context, suggesting that rigid, standardized procedures are not necessary or advisable. This aligns with the findings in Chapter 4.2 that effective co-learning is best achieved by following the guidance of Indigenous communities, since each community possesses distinct knowledge systems and traditions. As Riley remarked, "Context is so important. It's…not very helpful [to generalize]; each situation is different."

5.2. Common Challenges in E/TES Application

Undoubtedly, a significant barrier in cross-cultural research is understanding "differing worldviews and methodological approaches" (Riley). Specifically, non-Indigenous researchers often overlook the "values and relational side of Indigenous knowledge systems" (Jesse). These philosophies emphasize shifting away from the Western mindset of overconsumption and cultivating harmonious relationships with Mother Earth—principles crucial to addressing the climate crisis. Notably, the quality of climate E/TES research relies on the attitudes of researchers and their openness to explore the richness of another culture. In addition, there is a "lack of meaningful and sustained funding for Indigenous leadership [in] research projects," with "Indigenous communities and peoples [often] included late in the research project and/or superficially" (Parker). Corroborating the epistemological marginalization pinpointed by Deranger et al. (2022) and Datta et al. (2024), Harper contends that this misunderstanding stems from "500+ years of racial capitalism and settler-colonialism" that delegitimized IK.

5.3. Key Strategies for Successful E/TES Application

In the context of E/TES research, securing adequate funding for research and Indigenous leadership is paramount. Skyler recommends "[investing] in programs that support and foster Indigenous leadership and training programs for E/TES." She further highlights the importance of institutional mechanisms, such as community advisory boards, to ensure that Indigenous voices remain central in the decision-making process. At the same time, these official bodies can help obtain funding and facilitate the flow of the research process. In accordance with Carolyn

Smith's (2013) perspective, Skyler stresses the need to uphold Indigenous leadership: "We have to accept that Western science has been 'exalted'…we need equitable (not equal) and intentional investments and support for [IK]."

Nevertheless, non-Indigenous researchers should abide by the protocols of the Indigenous communities involved, familiarizing themselves with the place-based IK. This ensures that IK is respected during research, reinforcing Indigenous knowledge and sovereignty. When these recommendations are neglected, IK risks being treated as supplementary knowledge, as warned by Latulippe and Klenk (2019). To prevent the domination of one knowledge system, all parties should aim to foster an inclusive research environment where all opinions are valued and freely expressed.

5.4. Clash of Knowledge Systems

There is consensus that E/TES should optimize IK and scientific knowledge to produce the most effective climate solution. However, participants differ on strategies for conflict resolution. In his response, Riley proposes that decision-making power should rest with the information user. Opposing this viewpoint, Harper argues that IK and science already exist in an "unequal relationship of power," as acknowledged by Comberti et al. (2019) and Latulippe and Klenk (2019). This imbalance indicates that non-Indigenous information users may lack the cultural competency to assess IK, often prioritizing scientific findings over Indigenous teachings. Therefore, in cases of conflict, non-Indigenous researchers need to first reflect on their biases, as Canada's colonial history has positioned Western science as the most credible source in academia (Harper). Given that this conflict is unresolved, the addition of an Indigenous panelist would provide valuable perspectives because, ultimately, E/TES was designed to honor IK—as corroborated by MacRitchie (2018) and Smith (2013).

5.5. Limitations

Firstly, non-response bias was a significant challenge during data collection. Despite contacting 30+ relevant organizations, professors, PhD students, and environmental experts by email, only five opted to participate in this study. Consequently, not all perspectives on the E/TES framework were gathered, especially as no Indigenous panelists were successfully recruited. To mitigate this effect, the researcher sent follow-up emails in an effort to recruit Indigenous scholars and ensured that most non-Indigenous panelists had prior experiences working with Indigenous communities.

Secondly, as this Delphi study involved three questionnaires lasting 10 to 15 minutes each, survey fatigue may lead to high drop-out rates and low-quality responses, compromising the validity of the findings (Beiderbeck et al., 2021). To address this challenge, the surveys were kept succinct to minimize time demands and participants had a one-week break between each round. Additionally, follow-up emails were sent out as reminders.

6. Conclusion

From rising sea levels to unprecedented climate patterns, the consequences of climate change are felt globally. As this crisis intensifies, the need for effective and sustainable environmental solutions becomes increasingly urgent—Western science alone is not enough to address the complexities of climate change. A promising resource is Indigenous knowledge (IK), rooted in generations of place-based understanding of the world (Datta et al., 2024; Filho et al., 2022; Petzold et al., 2020; Whitney et al., 2020). Given Canada's colonial legacy that marginalized Indigenous voices in academia and policymaking (Comberti et al., 2019; Deranger et al., 2021; Mach et al., 2020), Mi'kmaw Elder Albert Marshall developed the Etuaptmumk/Two-Eyed Seeing (E/TES) to encourage cross-cultural collaboration between IK and science.

This study highlights the key factors shaping the quality of E/TES application in environmental research and provides suggestions to overcome the mentioned structural barriers. Through a three-round Delphi study, panelists identified the primary obstacles in E/TES research as a lack of sustained research funding, tokenization of Indigenous knowledge and researchers, and clashing worldviews that hinder co-learning. The fundamental challenge is the failure of non-Indigenous researchers to grasp the essence of IK—a worldview centered on cultivating deep, reciprocal relationships with nature and fostering an intrinsic responsibility to care for Earth. This philosophy contrasts sharply with the Western paradigm that prioritizes resource extraction and exploitation, only retroactively addressing the environmental harms as an afterthought. As such, panelists stress that non-Indigenous researchers should approach E/TES research with openness and humility, thereby creating space for IK and deepening their understanding of its principles. Individuals unfamiliar with the framework should undergo training or mentorship programs before participating in such collaborative studies. Furthermore, establishing more robust institutional support, such as academic and community advisory boards, ensures that Indigenous insights are included meaningfully throughout the research process.

6.1. Future Research

The insights gathered from this Delphi study can inform policymaking, guide research funding, and contribute to the development of ethical guidelines in E/TES research that amplifies Indigenous voices. When utilized appropriately, E/TES can optimize IK and Western science, leading to more holistic, sustainable, and culturally appropriate climate solutions. Notably, future research can assess the quality and impacts of policies generated from E/TES research. To further the understanding of E/TES application, subsequent studies should also perform a Delphi study with Indigenous and non-Indigenous researchers from various E/TES programs and inquire them about how workplace relations, power dynamics, and advisory bodies impacted their research experience and team dynamics. This would allow researchers to compare different application strategies and identify the best practices for equitable collaboration. In addition, ethnography can provide direct observations of the E/TES research environment, reducing reliance on secondary participant recounts. Such an approach helps mitigate social-desirability biases, enhancing the accuracy and authenticity of the data.

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ChatGPT Usage among Teens in Humanities Courses

Harshavardan Gunasegaran

Abstract : The artificial intelligence program ChatGPT has become increasingly prominent within society, especially in education. It is known for its ability to generate quick responses to prompts, regardless of the discipline. Pre-existing research focuses on issues of AI-related academic integrity, but few identify the motivation for its usage and for what specific tasks. Correlational and thematic analysis was conducted through a mixed-methods approach, in the form of a survey and interviews, identifying the specific tasks for which high school students within the Greater Toronto Area use ChatGPT and their motivations. The results revealed that students primarily use it for writing essays and research papers, based on the time they have to complete an assignment, interest in the course, and character of the assignment (formative vs summative). This research helps humanities teachers and educational policymakers alter their coursework such that students continue to develop the necessary humanities skills despite the growing presence of AI.

Keywords: ChatGPT, high school, mixed method, humanities, GTA (Greater Toronto Area)

Introduction

Artificial Intelligence (AI) is a computer system that can simulate a human's ability to think, learn, and adapt (Stryker et al., 2024). Though the pace of innovation in artificial intelligence grew significantly during the 20th century, it only gained major recognition in the past five years, following the introduction of OpenAI's third-generation ChatGPT, which attracted a million users in less than five days (Reynoso, 2019; Marr, 2023). From creating essays to debugging Python code, its versatility is increasing. However, its unprecedented abilities have given rise to many questions throughout the educational community on the ethics of its usage and its acceptance in academic institutions. Many sources highlight the significant increase in the usage of AI, specifically in educational contexts. Prothero's (2024) findings indicate that 63% of American undergraduates were accused of using generative AI like ChatGPT in 2023, and 1 in 10 assignments submitted through Turnitin contained some traces of AI-generated content. However, there is not enough emphasis on what specific fields of study (STEM, Humanities, Arts, etc) utilize these tools. Knowing which fields rely on AI the most can help conclude what ChatGPT is most capable of based on what it's being used for the most. This data can then be used to inform educators about what they would change about the coursework to ensure the students make the most out of their classes. Due to the majority of AI studies focusing on AI's ability to code and its ability to write thought-provoking essays, this paper will focus on ChatGPT's impact on humanities courses. There is also another gap regarding the focus on largely post-secondary populations, with studies like Wood and Moss (2024) examining graduate students' usage of generative AI without addressing its impact on younger populations, especially today's

teenagers, who were introduced to such resources earlier on. Finally, a majority of these studies are based in the USA, like Ngo and Chng et al.'s 2023 studies. As a result, this study will be conducted on Canadian teenagers, specifically those in the Greater Toronto Area (GTA). This leads to the research question: To what extent is ChatGPT utilized by high school students in humanities courses across the Greater Toronto Area to complete schoolwork? Correlational research will be done through surveys to gather data on usage patterns between those who take humanities courses. Qualitative analysis will be conducted through interviews with humanities students who will be asked questions on whether they use ChatGPT for problem-solving, coding assistance, etc. In contrast, humanities students will be asked questions on how they use it to analyze texts, generate essay topics, etc. Understanding the extent to which ChatGPT is integrated into the academic practices of high school students in the humanities provides educators with insights so they can tailor their teaching strategies accordingly.

Literature Review

The Role of ChatGPT in Education: Applications and Prevalence

ChatGPT and other learning AI tools are increasingly prevalent in recent times due to their expansive applications in education. Gonzalez (2024) states that nearly half of the students polled in the Tri-State Area indicated that they used ChatGPT for schoolwork. ChatGPT is beneficial in offering instant responses to learning questions, aiding in writing essays, and summarizing content, thus making it a viable option for students who would like to do academic work with ease. Research by Sumakul et al. (2022) indicates that AI tools like ChatGPT are used extensively for various learning activities such as tutoring, grammar checking, and improving writing structure. As Fitria (2023) explains, ChatGPT has also impacted English as a Foreign Language (EFL) classes, with students using the AI tool to improve writing and avoid grammar errors. Moreover, ChatGPT is also useful for lecturers, as it can prepare course materials, offer ideas for syllabi, and even construct tests or activities (Lo, 2023). As good as these benefits are, greater dependence on AI in academia evokes concerns about whether there is a possibility for excessive use of such tools. Despite ChatGPT's benefits, there is increasing doubt about its impact on student participation in learning and intellectual development. As per Chen et al. (2020), while it may make students more efficient, it may in turn lower students' motivation to study content thoroughly, especially if they would rather use AI to do the work.

Student Perceptions of ChatGPT and Its Impact on Learning

Students have completely different attitudes regarding the effectiveness and perceived value of ChatGPT as a learning tool. Studies show that several students find the AI tool beneficial, but others are worried about its ability to disengage students and deter them from critical thinking. In Tossell et al.'s study (2024), students using ChatGPT to complete a college essay assignment expressed initial skepticism because of the perceived risks of cheating and academic laziness associated with the use of the tool. After completing assignments using ChatGPT, a considerable number of students did, in fact, report a positive attitude shift, claiming they started viewing the tool as a collaborator rather than a shortcut. This positive attitude shift is consistent with the study conducted by Jen and Rahim (2024), as they report that students taking writing classes appreciated having the support of AI in enhancing their essays as well as argument structure, but were also worried about the over-reliance on AI. Students have expressed appreciation for ChatGPT as a resource that can enhance writing skills, but doubts about its impact on self-learning capabilities remain unsolved. Fitria (2023) notes that some students feel less driven to engage with materials or assignments that require lots of reading when there is an option to just rely on ChatGPT's generative ability to provide responses or summaries. Such reliance can impede students' ability to engage in higher order reasoning as well as develop critical thinking skills, both of which are important for learning and achieving academic success. While students tend to view ChatGPT positively as a convenience, they also regard it with some degree of skepticism since a significant number of students are concerned that it would negatively affect their level of intellectual engagement. The problem remains as to how to apply AI to improve learning while

preventing the erosion of critical and independent thinking skills among learners.

Ethical Implications and Academic Integrity in the Use of ChatGPT

As ChatGPT becomes adopted more in students' academic-related activities, major ethical concerns associated with its usage, particularly around academic integrity, are increasingly emerging. With AI being used in composing essays, calculating math questions, etc., numerous teachers are concerned about the misuse of this technology (Chounta et al., 2021). Studies suggest that AI applications such as ChatGPT can generate essays at par with human writers, which raises the worry of academic fraud. Research by Jen and Rahim (2024) found that 40% of the students had actually utilized AI tools for homework and viewed it as the new norm, with some stating it as a normal routine. This proves that the enforcement of not using AI tools is becoming a challenge. Waltzer et al. (2024), along with Liu et al. (2024), both found that teachers can only confirm ChatGPT-written essays as such 70% of the time, and that students did worse at an accuracy of only 60%. Gonzalez's (2024) study and Baskara's (2023) study discover a paradox: students claim to know about academic integrity, yet students use ChatGPT for academic work. Similarly, Abdelaal et al. (2019) learned that the majority of students do not see AI-assisted writing as misconduct. Many students also justified their ChatGPT usage. They stated it enhances the quality of their work and saves time, proving how AI is being normalized within education (Abdelaal et al, 2019). This shift is giving concern to instructors who are tasked with maintaining academic integrity. Haleem et al. (2022) suggest that schools use AI detection software to prevent further unethical AI usage. He says it is necessary for educators to have conversations with students about the unethical usage of AI to increase their awareness. As AI usage increases, traditional assumptions about plagiarism and cheating are being questioned on a continuous basis. Al Fraidan (2024) advocates for a balanced strategy for AI in education, suggesting that we focus on making our technology more suitable for detecting AI while also making classwork that is not easily replicable by an AI. This ensures that students are still able to benefit from their classwork.

Gap

The increasing presence of artificial intelligence writing tools in today's classrooms has sparked considerable academic discussion, particularly as students incorporate platforms like ChatGPT into their daily schoolwork. The body of literature explores ChatGPT's usage in the context of education prevalence among students, its impact on their writing and learning abilities, and whether it is inducing any academic dishonesty. Many of the sources focus on how university students use these AI tools, and the academic dishonesty issues that come with it, but there is little research done on the specific tasks and motivations behind students, specifically on the usage patterns of high school students. Furthermore, since many humanities courses are vital for developing writing and critical thinking skills, we need to understand how ChatGPT is influencing the way students approach their assignments and how much they are actually learning. A study has yet to be conducted which truly showcases any potential correlation between ChatGPT's explicit integration into the academic practices of high school humanities students. This leads to the research question: To what extent is ChatGPT utilized by high school students in humanities courses across the Greater Toronto Area to complete schoolwork?

Methodology

This mixed method approach began with a review of the survey and interview questions by an expert advisor, followed by approval by an ethics board to ensure the mental health of the participants would not be harmed. The quantitative data involved surveying high school students (grades 9-12) from various schools to mitigate socio-economic limitations. As minimal pre-existing studies relate to this study, their survey questions did not align with this paper's research. As a result, unique questions were created such that they would fit the study while being easily comprehensible to the young demographic. The survey contained Likert scale and multiple-choice questions to gather data on the frequency of ChatGPT usage for academic purposes (both summative and formative work) and the specific tasks for which it was used in a humanities context (e.g., generating ideas, summarizing content, writing essays). The survey also

assessed ChatGPT's perceived effectiveness (e.g., clarity on topics, marks received for AI-generated content and awareness of academic integrity issues related to posing AI writing as their own). Correlation analysis was conducted between the frequency of use and perceived reliability, teacher communication and formative use, as comparing the frequency with these three helps determine whether ChatGPT substitutes or supplements traditional learning, and how trust in AI impacts usage patterns. The other questions like those regarding guilt or student experiences with inaccuracy within ChatGPT were created to give a broader understanding of the student perspective. The Spearman coefficient was calculated for all three comparisons to accurately determine any correlation through Google Sheets. Students who indicated on the survey that they used ChatGPT for academic tasks and were willing to be interviewed were asked to provide their contact information. This was the final question on the survey, and participants were reminded that their personal information would not be shared in the study. I would contact them through email within one day to set an interview date with them and send them the consent form so that they could agree to all terms before the interview. Interviews were conducted via recorded Google Meets, with questions tailored to uncover the root causes of ChatGPT usage. It focused on three open-ended questions that explored when they started using AI, specific tasks that made AI an attractive option, and whether their reasons for using it evolved, keeping the questions neutral to prevent leading them toward predetermined answers. A thematic analysis was conducted to identify common themes (if any) among the interviewed participants. Taguette, a free software tool, was used to analyze the qualitative data. This application was used to encode specific sections of text, simplifying the process of identifying recurring patterns or themes. To encode a segment, the text is highlighted, and a tag is assigned. New tags (or codes) can be created as needed. Taguette also supports assigning multiple tags to a single text segment if it contains diverse ideas. Once the tagging was complete, the data was exported to Google Sheets to be viewed with clarity.

Sample

The study included 75 participants for the quantitative data (surveys) and 15 participants for the qualitative data (interviews). Participants were recruited from single-sex and co-ed schools throughout the GTA. Recruitment was conducted through social media platforms like Instagram and Discord, email outreach to various schools, and a few of these students shared this link into their own social accounts with my permission. This allowed me to meet my sample size and get a variable audience from multiple schools throughout the GTA.

Justification

Lo (2023) conducted a similar study on American university students in a virtual school, without focusing on specific subjects and without identifying key variables. His study included 75 participants for surveys and 10 participants for interviews. His sample size was used as his study is most similar to my own study, but he stated that he did not find many themes with just 10 participants; thus, this study has five extra qualitative participants given the data-gathering period. The quantitative data was examined by calculating the Spearman coefficient. Gonzalez (2024) used the Pearson coefficient to compare between age and other variables. As this study was different, the correlation between the frequency of usage and the other variables listed in the previous sections were found. She suspected a linear relationship between two variables and that the data were normally distributed. However, in my case, it was not necessary for the relationship between my variables to be monotonic, as the variables tended to change together rather than at a constant rate (Schober et al., 2018). Thus, I still conducted a correlational analysis but calculated the Spearman coefficient rather than its counterparts, as it was more fitting for my data. The qualitative responses were analyzed thematically, similar to Shoufan (2023) who did a thematic analysis of student opinions related to ChatGPT. She utilized Taguette, which was also utilized in this study as a tool to encode and organize qualitative data by tagging specific text segments, thereby enabling the identification of patterns and themes for further analysis in Google Sheets. As a free tool with an understandable user-friendly interface, it proves to be the best tool for this research.

Findings

Quantitative

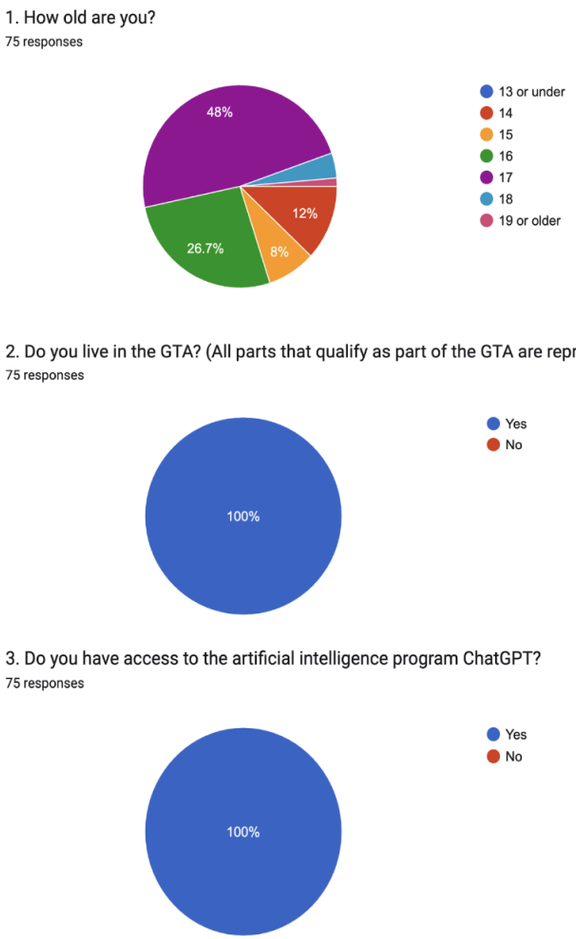
Of the 75 participants who completed the survey, one of them was 19 or older, thus, not fitting the demographic. All other participants fit the demographic, stating that they had access to ChatGPT and resided in the GTA. Note that the numbers below the bar graphs represent Likert scale values, with 5 being always, and 1 being never. This convention is used throughout this report whenever discussing Likert scores.

Incidence of ChatGPT Utilization in Humanities Homework

Many high school students in the GTA area use ChatGPT in humanities homework. Of these, 23.7% either never use it or use it sometimes, while 21.1% use it rarely or most of the time. Students most commonly

Figure 1

Demographic Questions (age, where they reside, access to ChatGPT)



employ ChatGPT in essays (53.3%), research papers (37.3%), and creative writing assignments (28%). Among those who employ ChatGPT for essays, 88% use it to brainstorm ideas, 68% turn to ChatGPT to summarize lengthy texts, and 50% use it to proofread/grammar check. Of those who use it for research papers, 76.5% use it to brainstorm ideas, 64.7% to summarize lengthy texts, and 47.1% to grammar check. In creative writing, 80.8% use it to brainstorm ideas, while 50% use it to summarize lengthy texts, analyze course materials, and resolve writer's block.

Figure 2

Frequency of Usage and Specific Tasks Used For

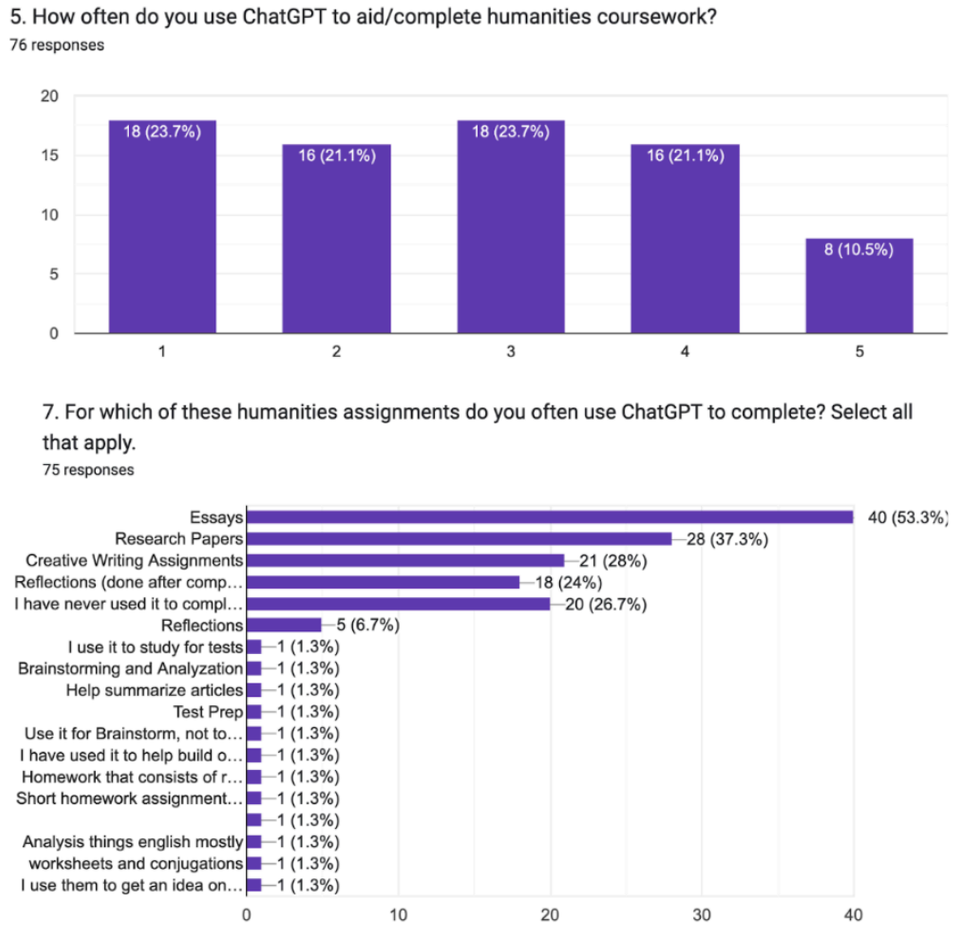
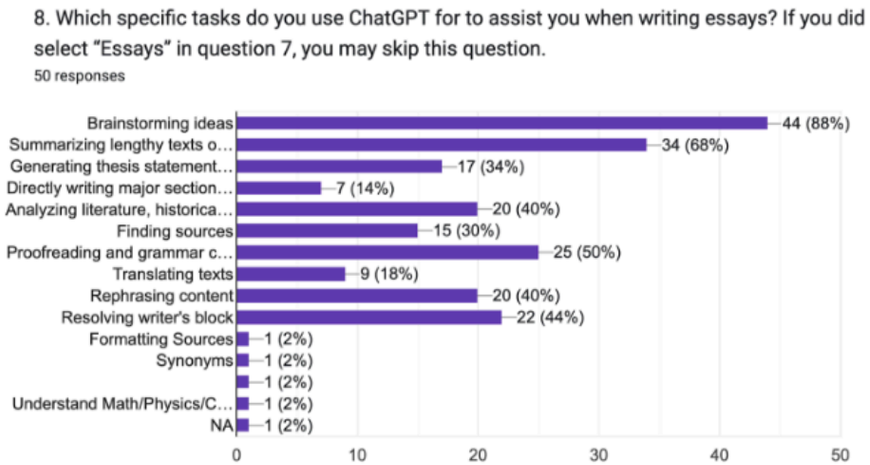
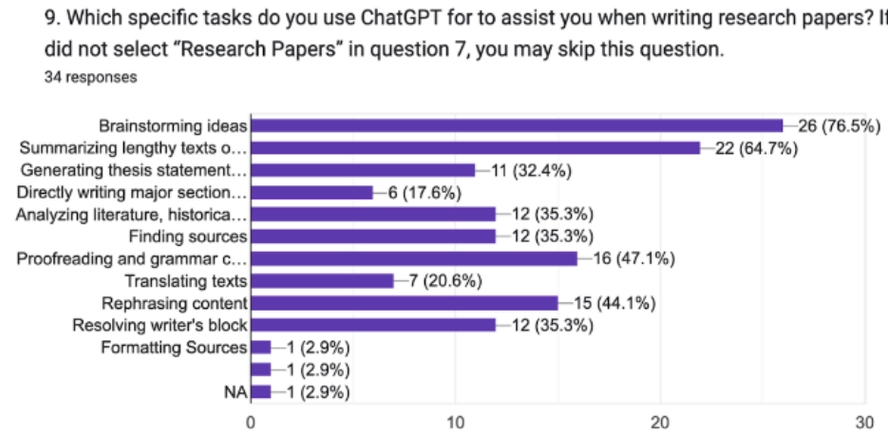
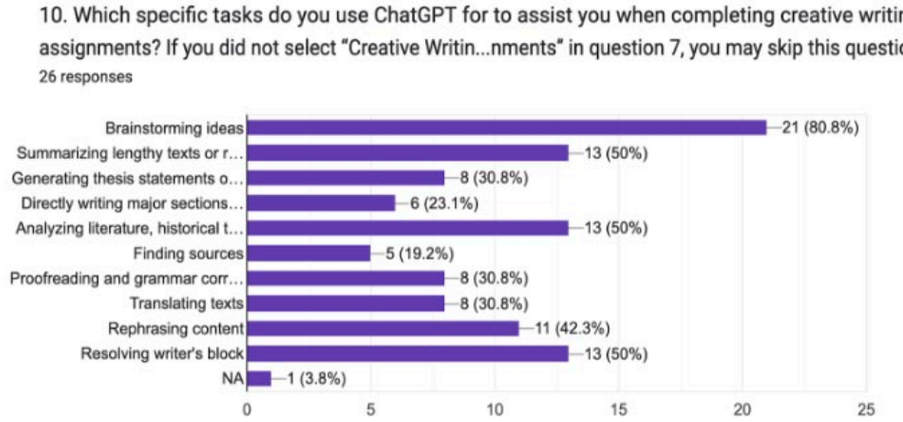


Figure 3
How Used for Writing Essays, Research Papers, & Creative Writing





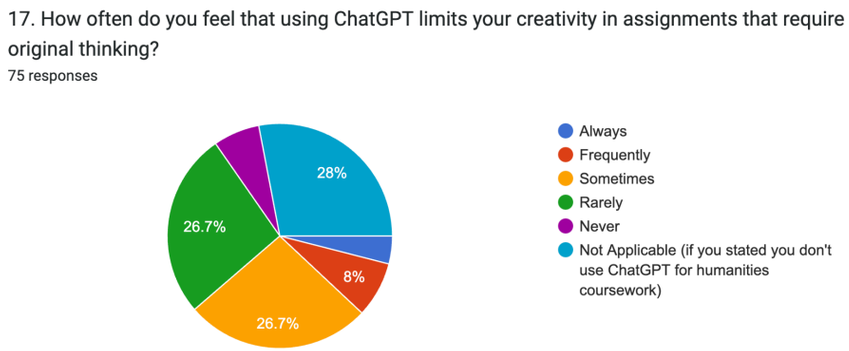


Grades, Perceived Confidence, and Creativity in ChatGPT-Assisted Work

Approximately 9.3% of students indicate that work generated by ChatGPT always scores better than work done by themselves independently, while 17.3% indicate this happens rarely, and 28% indicate this happens sometimes. Meanwhile, approximately 14.7% always have greater confidence in work done through ChatGPT, while 30.7% have greater confidence most of the time. It's also important to note that around 26-30% of students indicated they do not use ChatGPT for humanities courses. Approximately 28% of students feel ChatGPT always limits their creativity in assignments requiring original thinking, while 26.7% say it sometimes does.

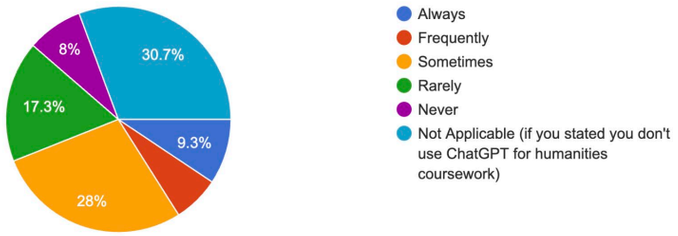
Most students use it for formative tasks (26%) compared to summative tasks, whereas 23.7% of students selected a 1, indicating they use it for summative tasks more than formative ones, and 22.4% also selected 3, indicating they use it for both formative and summative tasks.

Figure 4
Grades and Confidence of ChatGPT



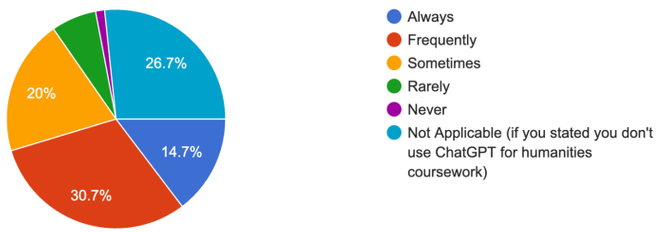
18. How often does ChatGPT-generated content receive better marks than your independently written work in humanities courses?

75 responses



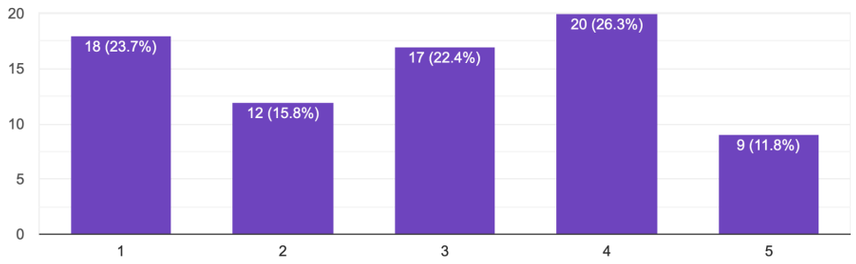
19. How often do you feel more confident in your humanities assignments when you use ChatGPT to assist with them?

75 responses



6. How often do you use ChatGPT for formative tasks (coursework used to check your understanding) rather than summative tasks (cours...butes to your grade) in your humanities courses?

76 responses



Efficiency and Reliability of ChatGPT in Humanities Homework

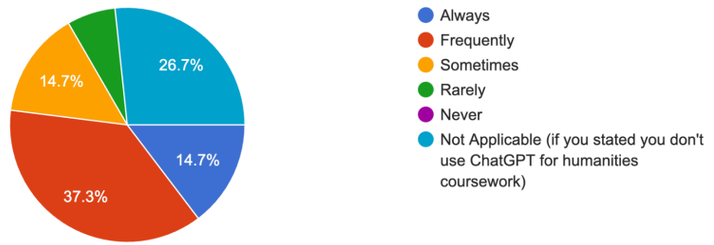
Around 37.5% of students regularly employ ChatGPT to work through homework faster without sacrificing work quality, while 14.7% always think this is helpful in this respect. On the other hand, 6.7% rarely ever think this helps improve efficiency. Regarding reliability, while 6.7% think ChatGPT is extremely reliable, 25.3% think ChatGPT is a 4 in reliability, while 33.3% think ChatGPT is a 3 in reliability.

Figure 5

Reliability and Productivity of ChatGPT

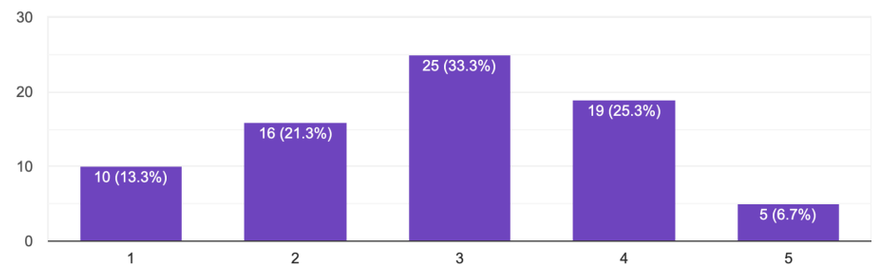
20. How often does using ChatGPT help you complete your assignments faster without compromising on the quality of your work?

75 responses



21. How often do you perceive ChatGPT as a reliable resource for generating accurate and relevant information for humanities tasks?

75 responses

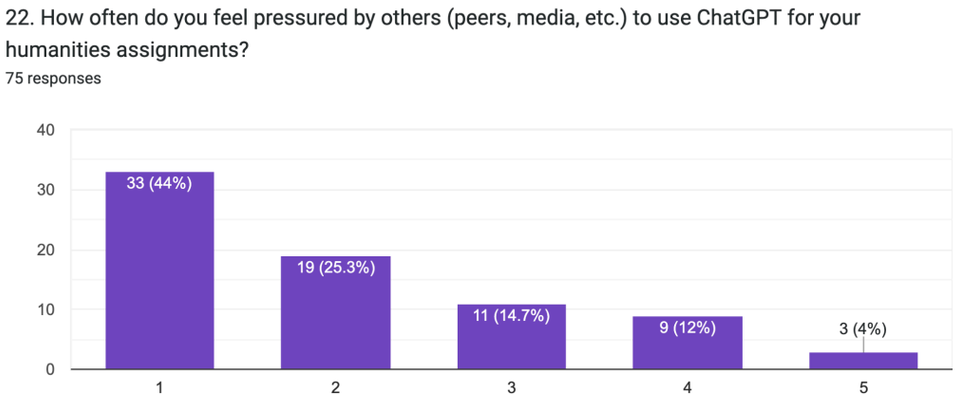


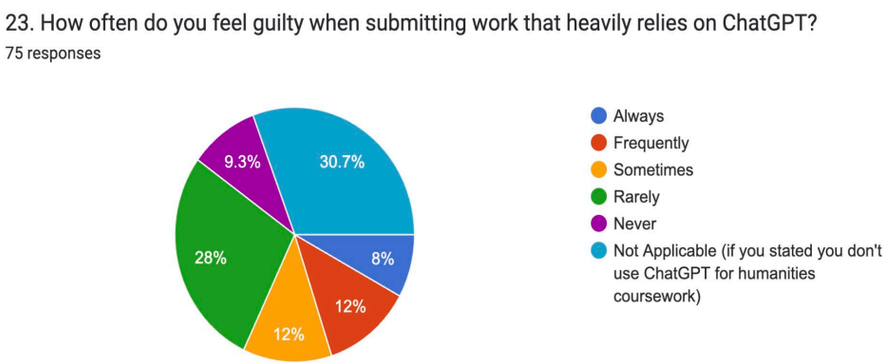
Social and Ethical Considerations

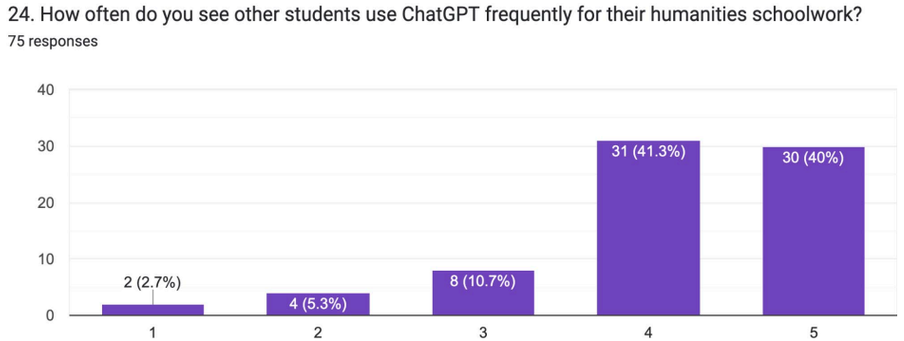
Among students, 44% indicate that they never feel pressured by others to employ ChatGPT, while 25.3% sometimes feel pressured to employ ChatGPT, and only 4% always feel pressured to employ ChatGPT. Additionally, while only 8% always experience guilt in turning in work that is heavily reliant upon ChatGPT, 12% frequently experience guilt in this respect. On the other hand, 28% rarely ever experience guilt in this respect. Regarding peer usage, 41.3% of students frequently see their classmates use ChatGPT for humanities coursework, rating its usage as 4 on a scale of 1 to 5, while 40% rate it as 5, indicating very frequent use.

Figure 6

Peer Pressure and Guilt through ChatGPT Usage





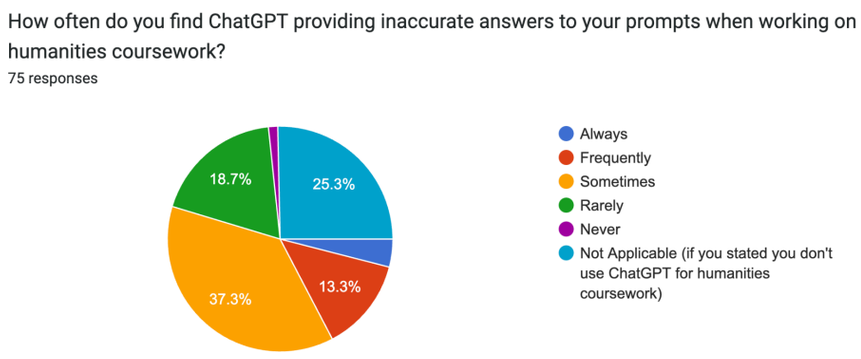


Accuracy and Behaviour in Checking Facts

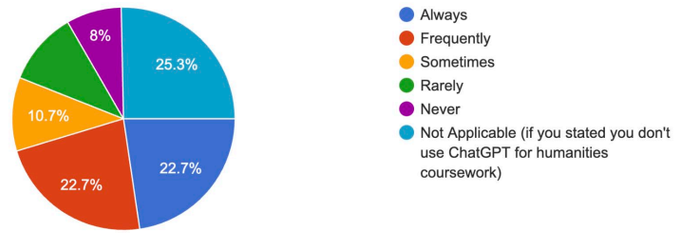
Around 18.7% rarely experience inaccuracies in ChatGPT use in humanities courses, while 13.3% experience this frequently and 37.3% experience this sometimes. However, only 22.7% always or frequently check ChatGPT responses to be accurate, while 8% never check content produced by ChatGPT to be accurate. Around 32% of students report that ChatGPT sometimes reduces their motivation to seek help from teachers or peers, while 21.3% say it always does.

Figure 7

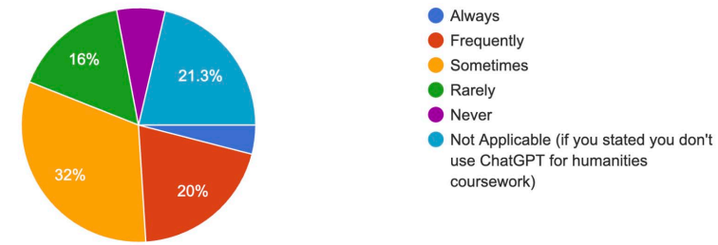
Accuracy and Students' Willingness to Fact-check ChatGPT Content



26. Based on the previous question, how often do you verify or fact-check the information provided by ChatGPT?
75 responses



14. How often has the use of ChatGPT reduced your motivation to seek help from teachers or peers?
75 responses

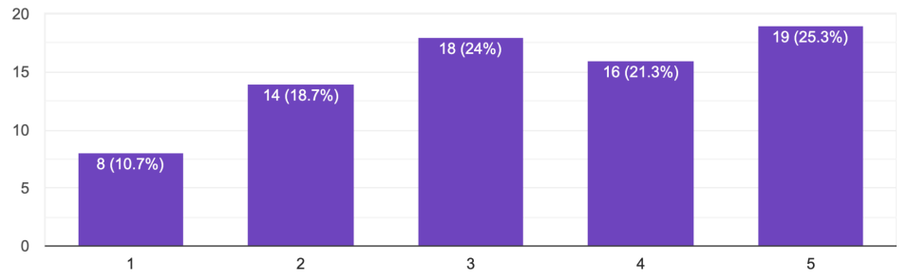


Clarity in Teacher Guidelines regarding ChatGPT Utilization

In terms of guidance by teachers regarding ChatGPT utilization, 25.3% report that rules have been explained by teachers clearly (a grade of 5), while 21.3% grade transparency an overall grade of 4, 24% grade an overall grade of 3, while 29.4% grade an overall grade of 2 or below, an indicator of mixed awareness regarding proper AI utilization in humanities courses.

Figure 8
Teacher Communication of ChatGPT

27. How often have your humanities teachers clearly communicated the acceptable uses of ChatGPT in their courses?
75 responses

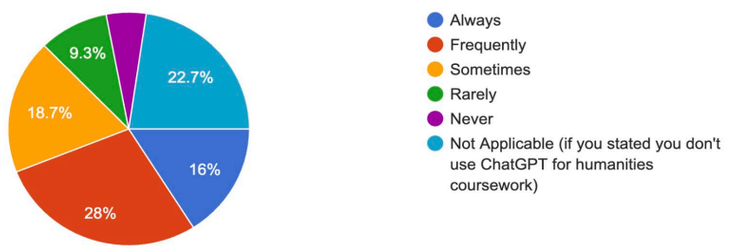


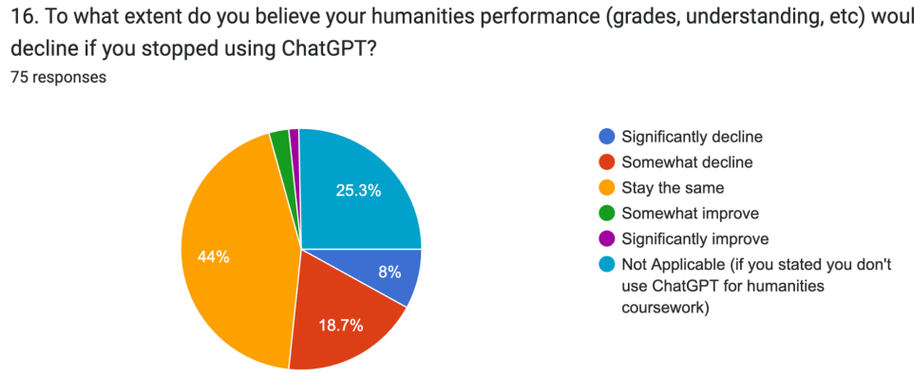
Over Reliance on ChatGPT and Impact on Performance

Regarding the use of ChatGPT despite being able to complete a humanities task without it, 28% of students say they sometimes choose to use it, while 22.7% say they always do. Regarding performance, 44% of students believe their humanities performance would stay the same without ChatGPT, while 25.3% believe it would significantly decline.

Figure 9
Overreliance and Impact on the Performance of ChatGPT

15. How often do you feel you could complete a humanities task without ChatGPT, but still choose to use it?
75 responses





Qualitative

Below are direct quotations from the 15 interviews conducted, from a variety of high school students from different schools and ages. They have been filtered such that each quote represents a unique idea rather than having multiple quotes that state the same thing. They are organized into three main themes: Factors Influencing ChatGPT Usage, Reliance on ChatGPT, and Challenges of ChatGPT.

Figure 10
Qualitative Themes and Corresponding Quotes

Theme	Direct Quotes from Interview
Factors Influencing ChatGPT Usage	- "It mostly depends on whether the assignment is summative or not." - "If the assignment specifically says not to use any AI, I might use ChatGPT to generate some initial ideas to get started, but I won't rely on it for the actual content." - "One key factor is whether or not I'm allowed to use it. If I'm not allowed and I get caught, that's not good." - "If a teacher, like, openly says that they'd allow ChatGPT, then I'd probably use it to help me a little bit." - "I use ChatGPT when I'm stuck or need help brainstorming ideas for writing." - "It really just depends on how long I think something will take." - "It's more just, like, how confident am I? What mood am I in? Do I want to put in all the work, or do I, like, want to maybe use it for some brainstorming?"
Reliance on ChatGPT	- "I feel less reliant on it now that I'm in grade 12, but definitely earlier in high school, I relied on it more." - "I think when it first came out, I was a little skeptical. Some people took to it more quickly than I did." - "At first, it was kind of a joke, like people would say they wrote everything with ChatGPT, but now it's normalized, and everyone talks about it." - "I feel like I rely on it more now, especially for humanities courses, like English. I'm not a very strong English student, so it's harder for me to come up with ideas on my own." - "Yeah, I'd say I've become more dependent on it, not because I can't do my work without it, but because it's become more popular." - "My use of ChatGPT for humanities has stayed pretty low. I haven't become too reliant on it, and it hasn't really evolved for me." - "So, I'd say I'm more reliant on it now than when I first started using it."
Limitations and Challenges	- "A challenge is that it can make you too reliant on it, which means you might lose the ability to come up with ideas on your own." - "One challenge I've encountered is that when I ask ChatGPT for quotes on a specific theme or character from a book, it often messes up the page numbers and line numbers." - "I think the biggest challenge is that sometimes it can be inaccurate or contradict itself. When I ask it to clarify, it admits that it's wrong." - "It's not going to be perfect and might not provide the best information or writing structure. So, it's important to review it carefully." - "For me, uh, I don't think it has improved the quality of my work. It mainly helps with efficiency—speeding things up." - "ChatGPT does a really good job of, like, coming up with ideas. But it might use really overly complicated words where, like, you kind of don't understand what's going on." - "Sometimes it straight-up makes things up, and you really can't have that in humanities courses. Accuracy is really important." - "I personally don't think it writes very well, so I don't rely on it for writing."

Discussion

Quantitative

In order to ascertain the extent to which students in humanities classes in the Greater Toronto Area use ChatGPT, several factors were examined related to the frequency of its use. These factors give us an understanding of how students implement the use of ChatGPT in their work as well as the type of use. Specifically, I examined the correlation between frequency of use and perceived reliability, teacher communication and formative use through Google Sheets, and calculated the Spearman coefficient.

First, usage frequency vs. perceived reliability indicates how the perception of the tool by the students can impact their usage pattern. Whether the students use ChatGPT frequently and think it is reliable can reflect whether the tool is appreciated as a valuable and reliable tool for completing schoolwork. The moderate positive correlation of 0.527 suggests that the higher the usage frequency of the tool by the students, the higher the perception of reliability. This suggests the frequent users of the tool can be more confident in its capability, which can encourage them to use it more. Second, usage frequency vs. teacher communication investigates the frequency of communication by teachers about the acceptable use of ChatGPT in the classroom. The relationship between the two variables can be used to ascertain whether increased usage of ChatGPT is related to decreased direct instruction by teachers. The moderate negative relationship of -0.527 shows that students with greater usage of ChatGPT are less likely to have been given clear instructions or rules by teachers about its acceptable use. This can be taken to signify that teachers are not talking about its use in the classroom, or students with greater exposure to the tool are less likely to be looking for such instructions. Finally, usage frequency vs. formative use examines whether students are using ChatGPT for more formative (developmental, continuous) or summative (end-of-unit, evaluation) work. This relationship is important in understanding the nature of the role of the use of ChatGPT in the learning process. The negative correlation of -0.527 shows that the more students use ChatGPT, the less likely they are to use it for formative work (checking for understanding) and the more likely they are to use it for summative work (end-of-unit work for grade contribution). After calculating the p-value for all three correlations, they were all found to be less than 0.05, demonstrating that the results are statistically significant and are unlikely to have occurred by chance.

Qualitative

Factors Influencing ChatGPT Usage

Students have listed a series of determining circumstances that determine whether they will use ChatGPT on a particular assignment. The common ground is that time is a key consideration, with students expressing that they turn to ChatGPT whenever they have a deadline. In circumstances with deadlines, students employ the application in a bid to summarize, do quick research, and format assignments. Another key consideration is the character of the assignment. Students employ ChatGPT to brainstorm and for formative work (work used to check their understanding), but not for summative work (work counting towards their grade), as they do not want to be

involved in academic dishonesty. Some respondents also noted that whether AI tools are allowed or not is a determining factor in whether they will use ChatGPT. Lastly, students' interest in a course also determines whether or not they will use ChatGPT. Assignments that are considered dull or repetitive are likely to be worked on with AI assistance, as students will work on subjects that are of interest to them on their own. Overall, research points out that although ChatGPT is utilized to some extent by a majority of the students for humanities, its usage is variable with students' inclinations, assignment structures, and academic institution requirements. The software is valued in terms of its efficacy, though students are aware of its limitations.

Reliance on ChatGPT

Students' application of ChatGPT is also observed to have evolved. The majority reported increased application from the time that they originally got accustomed to it. Students originally utilized ChatGPT as a novelty, though over time, it more frequently became a more integral part of students' academic work, particularly in formative work as well as preparation.

However, not all students have developed a greater reliance on ChatGPT. A minority of respondents noted that their reliance on the instrument had decreased as they gained more confidence in writing. Some students noted a shift in attitude, realizing that while ChatGPT is efficient, it does not necessarily enhance work quality.

Challenges of ChatGPT

While recognizing its strengths, students identified a series of limitations in ChatGPT in humanities course work. The first is that ChatGPT is not that in-depth in its responses. Some students noted that though ChatGPT is able to generate broad ideas, its answers are shallow or repetitive in a sense, which makes it not as effective in courses that require critical analysis. Another restriction stated by more than half of the participants is that ChatGPT is not necessarily always accurate. Some students observed that ChatGPT can produce incorrect or misleading information, particularly in literary or historical interpretation. In many cases, they say that if you challenge it, it admits its mistake, raising questions on its reliability. Some also observed that AI-written material can be unnatural-sounding or robotic-sounding, making it identifiable as AI-written material by both teachers and examiners, thus not making it a popular choice for writing-driven courses. Numerous students reported exercising caution in using ChatGPT on graded work because they did not intend to be caught committing plagiarism. Some students also confessed that they had a personal aversion to over-dependence on AI because they did not intend it to undermine their critical thinking or independent reasoning ability in the long run.

Limitations

While I recruited participants with diverse backgrounds from various schools, my study still faced certain limitations in the data-gathering process. I recruited participants through my school connections, social media platforms, and personal contacts, but being an individual high school student, my influence was limited. This limited the capacity to get a fully representative sample of the wide demographic range. Additionally, my recruitment approach—through the utilization of social media platforms such as Instagram and Discord—may have inadvertently recruited students already knowledgeable about or using digital learning tools. The sample may thus be biased towards AI tool users such as those familiar with the use of ChatGPT, which constrains the ability to generalize the results to students who do not typically use such tools. Finally, being a high school student with limited time for the study, I could not further increase the sample size or explore other qualitative questions in more detail, constraining the range of information I could analyze.

Future Direction

Despite the constraints, the data obtained in this study provides valuable insight into the application of ChatGPT by high school students in the Greater Toronto Area. The data reveals that the students use ChatGPT for several purposes in their studies, including the generation of ideas, summarizing text, and writing essays. The widespread application of the tool indicates the extent to which AI tools are part of the students' learning process. The teachers can apply the data to alter their teaching strategy by indicating how AI can be applied to enhance the critical thinking skills and writing skills of the students while ensuring the students are not too dependent on AI-based content. The study also highlights the requirement for teachers to make the rules for the acceptable usage of AI tools clear. Most of the students indicated that the teachers did not make the rules clear. This is an area for the teachers to devise effective policies for the responsible usage of AI while maintaining the integrity of academics.

Conclusion

From the study results, it can be asserted that the students in the Greater Toronto Area have a significant but diverse application of ChatGPT in their humanities courses. While the majority of the students see the tool as useful for brainstorming, summarizing, and outlining, the application is mostly dictated by the deadline, assignment type, and course nature. While it is considered useful to many, there are still a few students who are reluctant to be too reliant on AI, particularly in the area of critical thinking. This study points towards the growing use of AI tools like ChatGPT in the lives of students, with broad-reaching ramifications for education and learning. However, many variables are still yet to be studied, such as the use of AI in other creative subjects such as artwork or scriptwriting in drama. How AI tools like ChatGPT affect the creativity of students in such subjects may provide a broader insight into the use of AI in education. Further study could also examine how students use AI outside the classroom, such as in extracurricular activities or independent projects, which may provide insight into its use outside the school setting. This gives us deeper insights into the impact of AI tools on teenage lives.

Acknowledgements

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CHATGPT USAGE AMONG TEENS IN HUMANITIES COURSES

Molecular Diagnostics and Genetic Testing in Cancer: An Experimental Study of KRAS Mutations and a Case of CHEK2-Linked Breast Cancer

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Abstract: Hereditary factors significantly influence cancer risk, particularly through mutations in tumor suppressor genes and oncogenes. This study investigates the role of inherited gene mutations in cancer development, focusing on familial cancer syndromes such as those involving *BRCA1/2* and *KRAS* genes. A *KRAS* mutation analysis was conducted using RT-PCR on tumor samples, revealing mutations in codon 12 in 3 of 12 cases. Additionally, a case study using whole-exome sequencing of a BRCA1/2-negative breast cancer patient identified a *CHEK2* mutation, emphasizing the diagnostic value of extended genetic screening. These findings underscore the clinical relevance of identifying hereditary mutations for early diagnosis, treatment planning, and genetic counseling. The research also explores ethical considerations and the psychological impacts of genetic testing. By combining molecular diagnostics and familial history, the study supports a personalized medicine approach for cancer prevention and management.

Keywords: Hereditary cancers; molecular diagnostics; genetic testing; KRAS mutations; RT PCR

1. Introduction:

Overview

Cancer is caused by the accumulation of gene mutations that control the cell's growth and multiplication, leading to cancerous growth over the years. Aberrant gene function and altered patterns of gene expression are key factors responsible for cancers. These alterations can happen because of random modifications of the genetic code, carcinogens in the environment that change the DNA code, or mutations can be inherited from the previous generation. Cancer is among the leading causes of death worldwide. There were almost 20 million new cases of cancer and 9.7 million deaths due to cancer in 2022. By 2040, the number of new cancer cases per year is expected to increase to 29.9 million, while the number of deaths is expected to rise to 15.3 million (International Agency for Research on Cancer, WHO).

The genetic changes that contribute to the existence of cancer mainly tend to affect three types of genes: tumor suppressor genes, DNA repair genes, and proto-oncogenes. Proto-oncogenes are a part of normal cell growth and division. However, when these genes are mutated in certain ways or become more active than they normally would be, they may become cancer-causing genes (or oncogenes) that allow cells to survive and grow when they should not.

Tumor suppressor genes are also involved in the control of cell growth and division. If tumor suppressor genes are altered, their cells may divide uncontrollably. Additionally, damaged DNA is fixed by DNA repair genes. Cells with mutated versions of these genes have a tendency to develop changes in their chromosomes, for example, deletions and duplications of chromosome parts, as well as additional mutations in other genes. All of these mutations together can lead to cells becoming cancerous.

In this study, I delve into understanding the role of hereditary factors in cancer risk by summarizing the research on familial cancer syndrome. Further, I have performed molecular methods (RT-PCR) to identify gene mutation on a dummy tumor sample to comprehend the role of diagnostics of gene mutations and their application in cancer treatment.

1.1 Familial and sporadic cancer

Cancers are generally categorized as hereditary (familial) and sporadic (non-hereditary) types (Roukos et al., 2007). This categorization was made when researchers identified highly penetrant and rare germline mutations. These germline mutations are known to cause hereditary cancer. In contrast, most cases of cancer in the general population are known as sporadic as they occur at random and have no germline genetic component. These are not heritable (Lu et al., 2014).

1.2 Implications of Hereditary Cancer Syndrome

Inheriting genes with mutations is called hereditary cancer syndrome which increases the chances of developing cancer (Imyanitov et al., 2023). Hereditary cancer syndromes are the most common type of vertically transmitted diseases that result in a higher risk of cancer development. Since the only difference between people with hereditary cancer syndromes and healthy people is the higher chance of developing cancer, they generally don't have any visible phenotypic problems. Most hereditary cancer syndromes are transmitted through autosomal dominant mechanisms, meaning they have a Mendelian mode of inheritance (Imyanitov et al., 2023).

The highest contributors to cancer morbidities are Lynch syndrome and hereditary breast cancer. **Lynch syndrome** is a classic example of hereditary cancer syndrome, also known as hereditary non-polyposis colorectal cancer, and is the most predominant cause of predisposition to colorectal cancer. There is convincing data that highlight that patients with Lynch syndrome, a hereditary predisposition to endometrial and colorectal cancer, will develop ovarian cancer more frequently than in the general population. (Pietragalla et al., 2020).

1.3 Role of genetic mutations and their significance in cancer risk

Chemicals such as benzene or biological factors such as viruses can induce mutations that occur spontaneously. Not all mutations will cause observable alterations in cellular functions. However, there are certain key cellular genes, and mutations in them can cause developmental disorders. This acts as one of the main ways by which proto-oncogenes can change to their oncogenic state. The progressive accumulation of many genetic variations throughout one's life causes cancer. In the last few decades, there has been extensive research on cancer biology which has found that many pathways and genes play a role in the development of cancer. Some of the most common mutations are because of alterations to members of the KRAS, ErbB family, BRCA1/2, TP53, BRAF, p16, PIK3CA, FGFR2, AKT, and MAP2K1 gene. (Paul et al., 2019).

1.3.1 BRCA1 (BReast CAncer gene 1) and BRCA2 (BReast CAncer gene 2) are tumor suppressor genes. Inheritance of a mutated copy of either one or both genes increases the risk of ovarian and breast cancer. People who inherit a mutation in the BRCA1 or BRCA2 gene show a tendency to develop cancer at younger ages than people who do not possess a variant such as this.

Hereditary ovarian and breast cancer syndrome, also known as HBOC because of a mutation in the BRCA1 and BRCA2 genes, are inherited in an autosomal manner and make up around half of the cancer cases that are related to an inherited genetic risk (Petrucelli et al., 1998). Approximately 3% of breast cancers and 10% of ovarian cancers result from mutations in BRCA genes (CDC report 2023). It is estimated that the lifetime breast cancer risk is between 43–55% for BRCA2 carriers and 46–60% for carriers of a BRCA1 mutation (Rich et al., 2015).

1.3.2 RAS mutation is the most frequent oncogenic alteration in human cancers. All mammalian cells ex-

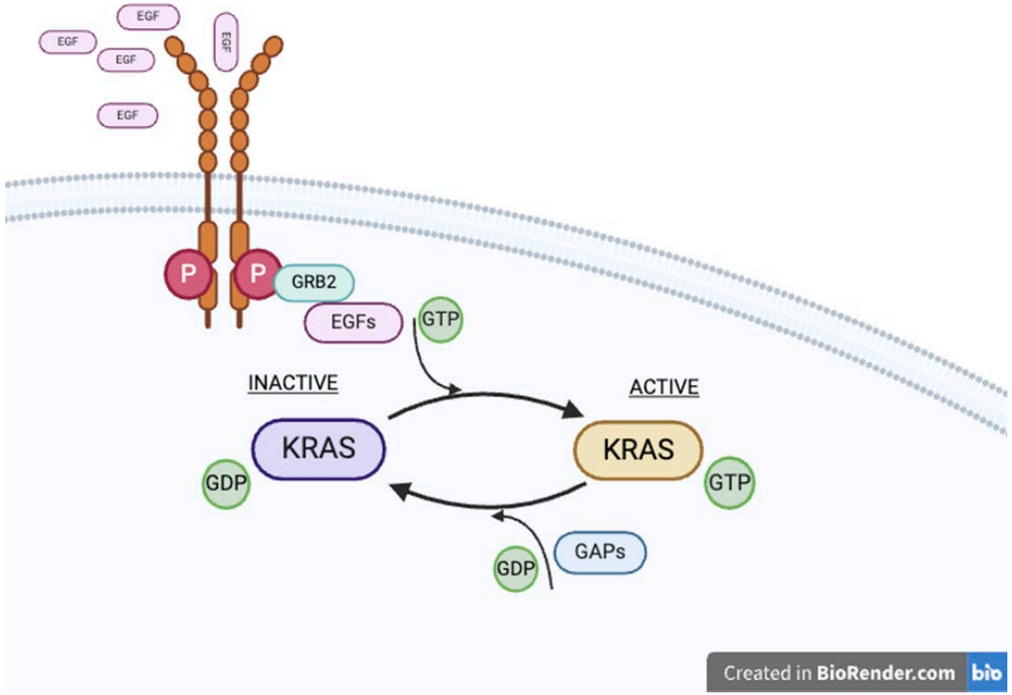


Figure 1: *Schematic representation of KRAS as molecular switch in regulating in EGFR (epidermal growth factor receptor) pathway*

press three very closely related Ras proteins: K-Ras, H-Ras, and N-Ras, which promote oncogenesis if they have mutations at codons 12, 13, or 61 (Quinlan & Settleman, 2009). These proteins are GTPases that function as molecular switches regulating the pathways of growth factor receptors such as EGFR (epidermal growth factor receptor) and tyrosine kinase receptors for HGF (MET) responsible for proliferation and cell survival (Shimanshu et al., 2017) **(Fig 1).** Since it is involved in transducing signals from epidermal growth factor receptors, mutant forms of these genes are less likely to respond to anti-EGFR antibody therapy.

1.3.3 The KRAS gene has been recognized as a homolog of the Kirsten rat sarcoma virus responsible for the malignant transformation of rodent cells. In humans, the KRAS gene is located on chromosome 12p12.1, which is encoded by 6 exons. Despite a high level of similarity between the isoforms (KRAS, HRAS, NRAS), K-RAS mutations are significantly more frequently observed in cancer. Each isoform displays preferential coupling to particular cancer types (Prior et al., 2012). The HRAS gene in humans is located on chromosome 11p15.5, and the protein it encodes is expressed by almost all tissues at low levels and is overexpressed only by uterine and muscle tissues, Langerhans islets of the pancreas, and bronchial epithelium (Gupta et al., 2011). N-RAS is the third member of the family. Its gene is located on chromosome 1p13.2. The expression NRAS is high in the bone marrow, GI tract, and brain and endocrine tissues.

The KRAS mutation is a gremlin variant that leads to an increased risk of breast, ovarian, pancreatic, colorectal, and lung cancers (Keane et al., 2010; Poorebrahm et al., 2022). The KRAS gene in humans has two space variants: KRAS4B (highly expressed) and KRAS4A (weakly expressed) (Jancik et al. 2010). In patients with a family history of breast and ovarian cancer who were evaluated for the BRCA1 and BRCA2 genes, 61% had the KRAS variant present (Ratner et al., 2011).

1.4 The implications of genetic testing for individuals with a family history of cancer

Identifying genetic changes and mutations related to cancer has greatly ameliorated the ability to identify individuals at risk of developing cancer, the interventions reducing the risk of cancer, improving screening, treatment, and dose, and finding optimal treatments. Furthermore, rather than preventing single gene disorders, cancer inhibition has moved to understanding the entire genome and its interactions with the surrounding environment and other factors. Once the family history is assessed, individuals can find any cancer-related inherited mutations they have (Calzone et al., 2023). This study presents a case of a BRCA1/BRCA2-negative breast cancer patient undergoing genetic testing. Whole-exome sequencing (WES) was used to identify hereditary breast cancer-related genes beyond BRCA1/2 and to detect the same mutation in a relative, helping assess their breast cancer risk.

Genetic counseling: Genetic testing can cause psychological distress in those being tested, both before the testing and after if they are identified as being carriers of certain genes. Generally, this distress reduces over the first year; however, in some cases, long-term studies have shown that some carriers of mutation continue having greater stress levels (Hirschberg et al., 2015).

In this study, I have reviewed research work in the field of hereditary cancer by analyzing papers from peer-reviewed journals, government health reports, and authoritative sources like the World Health Organization, PubMed, PMC, and Google Scholar. I also used Elicit AI to compare different studies for diagnostics and prognostic values. I then collated my experience and understanding of molecular diagnosis with the existing research.

2. Objectives

To study the diagnostics of KRAS mutations in human cancer and its role in cancer treatment

To understand the role of genetic counseling and its application through a case study of Breast cancer

3. Methodology

In RT-PCR, fluorescent probes (e.g., FAM and HEX) are used to detect specific DNA sequences in real time. Each probe has a fluorescent dye at one end and a quencher at the other. When bound to the target DNA, Taq polymerase cleaves the probe during amplification, separating the dye from the quencher and generating a fluorescent signal. This fluorescence increases with each cycle, proportional to the number of DNA copies. FAM targets the gene of interest, while HEX serves as an internal control to ensure proper amplification and detect potential inhibitors.

3.1 Procedure:

Case 1: KRAS mutation analysis in a sample

Human genomic DNA was extracted from a paraffin-embedded tumor sample fixed in formalin.

QIAamp® DNA FFPE Tissue protocol (QIAGEN, USA) was used to extract DNA for KRAS mutation analysis

a. Using a scalpel, excess paraffin was trimmed off the sample block

b. Up to 8 sections 5–10 µm thick were cut. If the sample surface has been exposed to air, the first 2–3 sections were discarded.

c. the sections were immediately placed a 1.5 or 2 ml microcentrifuge tube (not supplied), and 1 ml xylene was added to the sample. The lid was closed and the sample was vortexed vigorously for 10 seconds.

d. The sample was centrifuged at full speed for 2 min at room temperature (15–25°C).

e. The supernatant was removed by pipetting. None of the pellets were removed.

f. Further steps were followed as given in the protocol of the manual - QIAamp® DNA FFPE Tissue protocol QIAGEN, USA (protocol)

Steps involved in PCR:

1. The **Master Mix** was prepared:
   - Reaction Buffer
   - MgCl2 and stabilizers
   - Hot-start DNA polymerase
   - dNTPs (dATP, dCTP, dGTP, dTTP)

2. Primer and probe mix of different detectable KRAS mutations was used **(Table 1)** as given in the kit. The sample was tested for each mutation separately.

Table 1: List of detectable KRAS mutations

Mutation	exon	codon	Nucleotide change
G12C	2	12	c.34G>T
G12S			c.34G>A
G12R			c.34G>C
G12V			c.35G>T
G12D			c.35G>A
G12A			c.35G>C
G13D	2	13	c.38G>A
A59T	3	59	c.175G>A
A59E			c.176 C>A
A59G			c. 176 C>G
Q61K	3	61	c.181C>A
Q61L			c.182A>T c.182A>G
Q61R			c.183A>T c.183A>C
Q61H			
Q61H			
K117E	4	117	c.349A>G c.350A>G
K117R			c.351A>C
K117N			c.351A>T
K117N			
A146T	4	146	c.436G>A
A146P			c.436G>C
A146V			c.437C>T

3. Primer and probe mix of **Reference control gene** of the KRAS region without any known polymorphism/mutation was used.

4. A primer and probe mix of **internal control gene- HEX** was used to verify the amplification procedure and the possible presence of inhibitors, which may cause false negative results.

5. **Positive control** comprised Master mix + 5 µl KRAS-positive sample

6. **Negative control** comprised Master mix + 5 µl sterile water

Table 2: *PCR mix for one reaction*

1.	Master mix	10µl
2.	Primer & probe mix of any one mutation and Reference control gene	2.5µl each
3.	Primer and probe mix of internal control	2.5µl
Total:		15µl

4. The above-prepared PCR Master Mix (15 µl) was transferred in 0.2 ml PCR tubes and the tubes were closed.

5. Negative Control comprised 5µl Sterile Water

6. Sample comprised 150-200 ng DNA (up to 5µl)

7. Positive Control comprised 5µl KRAS-Positive Control sample

Reaction Cycle:

Initial denaturation was conducted at 94°C for 10 minutes for 1 cycle.

Denaturation was conducted at 94°C for 15 sec (40 cycles).

Annealing and extension was conducted at 60°C for 60 sec (40 cycles).

8. The BIORAD CFX Maestro software was used to analyze the data and interpret the graphs

9. Data Analysis: the cycle threshold (Ct) value was determined, which indicates the cycle number at which fluorescence exceeds the threshold, reflecting the presence of the target gene.

Case 2- A clinical case of diagnosis of breast cancer in patients with a family history of non *BRACA1/2* mutation

1. Study Objective and Case Presentation

This study highlights a rare case of *BRCA1/BRACA2*-negative breast cancer in a young patient with a strong family history of the disease.

2. Case Presentation

I present the case of a 32-year-old woman diagnosed with left breast carcinoma, confirmed through genetic testing using whole-exome sequencing.

Genomic DNA was isolated from blood using the QIAamp DNA Blood Mini Kit (Qiagen), following the manufacturer's instructions. Samples for sequencing were prepared using the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, CA, USA), following library preparation and enrichment. Paired-end sequencing (2×150 bp) was outsourced and conducted on the Illumina NovaSeq 6000 platform.

4. Results

KRAS mutations are observed in several human cancers. KRAS codon 12 mutations have been detected in almost 67% of all the tumours. The clinical material used in this experiment was from a tissue of the tumour sample embedded in paraffin, which was used to extract genomic DNA. This genomic DNA sample was used as a template for the RT PCR reaction.

The sample was studied for all 11 assays given in the TRUPCR® KRAS Kit. The results from the RT-PCR were positive for three assays, while the rest were negative.

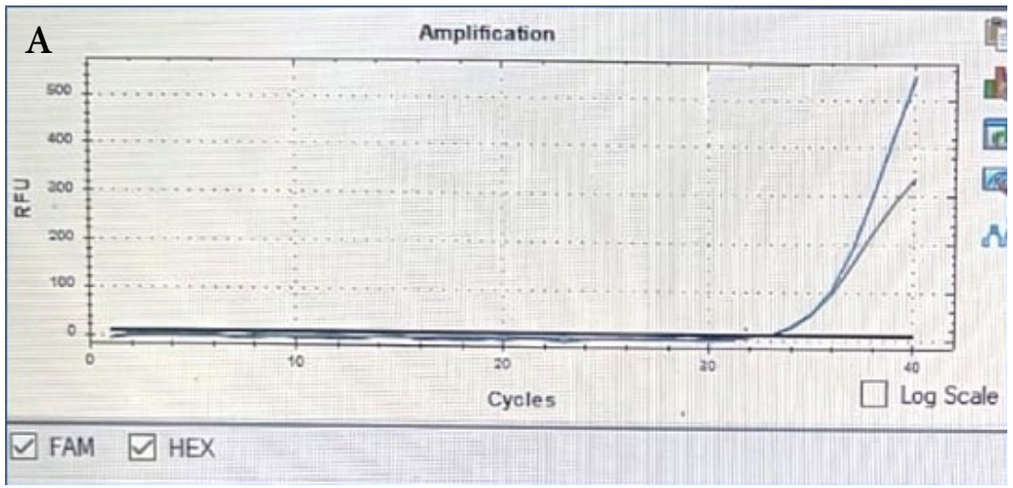
INTERPRETATION OF THE GRAPH:

The samples showing a peak above the threshold value (the point up to which no fluorescence is generated) were positive and indicated the presence of mutated copies of the gene present in these samples. The mechanism behind the development of the peak is when fluorescence is generated above the threshold value. The y-axis of the graph is RFU, which is the relative fluorescence unit that quantifies the intensity of fluorescence emitted by the sample. The x-axis is the number of cycles.

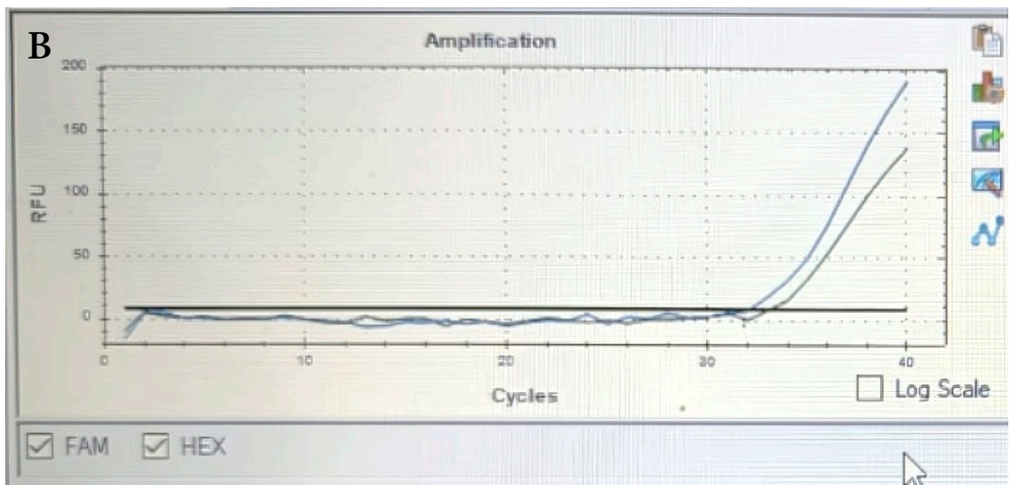
The development of fluorescence is a result of the distance between the quencher dye and the reporter dye. The reporter and quencher dyes are attached to a primer. Before the primer attaches to its target DNA sequence, the dyes are in close proximity to each other, and no fluorescence is generated. However, if the primer finds its DNA template and binds to it, then the two dyes will move away from each other. This generates fluorescence. Due to this, the number of copies of the mutation on the template is directly proportional to the number of copies of the target gene.

The Ct value is the number of cycles after which the fluorescence levels cross the threshold value. Therefore, the higher the Ct value, the less pathogenic material load as more cycles are needed to generate enough fluorescence to cross the threshold value. Moreover, one unit change in the Ct value shows the doubling of the viral target. I analyzed the sample for mutation in the G12 codon as per the kit manual. The delta Ct value of the G12 codon of the KRAS gene for assays G12C, G12A, and G12V was then calculated using the formula:

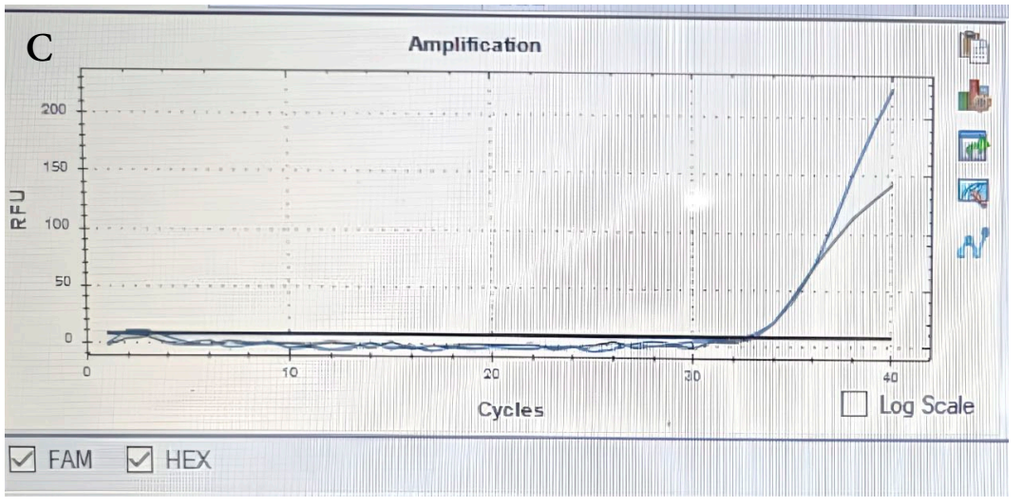
$$\Delta \text{ Ct} = \text{Ct Mutation} - \text{Ct Reference}$$



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	31.65
HEX	KRAS	2	32.95



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	31.79
HEX	KRAS	2	32.27



Fluorescence	Target gene	Sample	Ct value
FAM	KRAS	1	32.15
HEX	KRAS	2	33.01

Figure 2: RT-PCR results for identification of KRAS mutation in the sample. **A.** Graph showing two distinct curves = dual-target detection, mutation (KRAS= G12 C) and a control; **B.** G12 V; **C.** G12 A. FAM dye (green) was used for KRAS mutation and HEX (blue) was used for internal control gene.

Table 3: Results from RT PCR study of a sample for all 11 assays using TRUPCR® KRAS Kit.

Codon	Ct FAM	Ct HEX	ΔCt calculated	ΔCt Reference	Result
G12C	31.65	32.95	**0.94**	≤ 3.5	Positive
G12S		32.41		≤ 7.0	Negative
G12R		33.31		≤ 8.5	Negative
G12V	31.79	32.27	**1.09**	≤ 6.5	Positive
G12D		33.61		≤ 4.5	Negative
G12A	32.15	33.01	**1.44**	≤ 7.5	Positive
G13D		33.13		≤ 5,5	Negative
Q61x		32.80		≤ 4.5	Negative
A59x		32.67		≤ 4.0	Negative
K117x		32.63		≤ 5.5	Negative
A146x		32.62		≤ 8.0	Negative

Delta Ct was compared with the mutation analysis table in the TRUPCR manual. Both curves crossing the threshold indicates successful amplification (Fig 2).

The presence of a FAM signal implies that the mutation-specific probe bound and was amplified. Ct values of 30–35 suggest a low to moderate amount of starting target — likely a heterozygous mutation or low-level expression. The HEX curve ensures that it is not a false negative. KRAS mutation was detected in the sample, with Ct 31.65; 31.79; 32.15 (FAM), respectively, and internal control amplification confirmed with Ct 32.95; 32.27; 33.01 (HEX), respectively, indicating a valid and positive qPCR reaction.

Case study for genetic testing: Exome sequencing of the 32-year-old patient, who previously tested negative for BRCA1/2 mutation, was performed to identify the mutation in other breast cancer susceptibility genes. Our findings suggested a mutation in the CHEK2 gene (Checkpoint Kinase 2). The CHEK2 gene variant identified was del5395. The 29-year-old patient's sibling was tested for the same variant using the PCR technique. The result showed that she was negative for the BRCA1/2 and CHEK2 variants.

5. Discussion

In this study, I explored the role of hereditary factors in cancer risk by investigating mutations in genes like KRAS. KRAS mutation plays a major role in ovarian (50%), pancreatic (80%), and breast (10%) cancers, which are hereditary. By understanding the molecular diagnostics of samples for KRAS mutations, I highlight the importance of these mutations in the prognosis and treatment of cancer.

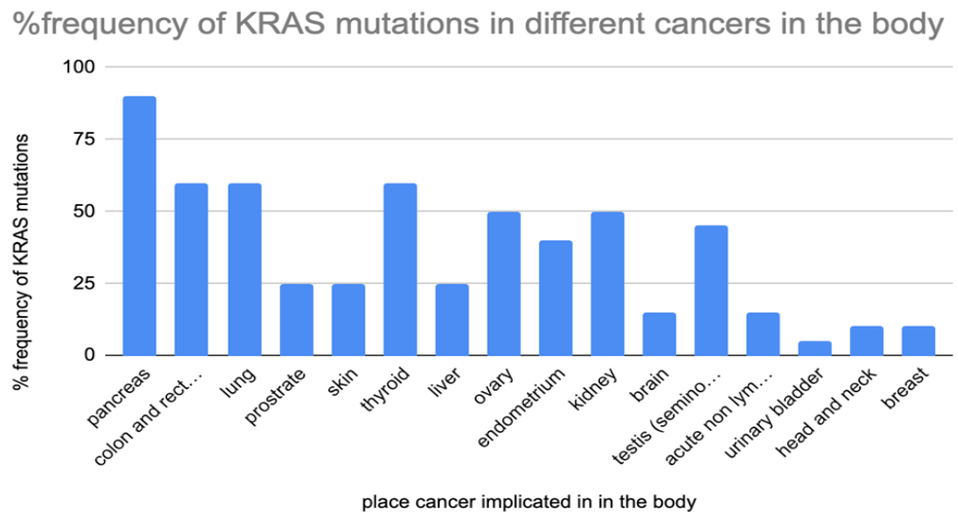
As seen in **Table 4**, the existence of KRAS mutations increases the risk of lung, colorectal, pancreatic, breast, and ovarian cancer. More than 80% of all cases

Table 4: List of major cancers involving KRAS mutations and the diagnostic methods used for their identification

Type of cancer	Codon	% frequency of KRAS mutations	Diagnostic method	Sporadic or hereditary	References
Pancreatic	12 and 13	>80%	Restriction-fragment length Polymorphism (RFLP) analysis, qPCR-based techniques, or next-generation sequencing	Both	Urban et al., 1993 Lang et al., 2011 Earl et al.2015
Colorectal	12, 13	>50%	ARMS PCR, pyrosequencing, and Luminex xMAP (multi-analyte profiling)	Both	Matsunaga et al., 2016 Liu et al., 2011
Lung	12, 13, 61	30%	qPCR, ddPCR, and NGS	sporadic	Bos, 1989 Timar & Kashofar 2020
Breast	-	2-10%	quantitative Allele-specific Competitive Blocker PCR (ACB-PCR)	Hereditary	Tokumaru et al., 2020 Myers et al., 2016
Ovarian (epithelial)	12 and 13	25-50%	quantitative allele-specific RT-PCR-	Hereditary	Keane & Ratner 2010 Sadlecki et al., 2016

The *KRAS*-variant is easily tested in a blood or saliva sample

Figure 3: Graphical representation of % frequency of KRAS mutation involved in different cancer types



of pancreatic cancer occur due to KRAS mutations, as do 50% of cases of colorectal cancer, 30% of lung cancer, less than 2% of breast cancer, and 25% of ovarian cancer (**Fig 3**). The KRAS gene encodes a GTPase that plays a crucial role in the RAS/MAPK signaling pathway, which governs cell proliferation, differentiation, and survival. Mutations in KRAS, especially at codons 12, 13, and 61, lead to persistent activation of the RAS protein, driving uncontrolled cell growth and tumor formation. KRAS mutations serve as important diagnostic markers, helping distinguish malignant from benign lesions, and have significant prognostic value, often being associated with therapy resistance and poorer clinical outcomes.

Literature data indicate that KRAS mutations predominantly occur in codons 13 and 12, accounting for 95% of all mutations—with 80% in codon 12 and 15% in codon 13. Mutations in other codons, such as 61, 146, and 154, are rare, comprising only 5% of cases. Among codon 12 mutations, G12V and G12D are the most common, while in codon 13, G13D is the most frequently observed variant (Knijn et al., 2011)

KRAS mutations, particularly in exon 2, play a critical role in cancer diagnosis and prognosis. These mutations not only help in identifying malignancies but also influence treatment decisions and patient survival.

1. Diagnostic Significance

KRAS mutations are frequently found in lung, colorectal, and pancreatic cancers, making them important targets for diagnosis and treatment strategies. Variations in KRAS mutational status can be due to several factors, such as the quality of extracted DNA, the type of tissue being processed, the testing method used, the proportion of cancerous cells in the sample, and the objective of the analysis (Dinu et al., 2014).

2. Predicting Therapy Response

Mutations in exon 2, particularly at codons 12 and 13, are strong predictors of resistance to anti-EGFR therapy in metastatic colorectal cancer (mCRC), impacting treatment effectiveness. The presence of KRAS gene variations has been linked to a lower response rate to certain chemotherapeutic agents, making the KRAS mutational status an important consideration when selecting targeted therapies. The connection between KRAS mutations and therapy re-

sistance was first identified in patients with metastatic colorectal cancer (mCRC) who received anti-epidermal growth factor receptor (EGFR) therapies. Lievre et al. (2006 were the first to establish this relationship, demonstrating that KRAS mutations are associated with reduced efficacy of anti-EGFR agents in mCRC treatment (Lievre et al., 2006).

3. Prognostic Significance

Association with Poor Prognosis- KRAS mutations are generally linked to worse clinical outcomes, particularly in colorectal and pancreatic cancer, where they correlate with aggressive tumor behavior (Dinu et al., 2014).

4. Impact on Survival

Patients with KRAS mutations often experience shorter overall survival (OS) and disease-free survival (DFS) than those with wild-type KRAS, making KRAS mutations a crucial prognostic marker (Safi et al., 2022; Dinu et al., 2014).

Certain subtypes of KRAS mutations affect prognosis differently:

1. KRAS G12D:

Studies suggest that patients with G12D mutations may have better overall survival (OS) than those with other KRAS mutations.

2. KRAS G12C:

In contrast, G12C mutations are often linked to poorer prognosis, especially in cancers like lung adenocarcinoma.

Understanding these KRAS variations is essential for personalized cancer treatment, guiding targeted therapies, and improving patient outcomes.

Genetic testing of tumors allows the identification of germline and somatic mutations that can cause the risk of cancer (Dubsky et al., 2024). Genetic testing can be utilized by people with or without cancer. If someone knows that they have inherited a germline variant, they can take steps to reduce the risk of cancer or can detect the risk early (Riley et al., 2011). This helps in identifying at-risk family members who may have the variant to increase surveillance and implement methods to treat cancer if identified. (Petrucelli et al., 1998). Additionally, they allow the prevention of cancer and the implementation of targeted therapies for more people. (Dubsky et al., 2024). If they already have cancer, the information from the genetic test will be important in selecting treatment. These results can also be shared with other relatives for their own cancer risk. After the genetic test, genetic counselling allows for discussion as well as giving informed consent for the processes to treat or prevent cancer. (Riley et al., 2011)

This case study identified a **gene variant** in a **BRCA1/2-negative** patient with **unilateral breast cancer**. The study aimed to explore the **role of genetic testing** in assessing cancer risk and its significance in predicting susceptibility among family members. Whole-exome sequencing identified a CHEK2 truncating variant (i.e., del5395). CHEK2 (Checkpoint Kinase 2) is a moderate-risk breast cancer susceptibility gene. It encodes a tumor suppressor protein that has a vital role in apoptosis in response to DNA damage, DNA repair, and cell cycle regulation. The patient's sibling was tested for the same variant and was found negative for BRCA1/2 and CHEK2 mutations, implying that she had a much lower probability of developing breast cancer. She was still advised to get periodic mammograms.

Genetic tests raise social and ethical issues for the field of medicine as well as public health and social policies. These issues regard the use, implementation, and application of results of the test, raising problems in the principles of confidentiality, equity, privacy, and autonomy. (Andrews et al., 1994). The importance of confidentiality differs between geneticists (Wertz and Fletcher, 1989) as there are a few different situations where geneticists would break patient confidentiality to disclose information without patient permission (Geller et al., 1993). However, this creates problems in the principle of confidentiality, which is why geneticists should highlight their policies to give out information before undertaking a genetic test (Andrews et al., 1994).

Genetic counselors have to make assessments on individual health risks by using daily history to analyze pedigree charts, which provide the information

required to decide treatment plans, strategies to prevent the incidence of disease, as well as the economic and social implications for patients. This requirement applies to information around individuals, their family history, risk of a genetic disease, and carrier status, which need to be kept confidential as they can be disparaged (Muthuswamy, 2011).

6. Future research:

The current trend in cancer treatment focuses on personalized medicine, which involves tailored therapeutic approaches, including minimally invasive surgery, precision chemotherapy (PCT), and monoclonal antibody therapies.

Targeting KRAS is a promising strategy due to its high mutation prevalence and key role in tumor growth. Continuous research has led to novel insights and drug development for cancers initially considered undruggable, particularly for KRAS (G12C).

Innovative techniques like NMR-based fragment screening, tethering, and in silico drug design have identified small molecules that bind directly to KRAS. Among them, KRAS (G12C) inhibitors, such as AMG510 (sotorasib) and MRTX849 (adagrasib), have shown encouraging clinical results (Canon et al., 2019; Papadopoulos et al., 2019). However, challenges remain, including clinical safety evaluation, efficacy optimization, and overcoming resistance.

Both intrinsic and acquired resistance pose significant hurdles. Some patients show limited response to KRAS (G12C) inhibitors, suggesting the need to identify biomarkers for patient selection. Additionally, acquired resistance remains a common issue in targeted therapies, requiring further research to develop effective treatment strategies. Identifying and analyzing KRAS gene mutations is crucial in advancing personalized treatment strategies. An individualized approach not only enhances patient outcomes by minimizing side effects and improving survival rates but also benefits the healthcare system by reducing overall treatment costs.

7. Conclusion:

To conclude, cancer can arise due to genetic mutations. These mutations can cause either hereditary or sporadic cancer. Hereditary cancer syndromes are the most frequently observed as a form of vertically transmitted diseases. Inheriting mutated genes augments the risk of developing cancer. This study confirms the diagnostic value of molecular techniques in assessing hereditary cancer risk. RT-PCR analysis detected KRAS codon 12 mutations (G12C, G12V, G12A) in 3 out of 12 tumor samples, indicating a low to moderate mutational load. Additionally, whole-exome sequencing of a patient with BRCA1/2-negative breast cancer revealed a pathogenic CHEK2 (del5395) variant, underscoring the importance of extended genetic screening beyond BRCA genes. These results reinforce the role of genetic testing and counseling in early detection, risk stratification, and personalized cancer management.

Acknowledgement

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Quick, Stop Scrolling: The Impact of Short-Form TikTok Video Characteristics on Video Engagement and Teenagers' Focused Attention

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Abstract: On social platforms, short-form content has grown to be one of the most dominant forms of media. This study investigated how the different content characteristics (video length, pacing, text overlay, format, music) of short-form videos influenced both video engagement and the focused attention of teenagers aged 14 to 18 years old. A content analysis was conducted to analyze how the characteristics influenced a video's engagement metrics. In the analysis, 100 TikTok videos were collected and a codebook was developed to assign characteristics to the videos. A TikTok Video Engagement Score (TVES) was then calculated for each video to standardize and compare their engagement metrics. To examine how the characteristics affected teenagers' attention, a quasi-experimental survey was taken by 438 respondents. Participants watched two TikTok videos–one with "desirable" characteristics and one with "undesirable" characteristics–to gauge how the characteristics differed in maintaining attention. The results of the content analysis and survey suggest that a medium video length, fast-pacing, present text overlay, vertical format, and music without creator speech are the most desirable short-form video characteristics for maximizing engagement and retaining teenagers' attention. These findings can be used by short-form content creators and advertisers to better optimize their videos for a teenage audience.

Keywords: Short-Form Video, Video Characteristics, Focused Attention, Engagement Metrics

Introduction

In the past decade, the popularity of short-form video applications has increased. TikTok (formerly Musical.ly) is a social media platform launched by ByteDance in 2018 (Bobrowsky et al., 2022). As the most downloaded app of 2022 with 2 billion users and 150 million daily users (Dong & Xie, 2022; Zhang et al., 2019), TikTok's popularity plays an essential role in driving cultural trends and influencing social developments (Minadeo & Pope, 2022). TikTok's exponential growth is partly attributable to TikTok's role as an important social space for youth, helping it to become an international mainstream rival to other short-form platforms such as Instagram, Facebook, and YouTube (Kaye et al., 2024). A recent Pew Research Center survey of 1453 United States teenagers aged 13 to 17 revealed that 95% of the participants had access to a smartphone, and 63% of them used TikTok (Anderson et al., 2023).

Given that the majority of TikTok's users are children and teenagers (Blackburn & Hogg, 2024), it is imperative to understand the characteristics of these short-form videos and their influences on the digitally engaged youth.

According to Shamloul (2024), short-form media is a type of "brief video content" that generally has a runtime of a few seconds or minutes (p. 48). On TikTok, short-form videos are shown to users through the platform's unique recommendation system and

algorithm, known as the "For You Page" (FYP), which distinguishes TikTok from its competitors (Kaye et al., 2024). Nevertheless, Shamloul (2024) warns that frequently consuming this "concise video content" has the possibility of altering the attention spans and other information-processing abilities of users (p. 48-49). In addition to weakened attentional functioning, Carstens et al. (2018) expands upon Shamloul's claim by stating that there has been an increase in "attention deficits" as a result of the rapid information overload individuals experience from consuming short-form media (p. 21).

With some adolescents using short-form applications for over 11 hours a day (Carstens et al., 2018), it is necessary to investigate how the varying characteristics of short-form videos on TikTok affect general user engagement and teenagers' focused attention to better understand the underlying implications associated with viewing short-form content.

Literature Review

This literature review identifies the five main characteristics of short-form videos (video length, pacing, text overlay, format, and music) and examines how each aspect influences the user's viewing experience and the video's engagement. In this study, video engagement is defined as the quantity of likes, comments, saves, shares, and views a video receives, and focused attention refers to an individual's ability to concentrate on a specific stimulus.

Content Algorithm

Similar to other social media platforms, TikTok's algorithm and FYP are shaped by a user's "digital identity," which is crafted by three factors: online behavior, content preferences, and provided personal information (Klug et al., 2021, p. 85). Compared to other research regarding short-form applications, TikTok's algorithm is insufficiently researched and is mostly based on assumptions about preexisting users (Klug et al., 2021). However, it is understood that the FYP can be personalized through user interactions, such as liking or commenting on related videos (Blackburn & Hogg, 2024). In addition to these factors, Lookingbill & Le (2024) argue that the algorithm is constantly learning, further factoring in video information and specific account settings to customize a user's feed. The algorithm is proficient at creating customized feeds, and this personalized content can contain addictive qualities for frequent viewers (Liu et al., 2021). However, Klug et al. (2024) believe that the majority of TikTok users understand the "algorithm's functionality" and can optimize their feed to mitigate addictive behaviors (p. 89).

By understanding how the content algorithm works, creators employ varying techniques and specific characteristics to increase their videos' engagement, popularity, and appearance on the FYP (Klug et al., 2021).

Content Characteristics

Video Length & Pacing

Short-form videos differ from other types of media due to their unique characteristics related to the design of the video. One of the most defining characteristics of short-form videos, compared to traditional long-form media, is their shortened length (Chen et al., 2024). Video length is an important aspect of media consumption, as it can influence a user's memory, attitudes, and intentions (Maenhout, 2022). In the existing literature, sources disagree on the most effective video length for user engagement. For instance, one study evaluated the most favorable characteristics of scientific short-form videos, concluding that video lengths longer than 60 seconds had higher engagement rates (Velarde-Camaqui et al., 2024). In contrast, Gu & Zhao (2024) determined that the relationship between video length and user engagement follows a "U-shaped curve," typically performing best at the 15-second duration (p. 15). Because the definition of a short video length is inconsistent across studies, media researchers declare that video length likely influences video performance only to some extent (Meng et al., 2024).

Additionally, pacing, or the speed of a short-form video's narrative, is another prominent characteristic of short-form videos, as they are typically faster-paced than traditional text-based media (Nguyen et al., 2024). Although fast-paced media is seen as more engaging (Nguyen et al., 2024), content too quick could also lead to varied attention, cognitive decline, and decreased performance of users (Qi et al., 2024).

Moreover, the quick speed of short-form videos can overwhelm and entrap users, leading to a loss of time management (Tan & Hu, 2022).

Text Overlay & Video Format

Few studies have evaluated the impacts of text overlay on the performance of short-form videos. The presence of on-screen text is a characteristic that creators take into consideration when creating short-form content, but typically, short-form videos contain purely visual information. Despite pictures attracting more attention from users than textual information (Qi et al., 2024), due to the short nature of TikTok videos, creators can include more information and content in their videos through the use of captions, video descriptions, or other forms of text overlay. According to Daele et al. (2024), maximizing video performance through captions is underdeveloped and users may negatively see it repetitive or it may interfere with the pace of short-form videos. Additionally, most on-screen text for short-form videos is typically used for extended information instead of transcribing the video's auditory information (Daele et al., 2024).

Similarly, there is little research regarding the impact of a short-form video's format on its performance and user engagement. On the TikTok platform, creators have the choice between uploading videos in a vertical or a horizontal format. Since the rise of short-form video applications, users are increasingly shifting from a horizontal format, typically viewed on computers, to a vertical format, typically viewed on hand-held devices (Zhu et al., 2022).

Furthermore, Shi (2024) claims that short-form video applications are designed for vertical content as they can capture attention easily due to the convenience of scrolling on a hand-held device. Of the few studies investigating short-form video format, there is a general agreement that vertical formats produce higher engagement than horizontal formats due to ease of accessibility, increased interest, and minimal effort to interact (Menon, 2022; Mulier et al., 2021). However, Mulier et al. (2021) argue that there is a discrepancy in video format preference across generations, with older mobile users, such as those of Generation X and Y, being less fluent in processing vertically formatted content than Generation Z. ***Music***

Finally, short-form video creators have the option of adding no music, background music, or music as the sole audio to their content. According to psychology researchers Salamé & Baddeley (1989), the presence of background music in advertising media can interfere with "immediate verbal memory" or the ability to quickly encode spoken information, and disrupt viewers' cognitive abilities (p. 119). Contradictorily, in a study investigating the most successful factors of short-form videos, the researchers discovered that the use of background music can increase the viewer's empathy, create an immersive experience, and gain more attraction

(Hsin-Cheng et al., 2024). Although both groups of researchers investigated the impacts of background music on viewer engagement, a plausible explanation for the difference in conclusions is likely based on the type of media that was analyzed. Notably, Mou et al. (2021) conclude that short-form content is especially different from other types of media due to the frequent inclusion of background music, meaning that viewers are likely more accustomed to the presence of music in short-form media. Additionally, users on TikTok believe that the inclusion of prominent trending music as the sole audio in short-form content can increase the video's chances of being watched on the FYP, furthering video engagement (Klug et al., 2021).

Therefore, it is reasonable to conclude that the existing literature agrees that both forms of music can increase short-form video engagement. However, the researcher was unable to find sources in the literature that evaluated how short-form videos without music would perform in contrast to videos that contained music.

Summary

A review of the literature demonstrates that the main characteristics influencing a short-form video's engagement are video length, pacing, text overlay, format, and music.

However, as seen in the pre-existing research, multiple inconsistencies exist over the most influential aspects of each characteristic, as scholars disagree as to what specific traits enhance video engagement most positively. Additionally, a majority of sources evaluating the attentional impacts of short-form videos overlook one of TikTok's largest populations, teenagers aged 14 to 18 years old, instead primarily exploring the impacts on users between the ages of 19 to 30

years old (Fillmore, 2015; Kies, 2018). Therefore, this study hopes to fill the research gap by investigating the question: How do the content characteristics of short-form videos on TikTok influence video engagement and the focused attention of teenagers aged 14 to 18 years old in the United States?

Hypotheses

After examining the literature, the researcher hypothesizes that the following characteristics are desirable for maximizing a short-form video's engagement: short video length, fast pacing, no text overlay, vertical format, and background music. This prediction was derived from the most consistent findings across the body of knowledge, as characteristics with a greater consensus among researchers were considered more desirable. Furthermore, the researcher hypothesizes that videos with these desirable characteristics will retain teenagers' focused attention more effectively than videos with undesirable traits, as prior research suggests that these characteristics are more likely to appeal to viewers.

Method

Overview

This study aims to identify which aspects of the characteristics are desirable or undesirable, to examine the relationship between specific content characteristics and video engagement, and ultimately investigate whether the desirable or undesirable characteristics sustain the focused attention of teenagers more effectively.

Study Design

To address each aspect of the research question, this study combined a statistical content analysis with a quasi-experimental survey, predominantly based on quantitative data. A quantitative content analysis was chosen because these analyses are the most common research method used by researchers investigating short-form video engagement (Zhang et al., 2022).

Through a quantitative approach, the researcher can calculate significant differences between variables and assess how individual characteristics could influence video engagement differently.

Additionally, to measure how the content characteristics influence teenagers' focused attention, a quasi-experimental survey was employed because the researcher could not control the participants' existing experience with short-form content. Short-form content researchers use surveys to understand the "preferences" and "consumption habits" of users (Rugrien, 2022, p.

32), which is an essential component of this study, as the researcher will be investigating how the respondents' perceptions of the content characteristics influence their focused attention.

Finally, TikTok was chosen as the primary source of analysis because of its widespread popularity and familiarity among American users compared to other, non-American short-form platforms, such as Douyin and Kuaishou. Although other American-owned social media platforms with short-form content were considered, such as YouTube Shorts and Instagram Reels, TikTok was selected because short-form content is the predominant form of media on the entire platform.

Content Analysis

Video Collection

Various video collection methods were considered for the study. For example, Minadeo & Pope (2022) collected videos through TikTok's hashtag system, purposefully selecting videos they deemed as relevant to their study by looking at videos under select hashtags. Another study collected videos and their information through a "scraping tool," selecting predominantly trending videos without the use of a TikTok account (Klug et al., 2021, p. 86). In both circumstances, the researchers used video collection methods that were not entirely random, instead relying on intentional choosing. By contrast, this study employed a modified version of Shutsko's (2020) method, where the researcher's video collection relied on sampling videos that appeared on the FYP, a more randomized method of video collection. Adapting Shutsko's video collection method is most beneficial for this study because it allows the researcher to gather TikTok videos in a moderately randomized way, which is more represen-

tative of the short-form videos on TikTok as a whole.

In adopting this method, a new TikTok account was created on December 23rd, 2024, to refresh the content algorithm and create an un-customized FYP. Collecting the videos on a newly created account by scrolling through the FYP imitated an authentic user's experience, further allowing the selected videos to be randomly sampled by means of TikTok's content algorithm instead of by nonrandomized selections. Videos were collected in succession by scrolling through the FYP, with the first video that appeared on the FYP being Video ID 1, the second video appearing being Video ID 2, and so forth. Since the researcher was working alone and relied on manual selection instead of using automated technological tools for video collection, such as the TikTok Application Programming Interface, only the first 100 videos on the FYP were collected to be coded. Collecting 100 videos was a reasonable, manageable number as it allowed for a variety of content characteristics to be considered, created a realistic sample size, and produced a practical number for statistical analysis. All 100 videos were collected on the same day that the account was created to limit confounding variables such as drastic changes in engagement, different trending content, and inconsistent platform versions. Since this study focused solely on short-form videos, the following types of content were not collected and were skipped altogether: advertisements (videos marked with a sponsored tag), slideshows (content that contained purely pictures), and TikTok Lives (real-time user broadcasts). After the first 100 videos of the FYP were collected, they were organized into a Google Spreadsheet for coding.

Ethical considerations were considered regarding the use of the collected videos and the creators' intellectual property (IP) rights. According to the University of Vermont Committee, TikTok videos are publicly available for analysis, meaning informed consent for the use of their videos can be waived (Minadeo & Pope, 2022). Additionally, the original links to the collected TikTok videos were recorded in the Google Spreadsheet to respect the creators' IP rights.

However, it is essential to note that the creators of the TikTok videos can unpublish their videos, either through deletion or privatization, meaning that some links may become obsolete, and certain collected videos may not be watchable in the future. To resolve this issue, the collected videos were coded the same day as the selection, allowing all 100 of the original collected videos to be usable in the content analysis.

Coding Scheme

After the 100 TikTok videos were collected into a Google Spreadsheet, a codebook was developed to allow for qualitative analysis and to assign characteristics to the collected videos.

Based on the literature review and the researcher's preexisting knowledge, Table 1 was created as the coding scheme. The coding scheme consists of video length, pacing, text overlay, format, and music, each divided into smaller subcategories. For this study, video length is defined as the duration of the video, pacing refers to the speed of the video's editing, text overlay describes the use of on-screen text, format indicates the video's orientation, and music represents the presence of accompanying audio.

		Explanation
Video Length	Short	0 to 20 seconds
	Medium	21 to 59 seconds
	Long	60 seconds or longer
Pacing	Slow	Relaxed pace, minimal or no frame changes
	Moderate	Average pace, some frame changes
	Fast	Quick pace, rapid frame changes
Text Overlay	Present	Text is shown at some point in the video
	Not Present	No text appears at any point in the video
Format	Vertical	Media that is taller than wide
	Horizontal	Media that is wider than tall
Music	None	No music is present
	Background	Music is present, overlaying the creator's actions
	Only	Music is present, without the audio of the creator's actions

Table 1. Short-Form Video Characteristics: Coding Scheme

$$\text{TikTok Video Engagement Score} = \left(\frac{[(\text{Likes} \times 0.4) + (\text{Comments} \times 0.3) + (\text{Saves} \times 0.2) + (\text{Shares} \times 0.1)]}{\text{Views}}\right) \times 100$$

Equation 1. TikTok Video Engagement Score (TVES) Calculator

All 100 videos were watched in their entirety and coded by a single coder, the researcher, so that intercoder reliability did not apply. Each video was assigned five sub-characteristics, or one option from each main characteristic. The video's engagement metrics were also recorded (number of likes, comments, saves, shares, and views) to allow for correlational analysis.

Finally, a TikTok Video Engagement Score (TVES) was calculated for each video based on the video's engagement metrics using Equation 1. Each metric was weighted according to the effort required by users to interact with it. More accessible metrics (e.g., liking a video) were given a higher weight due to their greater likelihood of being used by viewers. In contrast, metrics that required more effort (e.g., sharing a video with a friend) were given a lower weight due to the lessened likelihood of being used by the average viewer.

To the researcher's knowledge, no engagement score calculator exists specifically for TikTok videos; therefore, the researcher developed Equation 1, which took inspiration from other studies' video engagement score calculators (Chen et al., 2024; Liikkanen, 2014). By dividing the engagement metrics by the video's views, the TVESs would become normalized, effectively allowing posts' engagement metrics to be compared relative to one another and preventing biases in higher-viewed posts.

Survey

After the content analysis, a five-section survey was designed on Google Forms. The survey was open for approximately 8 weeks to maximize the number of responses. The first section of the survey contained an informed consent letter and a checkbox for participants to agree to take the survey. The second section used multiple-choice questions to gauge the demographics of the respondents, including questions regarding their TikTok usage. This section was implemented because it allowed the researcher to see who was eligible to take the survey, as participants who responded as being outside of the desired age range were deleted.

For the third section, the researcher selected two videos out of the 100 collected from the content analysis to be used in the survey, based on their levels of *desirability*. In this study, desirability refers to the degree to which a video characteristic enhances a video's appeal and ability to sustain viewers' attention. While desirability may also involve subjective factors such as aesthetic preference or emotional response, this study operationalizes the construct using engagement-based metrics. Specifically, for each content characteristic, the sub-characteristic option with the highest average TVES (see Table 3, Results) was designated as "desirable," and the option with the lowest average TVES was labeled as "undesirable." If a characteristic had a third option, the middle-value TVES was considered "neutral." These classifications assume that desirable characteristics enhance video performance, while undesirable characteristics reduce it. This operationalization is appropriate because the TVES equation was specifically designed to measure engagement outcomes relative to short-form TikTok videos, making it a practical and quantifiable proxy for desirability in this context. By assigning desirability through this method, each sub-characteristic is evaluated based on its actual performance, rather than subjective labeling. The desirability of each characteristic is summarized in Table 11 (see Discussion), where they serve as predeterminations that are subsequently tested and validated through the survey.

The first selected video, Video ID 11, was chosen as it contained the majority of the desirable characteristics identified from the TVES procedure (see Fig-

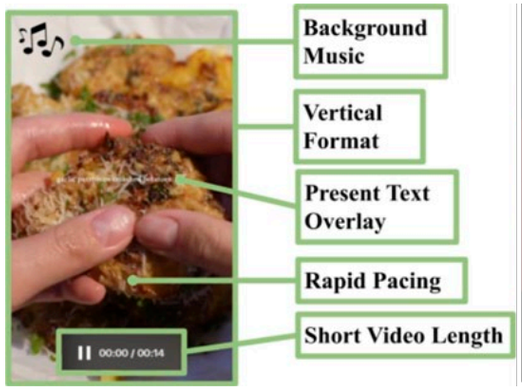

Figure 1. Characteristics of Video 1 (Video ID 11)

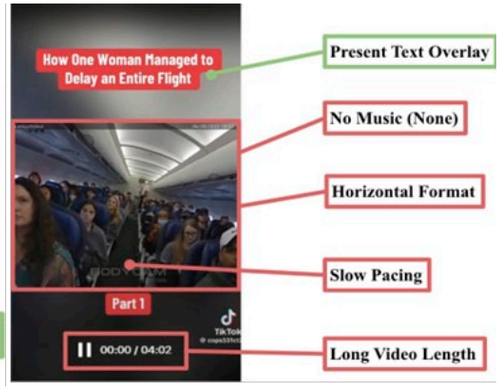

Figure 2. Characteristics of Video 2 (Video ID 94)

ure 1). The second selected video, Video ID 94, was contrarily chosen for containing the majority of the undesirable characteristics (see Figure 2). The selected videos were also chosen as they contained the majority of the desirable and undesirable options. None of the collected videos from the content analysis contained all five desirable or undesirable traits, so Video ID 11 and Video ID 94 were selected as they fit the requirements best. In section three, respondents were asked to watch each video and then answer four Likert scale questions for each to measure how well their attention was held comparatively. These questions took inspiration from Lehmann et al. (2022), as they used a similar method in which they evaluated participants' attention after watching a video. All of the questions had five response choices ranging from strongly disagree to strongly agree, including a neutral option, and two of the four Likert questions were designed to be reverse-scored to reduce response bias.

The fourth section directly asked respondents to choose which video they believed was more engaging and better retained their attention. Additionally, "select all that apply" (SATA) questions were implemented to better identify the characteristics respondents believed positively contributed to their preferred video's engagement. In the fifth section, respondents were asked to answer two SATA questions that were unrelated to the videos shown earlier in the survey. The SATA questions asked respondents to choose which characteristics (from Table 1) they believed positively impacted or negatively impacted a short-form video's engagement.

After the survey, a debriefing statement was provided to participants, as information regarding why the videos were selected was omitted from the respondents at the start to ensure unbiased responses.

Participants

The sample consisted of N = 438 teenagers aged 14 to 18 years old who lived in the United States. To gather a wide range of respondents, the researcher employed various methods.

First, the researcher emailed teachers at the high school they attended, asking them to share the survey with their students because high schoolers fit the researcher's demographic of investigation. Secondly, the researcher emailed teachers at high schools across the country to further diversify the geographic distribution of respondents. Finally, the researcher posted the survey in relevant online forums to maximize the number of responses.

Results

Content Analysis

A total of N = 100 TikTok videos were collected, coded, and analyzed on Google Sheets.

Table 2 demonstrates the descriptive engagement metrics of the videos as of December 23rd, 2024. The average video collected had about 2.1 million likes, 23K comments, 164.5K saves,

Engagement Metric	Min	Max	Mean	Standard Deviation	Standard Error
Likes	6585	21700000	2096983.76	3432456.91	343245.69
Comments	215	730000	23062.08	84556.68	8455.67
Saves	526	2100000	164498.73	346924.66	34692.47
Shares	74	3100000	142109.41	369832.38	36983.24
Views	121200	204700000	21819008	36117759.90	3611775.99
TVES	0.17	10.39	4.36	2.30	0.23

Table 2. Short-Form Video Engagement Metrics (N = 100)

142.1K shares, 21.8 million views, and a TVES of 4.36. The TVESs varied the least (STDEV ≈ 2.30), and the number of views varied the most (STDEV ≈ 36,117,759.90).

Table 3 depicts the frequencies of the video characteristics across the 100 collected videos. The most common characteristics were short video length, slow pacing, present text overlay, vertical formatting, and only music. The least common characteristics were medium video length, fast pacing, not-present text overlay, horizontal formatting, and background music. To calculate the average TVES of each characteris-

Characteristic	Option	Frequency	Average TVES
Video Length	Short	57	4.354473684
	Medium	14	4.885642857
	Long	29	4.118758621
Pacing	Slow	54	4.391685185
	Moderate	37	4.268405405
	Fast	9	4.551777778
Text Overlay	Present	73	4.439794521
	Not Present	27	4.146037037
Format	Vertical	97	4.370010309
	Horizontal	3	4.052333333
Music	None	42	3.699642857
	Background	14	5.126714286
	Only	44	4.747477273

Table 3. Short-Form Video Characteristics: Frequencies (N = 100)

tic, the researcher used Google Sheets'=AVERAGEIF function to compute the average TVES of each video with the associated characteristic. Videos with background music had the highest TVES on average, and videos without music (none) had the lowest TVES on average.

Next, five separate single-factor ANOVA tests were conducted, one for each content characteristic. Because the independent variables were primarily categorical, ANOVA was selected as the appropriate statistical test. These tests were used to evaluate whether each characteristic had a significant effect on short-form video engagement by comparing the characteristic categories to their corresponding TVES. All tests were conducted using a significance level of $\alpha = 0.05$. Tables 4 to 8 display the results of the ANOVA tests. Video length, pacing, text overlay, and format were found to be statistically insignificant ($p > 0.05$), meaning that in this specific TVES model (Equation 1), these characteristics did not have a measurable effect on engagement. By contrast, music produced a statistically significant result ($p < 0.05$), and therefore likely influences engagement to a measurable extent.

Survey

438 respondents in total completed the survey. All respondents were teenagers aged 14 to 18 years old who lived in the United States. Figure 3 illustrates

Source	SS	df	MS	F	p-value
Between Groups	5.56	2	2.78	0.52	0.597
Within Groups	518.93	97	5.35		
Total	524.49	99			

Table 4. ANOVA Results: Video Length (N = 100)

Source	SS	df	MS	F	p-value
Between Groups	0.7	2	0.35	0.06	0.938
Within Groups	523.79	97	5.4		
Total	524.49	99			

Table 5. ANOVA Results: Pacing (N = 100)

Source	SS	df	MS	F	p-value
Between Groups	1.7	1	1.7	0.32	0.574
Within Groups	522.79	98	5.33		
Total	524.49	99			

Table 6. ANOVA Results: Text Overlay (N = 100)

Source	SS	df	MS	F	p-value
Between Groups	0.29	1	0.29	0.05	0.815
Within Groups	524.2	98	5.35		
Total	524.49	99			

Table 7. ANOVA Results: Format (N = 100)

Source	SS	df	MS	F	p-value
Between Groups	33.15	2	16.58	3.27	0.042
Within Groups	491.34	97	5.07		
Total	524.49	99			

Table 8. ANOVA Results: Music (N = 100)

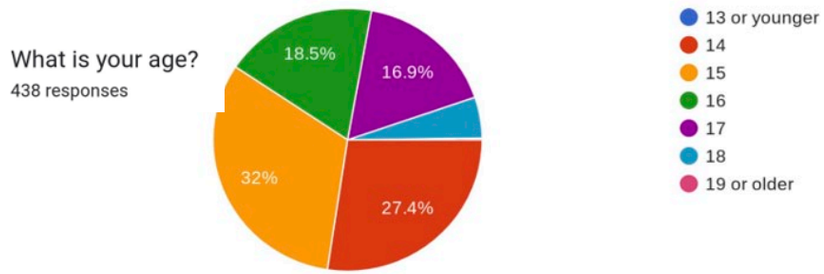

Figure 3. Ages of Respondents

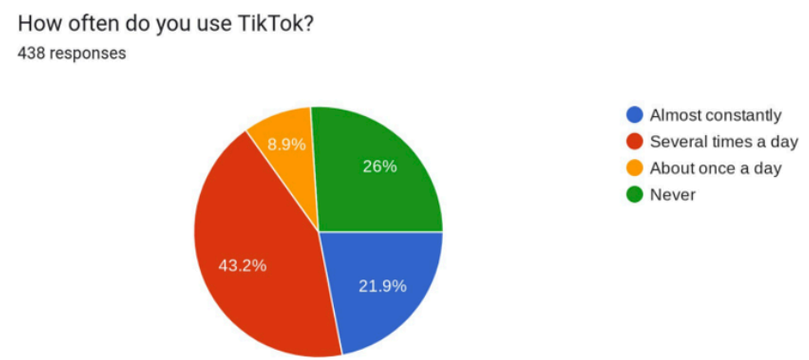

Figure 4. Respondent's TikTok Usage

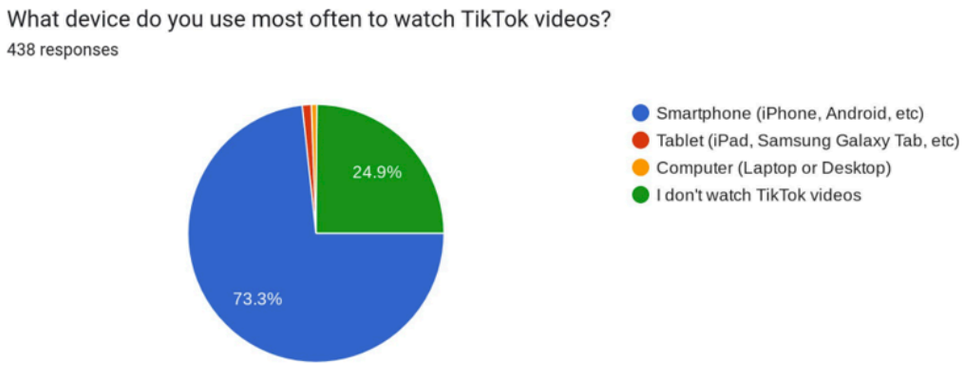

Figure 5. Respondent's Device Usage

the roughly equal age distribution of the participants aged 14 to 17 years old; however, 18-year-olds were underrepresented (5%). Figure 4 demonstrates that most participants used TikTok at least once a day (74%), and Figure 5 shows that smartphones were the most frequently used device to watch TikTok videos (73.3%). Participants who responded "I don't watch TikTok videos" (Figure 5) were still included in the analysis, as their lack of TikTok usage did not detract from this study's goals.

Table 9 displays the participants' varying focused attention levels between videos 1 and 2. Generally, participants exhibited a higher focused attention to Video 1, the desirable video, compared to the undesirable video, Video 2. Table 10 demonstrates that most participants believed that Video 1 was more engaging and effective at holding their attention than Video 2. Participants who responded with the option that stated the videos were equal in engagement and attention were omitted from the table for easier observational comparison.

Question	Video 1	Video 2
Which video was more engaging?	69.20%	19.90%
Which video held your attention better?	74.90%	16.70%

Table 10. Comparison of Engagement & Attention (Row 1: N = 390, Row 2: N= 401)

Figures 6 and 7 illustrate respondents' views of which characteristics contributed to their preferred video's engagement. The majority of participants who found Video 1 more engaging believed that fast pacing contributed the most to the video's engagement (81.7%). For participants who found Video 2 more engaging, most participants believed that on-screen text, a desirable short-form video characteristic, contributed most to the video's engagement (39.7%).

Question	Video	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
I found it easy to stay focused from start to finish	Video 1 (ID: 11)	43.40%	41.60%	8.40%	5.50%	1.10%
	Video 2 (ID: 94)	10%	26.30%	16.40%	32%	15%
I lost interest before it ended	Video 1 (ID: 11)	3%	14.80%	8.20%	42%	32%
	Video 2 (ID: 94)	22.60%	39%	11%	19.60%	7.80%
My full attention was held throughout its duration.	Video 1 (ID: 11)	27.60%	43.80%	11.60%	13.90%	3%
	Video 2 (ID: 94)	7.50%	18.90%	15.80%	36.50%	21.20%
I noticed that I missed some parts because my mind wandered.	Video 1 (ID: 11)	3.40%	16.20%	11.90%	39.30%	29.20%
	Video 2 (ID: 94)	14.40%	39%	13%	22.60%	11%

Table 9. Comparison of Participants' Focused Attention on Each Video (N = 438)

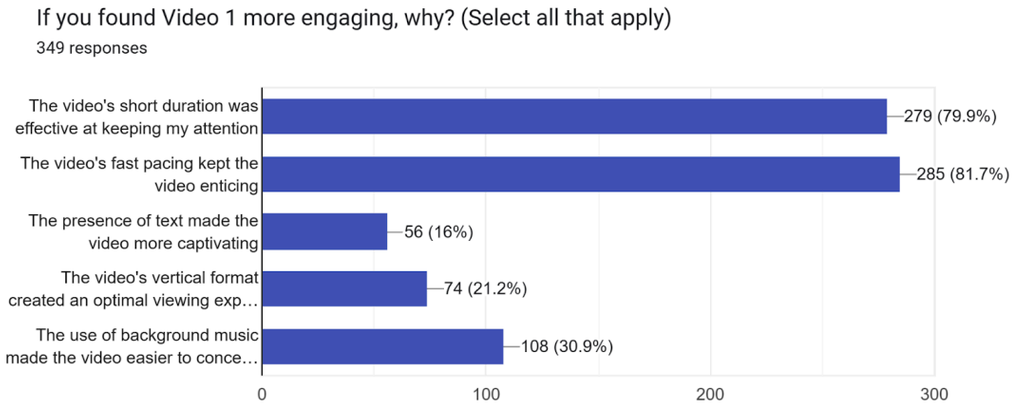

Figure 6. Respondent's Perceptions of Characteristics & Engagement: Video 1

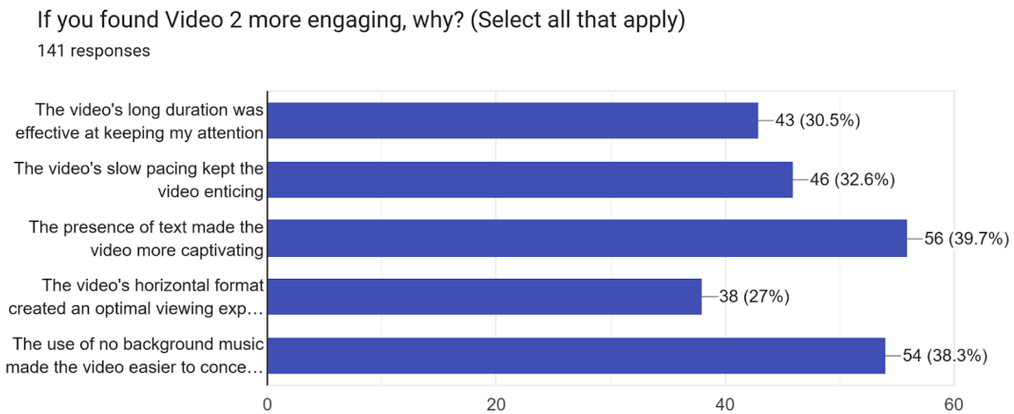

Figure 7. Respondent's Perceptions of Characteristics & Engagement: Video 2

Discussion

This study aimed to examine how the content characteristics differed in enhancing short-form video engagement and sustaining teenage viewers' attention. The findings shown in Table 11 are consistent with those of Liang et al. (2022), as their study similarly considered present text overlay and background music to be desirable traits in maximizing TikTok video engagement. Additionally, the desirability of vertical format aligns with Maenhout (2022), as he stated that users prefer short-form videos because of the seamless "vertical integration" of the content (p. 2). Finally, Table 11 contributes to the discussion in the literature over the most optimal video length and pace. These results suggest that a medium video length and fast pacing are most effective for maximizing engagement, but other studies disagree (Maenhout, 2022; Yang et al., 2024).

Next, it is important to acknowledge the relationships between each characteristic and its TVESs. According to Tables 4 to 7, video length, pacing, text overlay, and format, are statistically insignificant, meaning that each individual characteristic likely does not influence video engagement. Additionally, Table 8 reveals that music is the only statistically significant characteristic, and the other characteristics' results

may be due to chance. Overall, using this specific TVES formula, music seems to be the only characteristic that influences a video's statistical engagement, and the remaining characteristics (video length, pacing, text overlay, format) require further investigation from a different model of measuring short-form video engagement.

Finally, Table 10 demonstrates that Video 1, the video with desirable characteristics, was more favorable in terms of engagement and retaining attention than Video 2, the video with undesirable characteristics. In examining Figure 6, the majority of respondents believed that the short video length and fast pacing of Video 1 contributed most to the engagement. Only some respondents believed that the background music, vertical format, and text overlay contributed to maximizing Video 1's engagement. In Figure 7, respondents were more divided than in Video 1 over the most influential characteristics of Video 2's engagement. For instance, the largest group of respondents believed that the presence of text overlay was the most influential factor (39.7%), but present text overlay is a desirable characteristic, and so this result was expected over the remaining undesirable characteristics of Video 2. Therefore, the researcher accepts the hypothesis: teenagers' focused attention is better retained with short-form videos containing desirable characteristics rather than undesirable characteristics. However, the researcher's other hypothesis is only partially accepted: among the researcher's initial guesses, only fast pacing, vertical format, and background music were found to be most desirable.

Characteristic	Option	Desirability
Video Length	Short	Neutral
	Medium	Desirable
	Long	Undesirable
Pacing	Slow	Neutral
	Moderate	Undesirable
	Fast	Desirable
Text Overlay	Present	Desirable
	Not Present	Undesirable
Format	Vertical	Desirable
	Horizontal	Undesirable
Music	None	Undesirable
	Background	Desirable
	Only	Neutral

Table 11. Short-Form Video Characteristics: Desirability

Limitations

Although some portions of the data were statistically significant, several limitations reduced the effectiveness of the study. Firstly, content analysis is a highly subjective process, and without a team of coders, the reliability of the results may be undermined. Most notably, for coding the pacing of each video, what the researcher may have deemed as slow, another coder may have seen as moderate. Secondly, and most importantly, the characteristics that the researcher investigated do not represent the exhaustive list of qualities of short-form videos that can influence engagement and retention. For example, the content itself in each video is likely a larger contributor, and it should be taken into account that the investigated characteristics are only predictive correlations, not causations of engagement. Therefore, other factors may have influenced the results of both the content analysis and the survey. Lastly, the study was focused solely on videos from the TikTok platform and teenagers aged 14 to 18 years old in the United States. As a result, the findings may only be generalizable to short-form videos found on TikTok and populations that fit the researcher's demographic of investigation.

Implications and Future Research

This study offers valuable information for people who create short-form video content. For instance, by identifying the desirable and undesirable characteristics, casual content creators can learn more about how to optimize their videos' engagement. Additionally, with short-form video advertisements growing as a marketing practice (Meng et al., 2024), this study can inform advertisers on which characteristics to include in their short-form content to better maximize their video's engagement, retention of viewers, and profits for their advertisement. Finally, the creation of the TVES calculator could be used by other researchers investigating the engagement of short-form TikTok videos, as an equation specifically for the TikTok platform likely did not exist prior.

Future researchers could investigate and replicate this study on different short-form social media sites to see if the results change significantly. For instance, researchers can investigate whether the desirability of the characteristics changes on different platforms, such as on Instagram Reels or YouTube Shorts. Another area of investigation for future researchers is exploring characteristics that were not analyzed in this study using the same framework. Some characteristics in the body of literature that could be similarly investigated are voiceovers, the presence of hooks, and calls to action. Finally, different demographics should be examined to determine whether all populations prefer desirable characteristics over undesirable ones.

Conclusion

From the content analysis and survey, the researcher concludes the following. Firstly, in the realm of short-form content creation, a medium video length, fast pacing, present text overlay, vertical format, and background music are all desirable characteristics that likely contribute to maximizing a video's engagement and enhancing the retention of teenagers' focused attention. In contrast, a long video length, moderate pacing, not-present text overlay, horizontal format, and no music likely minimize engagement and retention. As mentioned in the discussion, in comparison to the body of literature, some of the desirable traits (present text overlay, vertical format, background music) correspond with other studies of short-form video engagement, while other traits (video length, pacing) enhance the debate and share a new perspective researchers can consider.

Secondly, the researcher determined that according to this specific TVES calculator (Equation 1), music is the only characteristic that can influence a short-form video's engagement. This conclusion supplements the study conducted by Radovanović (2022), as she claims that music is one of the most important factors in "optimiz[ing] content" and its virality (p. 56). The other characteristics were statistically insignificant (see Tables 4 to 7), meaning that a different model should be used by future researchers to better determine the characteristics' predictive and influential roles in video engagement.

Lastly, through the survey, the researcher determined that TikTok videos with desirable characteristics are more likely to be seen as engaging and to retain the attention of American teenagers more effectively than short-form videos with undesirable characteristics, as determined by the TVES calculator and Table 11.

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STOP SCROLLING: SHORT-FORM VIDEO ENGAGEMENT

The Effects of Post-Processing and Geometric Parameters on the Compressive Strength of Additively Manufactured Resin Lattice Structures

Chance Hattrick

Abstract: Compressive strength is a crucial property in load-bearing design, yet the combined effects of post-processing and geometry on resin-based lattice structures remain underexplored. This study investigates how these parameters influence the compressive strength of SLA-printed resin lattices. Using experimental testing and Bayesian Optimization, I examined the impact of cure time, cure temperature, wash time, pre-cure time, edge length, strut diameter, and relative density on mechanical performance. Fifty octet-truss lattices were fabricated and tested under static compression to measure stress, strain, and Young's modulus. Results show that relative density is the strongest predictor of compressive strength. Longer cure times, shorter wash and pre-cure times, and smaller edge lengths significantly improve structural performance. These findings offer a holistic view of how post-processing and design choices impact mechanical behaviour, and they provide predictive models to help researchers optimize lattice structures for specific performance goals in additive manufacturing applications.

Keywords: Bayesian Optimization, Lattice Structures, Additive Manufacturing, Compressive Strength

Introduction

When maximizing the safety, stability, and longevity of a structure, understanding its compressive strength properties is essential. Compressive strength is the ability of an object to resist a force that is attempting to deform that object. (Wattoo, 2016). Compressive strength is prevalent across various fields, from civil engineering, where it is used to ensure the integrity of bridges and buildings (Ramesh, 2021), to biomedical applications such as artificial implants (Tripathi, 2023). In all these applications, compressive strength is crucial for ensuring the safety of these structures, and negligence can lead to tragedy, as seen in the 1981 incident at the Hyatt Regency Hotel, where two suspended walkways collapsed, killing 114 people. Investigations revealed that a deviation from the original design reduced the load capacity of one of the walkways to only 53% of the maximum load capacity of Kansas City Building Code standards (Wattoo 2016). To prevent such tragedies, engineers must optimize compressive strength properties.

Optimizing these properties is challenging due to an expensive and time-consuming experimentation process. The large number of variables related to design, mass, and size in typical experiments requires a large sample size. In a comparison between Grid Search and Bayesian Optimization, a Boston University group led by Dr. Keith A. Brown required 1,800 samples for Grid Search (Riley 2020). Dr. Brown's paper demonstrated that using just 100 samples in Bayesian Optimization yielded results "superior to 1,800 experiments chosen on a grid" (Riley, 2020), demonstrating that Bayesian Optimization effectively reduces sample size while enhancing precision. To conduct many experiments, the research group used PLA (Polylactic Acid) filament to make the structures, allowing experimentation with numerous variables and designs. However, PLA, while accessible and easy to use, is brittle, which prevents the structure from exhibiting considerable compressive strength. A material that may exhibit greater compressive strength is resin printing. This resin material, while also easily attainable, is far less brittle than PLA, and its use of a UV laser allows for a greater amount of precision and detail in the complex lattice structure. Although these materials have been shown in the past to have significant tensile strength, there have been no studies on how altering the complete post-processing and geometric design of the lattice structure can impact its compressive strength. This could provide researchers with a greater understanding of the mechanical and material properties of the resin. This leads to my research question: How does post-processing and lattice geometry affect the compressive strength of the resin-printed lattice structure?

This study aims to answer this question through a quantitative correlation study. By experimenting with the curing time of roughly 50 resin prints, the displacement under compressive force was measured using a press machine. Using these measurements, a surrogate (initial model) was created to relate variables, such as curing time to ductility. After that, the Bayesian program determined the overall correlation between curing time and specific properties and suggested new experiments that it predicts will maximize the performance of the lattice (see Appendix 2).

The results of this study will broaden the applicability of autonomous 3d printing technologies in experimental applications. By using a 3d printing material closer to engineering-grade polymers, the findings will provide a more accurate depiction of how different structures perform under high compressive loads. This research aims to strengthen our understanding of how curing time affects the mechanical properties of resin-based structures. The findings could significantly improve the safety and reliability of structures across various fields.

Literature Review

Static Compression Tests

Static compression is a concern prevalent in many fields. In a study by the Central South University group led by Xibing Li (2017), researchers studied the causes of structural failures in major Chinese mines, such as the Erdaoigou gold mine and the Changba lead-zinc mine. These failures ranged from rock bursts to zonal disintegration, and future failures could pose a significant risk to any deep mining operations if the mine's construction is not structurally sound. By reproducing these events through subjecting granite and red stone to compression tests, the researchers determined that rock failure occurs when the confining pressure of the structure exceeds a certain threshold. This highlights the risks of disregarding the static compression behaviours of load-bearing structures. Due to these risks, researchers have been actively exploring methods to enhance the static compression strength of these structures. In a study at Western Sydney University led by Christophe Camille (2019), researchers examined the benefits of using fibres to reinforce concrete subjected to static and dynamic loadings. The study tested properties from compressive strength to flexural performance. While the impact of the fibres on the compressive strength was relatively insignificant, researchers found that in concrete samples with fibre, "the severity of the failure is drastically reduced" (Camille et. al., 2019). Therefore, while this does not demonstrate a significant impact on compressive strength, it does emphasize the activity in that field. However, the caveat of this study was to test these properties; the researchers utilized blocks of concrete, which both limited the number of samples they could produce and the level of detail their structures could have. These limitations point to a need for alternative methods which can offer greater flexibility and precision to the designing and testing of materials for static compression.

Additive Manufacturing

Additive Manufacturing (AM), the most common version being 3d printing, offers a new avenue for the experimentation of structures because of its ability to produce complex geometries and its reproducibility.

This capability has proven especially valuable in scientific research and engineering fields. For instance, at the University of Campania, Professor Riccio and Numan Khan studied the applicability of additive manufacturing in the design and implementation of lightweight aerospace parts (Khan & Ricio, 2024). They examined the increased interest in additive manufacturing in the aerospace field and determined that this is due to its ability to construct lattice structures. (Khan & Riccio, 2024). Lattice structures are a network-like design made up of repeating, interconnected patterns. These lattice structures provide strength through geometric arrangements that minimize the amount of material used, making lightweight, strong structures (Khan & Riccio, 2024). However, because of the complexity of the lattice structures, they are only achievable through AM. There are many versions of AM, ranging from metal additive manufacturing to fused filament fabrication. The form of additive manufacturing used in this paper was VAT polymerization. VAT polymerization is a method of creating 3d objects by curing liquid resin through UV light (Tosello, 2018). The most common type and the one used in this paper is Stereolithography (SLA), which uses a laser to cure the 3d structure. In a study by the University of Ostrava, researchers led by Marek Pagac found that VAT polymerization and SLA in particular were extremely accurate in producing detailed prints (Pagac et. al, 2021). The issue regarding additive manufacturing experiments is that the greater flexibility in design means that the experiment can consider an increased number of parameters, which in turn increases the complexity of the experimentation process. In addition, although 3d printer manufacturers do provide some data on how the post-processing of resin prints can impact their physical properties (Zguris 2025), the studies primarily focus on tensile strength and only in the context of cure time and temperature, meaning that further research must be done to create a holistic understanding of how alterations to the post-processing can impact the compressive strength of resin 3d printed lattices.

Bayesian Optimization

Bayesian optimization can be used to handle a large number of variables. According to a review of Bayesian optimization by Xilu Wang at the University of Surrey, Bayesian Optimization has "become popular for taking time-consuming and expensive problems due to its high data efficiency" (Wang, 2022). The process can be separated into two parts: the Gaussian process and the acquisition function. According to Professor Ryan P. Adams of Princeton University, the Gaussian process is a surrogate model used for estimating the true objective function in Bayesian Optimization, and the acquisition function takes this surrogate function and, based on the experimental goals, determines where the optimum next point would be (Snoek et al. 2012). Once this point is received, the researcher uses that point for the next iteration of the surrogate model (Wang, 2022). Bayesian Optimization has already been introduced to the energy absorption world with Dr. Keith Brown's paper on the use of Bayesian Optimization for dynamic impact loading. Dr. Brown's research team at the University of Boston compared results from Bayesian Optimization and Grid-Searching to find that Bayesian Optimization took fewer samples and less time. They found that five of the six optimized structures found by the experimental campaigns (Bayesian Optimization) outperformed the best structures predicted by grid searching. However, while this paper proved that it was possible to use Bayesian Optimization for energy absorption experiments, it was purely a proof of concept. The paper only used PLA, which, while easy to use, is extremely brittle, meaning its compressive properties are not similar to polymers commonly used in compressive strength scenarios. A material that may have a greater similarity to these polymers would be resin, which can print structures in greater detail and allows for varied strength depending on the curing conditions. If resin can accurately imitate these engineering-grade polymers, this approach, combined with machine learning, would open up a new avenue for experimentation with compressive loading structures. Though there have been studies on how post-processing affects the compressive strength of resin polymers, such as Professor Barne's paper from the University of Warwick, which experimented with cure temperature, none of these studies observe a cumulative effect of all aspects of the post-processing on compressive strength on the resin. Additionally, these studies do not test how the geometric properties of these resin lattice structures affect the performance. Therefore, the question is how do the post-processing and geometric parameters affect the compressive strength of resin-printed lattice structures?

Methodology

Type of Study (Quantitative, Correlation Design)

This study used a quantitative approach to investigate the relationship between the build conditions of resin prints and their static compressive behaviour. Quantitative data measured the mechanical properties of the resin prints and was then analyzed to find correlations between the different build variables and compressive performance. The experimental research method used for this study determined the correlation between certain processing conditions and the printed lattice's performance under static compression.

Tools Required

All samples were printed using the Formlabs Form 4 SLA printer, whose use of a laser to initiate the polymerization in the sample allowed for a greater amount of accuracy and complexity in the samples. Additionally, the Formlabs Form Wash and Form Cure were used in post-processing. The Form Cure is required to complete the polymerization process and ensure the sample has strong mechanical properties. This device was integral to the experimentation process as the parameters of the cure time and temperature could potentially have drastic effects on the object's mechanical strength and ability to perform in static compression. The Form Wash was the final step in the process. It was used to remove excess resin from the samples, which ensured that there were no imperfections in the structure that could interfere with the mechanical properties or the accuracy of the experimental results.

Procedure

All the samples of this study were generated using a Python program which created an STL file containing the dimensions for a 3 × 3 octet truss lattice structure (See Appendix 1), which is a lattice structure which

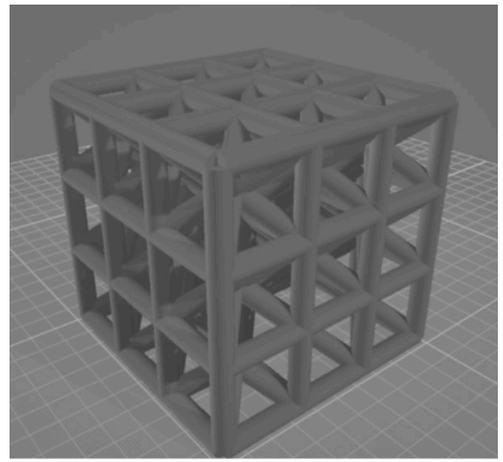

Figure 1: Octet-Truss Lattice Structure

uses 8 compressive-struts to divide the compressive force along each strut (Deshpande, 2001). To reduce variability, the geometry of the lattice was standardized across all samples. After the lattice was generated, all samples would be produced using the same Form Resin Printer. After printing the lattice and removing excess resin using the Form Wash, I waited a set amount of time before beginning the curing of the lattice through the Form Cure. This was to test whether oxidation of the resin prior to curing significantly affected its performance.

In this study, a preliminary set of 10 lattice batches was made to make the surrogate model that will be used in the experiment. Each batch contained five identical lattice structures which feature the same parameters. The surrogate model was fed into the optimization program and is also where the new experimental results were plotted. These 10 points were determined using Latin hypercube sampling (LHS). Head of Applied Research at Articul8 AI, Felipe Viana defines LHS as a statistical sampling method used to efficiently explore multidimensional parameter spaces. Essentially, it generated 10 sets of parameters with non-overlapping intervals to ensure that my initial data covers as many of the possible parameter values as possible (Viana, 2015). While this did not optimize the performance of the lattice structures, it ensured that my data is accurate for a larger scope of parameters and not a specific range.

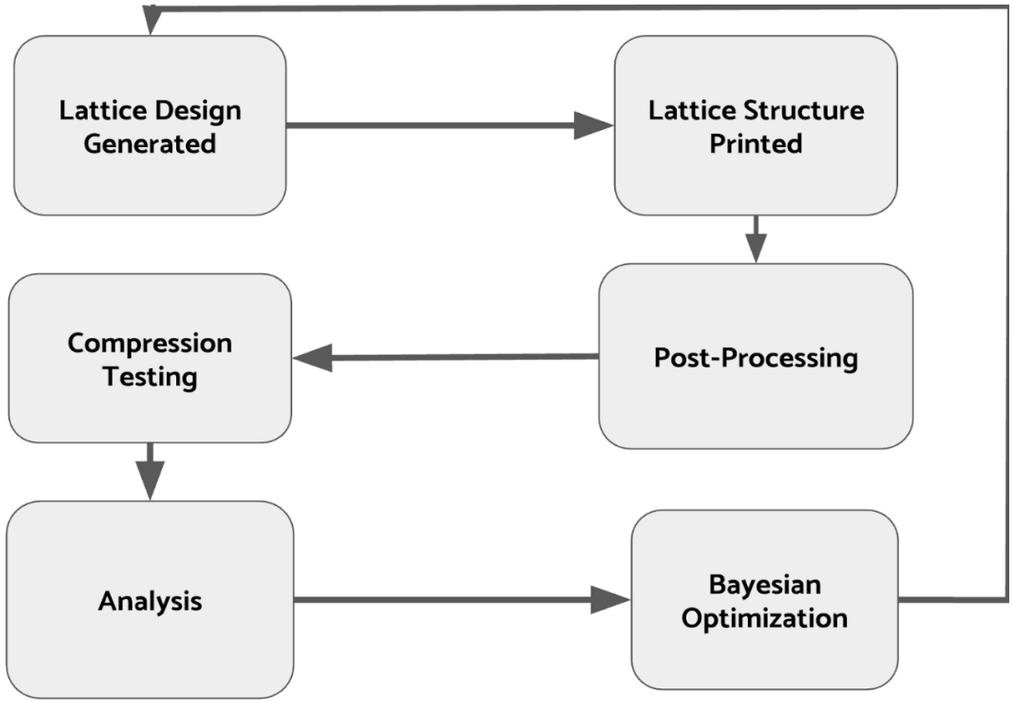

Chart 1: Flowchart of Methodology

The post-processing variables, cure time (mins), cure temperature (Celsius), pre-cure time (min), and wash time (min), were used to determine how altering the material properties of the resin could impact the compressive strength. At the same time, the geometric parameters edge length (mm), strut diameter (mm), and relative density (%) were examined to determine how altering the resin's size and density impacted its performance. The data was analyzed using correlation analysis, regression modelling (linear, polynomial, logarithmic), and machine learning-based feature importance ranking to identify which parameters most significantly influence compressive strength and how researchers could optimize these values.

For testing, the structures were placed on a universal testing machine, and the compressive force would be incrementally increased until the structure began to noticeably deform. This deformation and the force applied at that point were noted and used to calculate the compressive strength of the object in Megapascals (MPa). The performance of the lattice was then plotted on a Stress vs Strain plot, with Stress being the distribution of force on the object while Strain is the overall change in length of the object (Dong, 2015). These properties are commonly used to analyze an object's compressive or tensile strength as they accurately display the deformation of the structure. This can be seen in Professor Danial Rittel's study of the dynamic tension of ductile polymer, where stress vs. strain plots are used to effectively analyze the tension strength of the polymer structures (Tzibula et al., 2018). The details of the calculations will be explained in the Calculated Properties section; however, using the stress vs strain plots, I was able to find the compressive strength and Young's modulus of each lattice structure, creating a point for my Bayesian program (See Appendix 3).

This point was then input into the surrogate model and run through the Python Bayesian function coded in Python; this function takes the values from the surrogate model to use the acquisition function to predict the point at which the stiffness would be maximized. To limit the number of samples and time spent on this experiment, the Probability of Improvement acquisition function was used, as it provides a simple and fast way to maximize the probability of improving upon the current known objective value(Malu, 2021). Though this risks being too exploitative, meaning that the optimization focuses on making minor changes to values with good performance rather than exploring new combinations of variables, the limited time and resources of this experiment prevented me from undertaking a more exploration-focused path that would consider regions of high uncertainty in behaviour. Once the acquisition function suggested a point, this point would be tested through the same method and inputted back into the function, where the surrogate model would update itself and rerun the acquisition function.

Calculated Properties

To determine each lattice structure's performance, in addition to the compressive strength, the study also measured each lattice structure's measured stress, measured strain, Young's modulus and relative density.

The measured stress of each lattice structure was calculated using equation (1), where F is the force applied by the compression tester, and A is the cross-sectional area of the lattice structure. This calculates the distribution of force on the lattice structure, which is used to determine the compressive strength.

The measured strain of each lattice structure is calculated by equation (2), where ΔL represents the change in the length of the compressor, and L represents the original length. This is used to calculate the total displacement of the lattice structure, which was used to determine where the structure failed.

In their study of the durability of polymer composites under static loads, researchers at the Institute for Problems of Chemical and Energy Technologies defined Young's modulus as the mechanical property that measures the compressive stiffness of the structure. This modulus is defined as the ratio of stress to strain in the linear elastic region of the material. This means that when analyzing the Stress vs Strain graph, there is an initial linear trend between stress vs strain, which is referred to as the "linear elastic region," meaning that any deformation that occurs in this region will be recovered. The maximum extent of this linear region is the yield strength of the lattice structure, and the initial linear line is used to determine the stiffness of the lattice (Fig. 1). Following the yield strength the lattice begins to experience plastic deformation where the deformations will not recover after the force is no longer applied and the final maximum force applied to the lattice before it fails is called "ultimate strength" (Startsev, 2019)

$$\sigma = \frac{F}{A}$$

Equation 1: Stress Definition

$$\varepsilon = \frac{\Delta L}{L_0}$$

Equation 2: Strain Definition

The relative density of the lattice structure is the ratio of the lattice's apparent density to the density of the solid material. This is used to compare the density of the lattice to that of a solid cube of the same material, and is calculated through equation (3), where Vsolid is the volume of the lattice, and Vtotal is the volume of the solid structure. This is also used in the Bayesian Optimization section to minimize the density of the lattice.

Analysis

Once all of the data was gathered, the study found the average compressive strength of each batch of lattices and plotted how the compressive strength of each batch was affected by the post-processing and geometric parameters. After reviewing, a principal parameter was chosen as the key determining factor in the performance of the lattices. Using the graph of the parameter vs the compressive strength of the batch, a trendline was generated to represent the predicted change in compressive strength with relation to the principal parameter. This predicted value was then compared to the measured average compressive strength of lattices. These values were then used to plot the effect each parameter (excluding the principal parameter) had on the "excess strength" shown by the

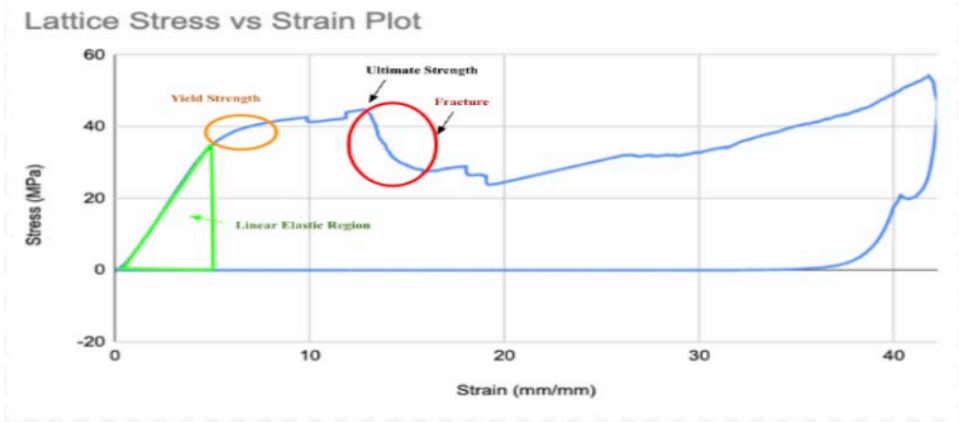

Figure 2: Compressive Stress vs. Strain Plot for SLA Resin Lattice

batches with respect to the predicted value. Using this method, even with the limitation of a small number of iterations, the study was still able to approximate the effect of each parameter on the lattices.

Justification

Dr. Keith Brown conducted a similar study for compressive strength testing when his group at the University of Boston was designing an autonomous experimental setup. In his study, his group found that the use of roughly 100 iterations with Bayesian optimization was more effective than the typical use of grid searching (Riley 2020). However, because I used resin rather than PLA, cost for materials was higher; therefore, I reduced the number of iterations to 50 so that I would still have a considerable sample size while conserving the material.

Findings

Table 1 displays the post-processing and geometric characteristics of each of the lattice structures; Table 2 displays the compressive strength, relative density, and Young's modulus of the respective lattice structures; and Figure 2 shows the average Stress vs Strain plot for all of the lattice structures.

Figure 2 shows the average stress vs strain plot for

$$\rho^* = \frac{V_{\text{solid}}}{V_{\text{total}}}$$

Equation 3: Young's Modulus Definition

each of the 9 batches of lattice structures. The initial batches, such as Lattices 1 and 2, feature much longer domains than later batches, suggesting that before the optimization, the lattices were more easily deformed, which, compared to their low maximum stress, suggests that these lattice structures were compressively weak, leading to structural failure. This can be seen clearly in Lattice 2, where the jagged lines are areas where the lattice experienced buckling failure as one layer of the lattice collapsed before the force was redistributed and the structure stiffened again.

Analysis

Due to the limitations in the sample size, the large number of parameters examined meant that it was difficult to determine how each individual parameter affects the compressive strength. However, one pa-

Characteristics	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9
Cure Time (min)	42	108	32	136	110	72	149	43	94.4
Cure Temp (Celsius)	35	78	71	36	74	53	47	35	35
Wash Time (min)	10	3	11	2	18	3	13	2	2
Pre-Cure Time (min)	225	69	29	22	39	25	21	6 mins	13
Edge-Length (mm)	4	2.21	3.16	2.21	3.09	2.7	2.11	2.40422	1.681772
Strut Diameter (mm)	0.2	0.21	0.38	0.263	0.377	0.371	0.338	0.263679	0.286151
Relative Density (%)	0.0245	0.089	0.156	0.142	0.16	0.203	0.271	0.1603	0.3044

Table 1: Compressive Strength and Parameters of Lattice Batches

Results	Young's Modulus (MPa)	Compressive Strength (MPa)	Standard Deviation	Relative Density(%)
Experiment 1	0.744016	0.2278755556	±0.0725	0.0245
Experiment 2	7.846	13.45	±3.00	0.089
Experiment 3	10.694	9.019997686	±0.706	0.156
Experiment 4	151.53	24.473	±0.272	0.142
Experiment 5	27.658	13.89796296	±7.50	0.16
Experiment 6	42.912	17.93688889	±0.590	0.203
Experiment 7	65.53	28.12555556	±3.12	0.271
Experiment 8	84.79	23.23	±0.0398	0.1603
Experiment 9	23.5	47.54	±0.0535	0.3044

Table 2: Average Compressive Strength and Young's Modulus of Lattice Batches

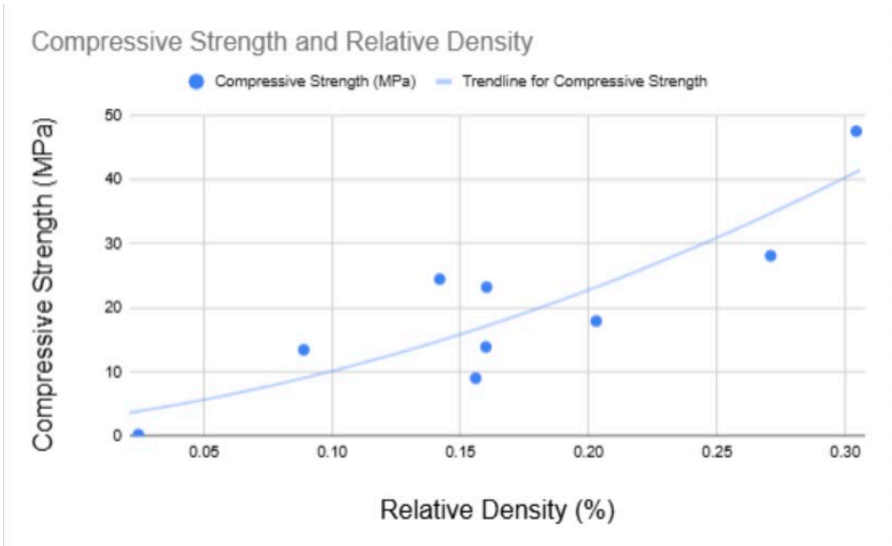

Figure 3: Compressive Strength vs Relative Density Plot for all Lattice Batches

rameter that showed a strong positive correlation and was relatively independent of other parameters was the relative density. When plotting the relative density of each batch vs its compressive strength, although a strong positive relationship is seen, some points show higher or lower compressive strength than the line of best fit predicts. By using the residuals of the relative density vs compressive strength plot, the study can

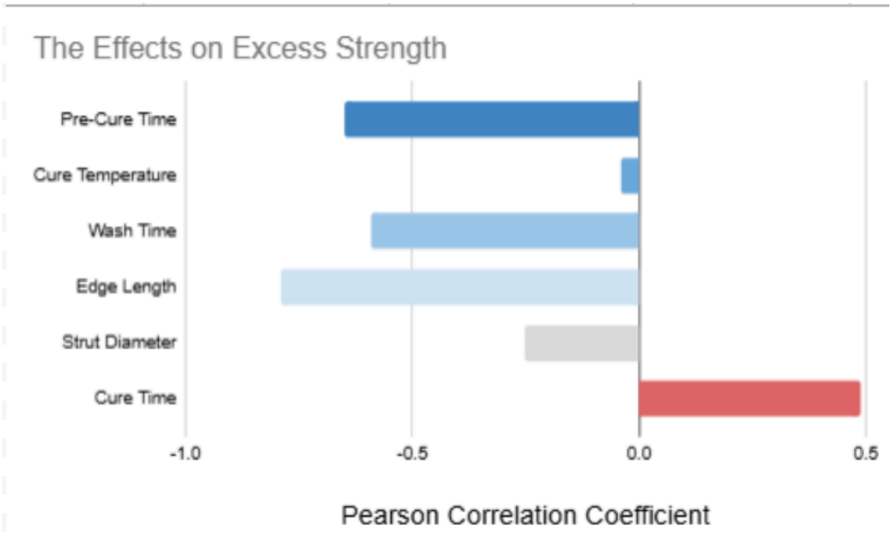

Figure 4: Pearson Coefficients of the Parameters vs Excess Strength

more clearly see how the other parameters, such as cure time and temperature, impact the excess compressive strength of the batches (See in Appendix 4).

When examining the Pearson coefficient (r-value) of the plots that compare each parameter to the excess strength of the lattice batches, it is seen that the edge length of the lattice has a strong negative correlation to the compressive strength while the wash time and pre-cure time exhibit a moderately strong negative correlation to the compressive strength. The only parameter to show a relatively strong positive correlation was the cure time.

Discussion

Summary

Through analyzing the plots, several main themes appear for optimizing the post-processing and geometric parameters. The first is that for post-processing, time is the most important parameter to optimize. When comparing the Cure Time and Cure Temperature plots in relation to compressive strength, it becomes clear that cure time has a much greater impact on the performance of the lattice structure. This plot shows that researchers looking to maximize the compressive strength should cure their lattices for extended periods of time to ensure that the curing process is fully complete. Additionally, the Pre-Cure Time to Compressive Strength plot exhibits a negative exponential relation, meaning that researchers should avoid leaving uncured lattices in oxygen-rich environments for extended periods of time, as the resin tends to oxidize, preventing the curer from fully polymerizing the lattice. Secondly, the geometric plots show that smaller and less dense lattice structures tend to perform better than larger lattices. While this seems counterintuitive, as larger lattices should have a greater distribution of the compressive force, it is possible that for larger lattices, the force is less evenly distributed, leading to localized failures. Finally, it shows that the geometric parameters, such as the edge length of the lattice, significantly affect the compressive strength of the lattice structure and that minimizing the size of the unit cells in the lattice will positively affect the compressive strength.

Through the use of Bayesian optimization and Pearson's coefficient, this study offers a foundational understanding of how the post-processing and geometric parameters of SLA resin lattices impact their compressive strength. By identifying which parameters strongly correlate with mechanical performance, such as the positive effect of cure time and the negative impact of delaying the curing of the lattices, this study allows researchers to make informed trade-offs with their parameters without compromising compressive strength. For instance, if a larger unit cell is required for functional or design restraints, the researcher can use this data to determine that they will need to increase the cure time or optimize the strut diameter to a certain amount to offset the negative effect of the larger unit cell. These insights give researchers and engineers the flexibility to fine-tune their parameters and still accurately predict the compressive strength behaviour of the lattice structure.

Interpretation

The results of this study support evidence from Dr. Brown's study on oxygen inhibition in resin printing in that a significant impact on performance was observed when the resin was left uncured for extended periods of time (Saygin 2023). Additionally, a study conducted by Professor Stuart Barnes at the University of Warwick confirms that at a certain temperature, resin compressive strength tends to decrease (Ateş 2011). However, unlike Professor Barne's experiment, this study found that cure temperature has minimal impact on the performance of the lattice and that cure time is a more important parameter to optimize. Finally, unlike studies conducted by Sholana Iffat at the Bangladesh University of Engineering (Iffat 2015), this study found that relative density does not have a positive linear relationship with compressive strength and is optimized at around 14.2% of the density of a solid cube with the same edge length.

Limitations

The accuracy of the plots is limited due to the small sample size of this study. Though this study did use Bayesian optimization to suggest optimized lattice structures to test, the small sample size means that the model's predictions may to be an accurate representation of how each parameter affects the performance of the structure. The models used in the study serve as a preliminary overview of the effects of each parameter on the lattice structure. Additionally, the mechanical limitations of the compression tester used meant that the height of the lattice structure was limited to 15 mm and had a maximum force of 1200 Newtons. This severely limited the edge-length parameter and meant that some structures could not reach failure, preventing me from measuring their actual compressive strength. Finally, due to budgetary and time constraints, this study only observed the effects of these parameters on Clear Resin; for resins with different properties, such as Rigid 10k Resin, the results may vary due to different material properties. Nonetheless, the results provide an accurate but general understanding of how post-processing and geometric properties come together to affect the compressive strength of resin lattice structures.

Recommendations

Future research studies could observe how these parameters affect the properties of the different types of resins used in additive manufacturing. Additionally, understanding how applying different methods of resin additive manufacturing changes the results could provide a greater number of researchers using different resin printers with how these parameters will affect their results. Further research could be done on how altering the post-processing of resin prints could allow researchers to mimic the compressive properties of more complex materials, such as PEEK polymers.

Conclusion

As additive manufacturing continues to progress, lattice structures will become more prevalent in day-to-day applications, from vehicles to buildings. Additionally, polymers provide a cost-effective way of producing high-performing materials for tensile and compressive applications. Thus, by assessing how post-processing and geometric parameters affect the compressive strength of resin lattice structures, this study establishes that to maximize compressive strength, researchers should aim to minimize the size of the unit cells, the wash time, and the pre-curing time, while maximizing the amount of time the lattice spends curing. Researchers can also use this data to optimize the performance of their resin lattice struc-

tures and predict how a large number of lattice samples will perform based on their post-processing and geometry. The field of compressive strength is a vital field for ensuring the safety of workers and civilians alike, and with these models, researchers will be able to more effectively test and analyze the performance of countless lattice structures in their research to advance our understanding of compressive strength.

Acknowledgements

I would like to thank Dr. Keith Brown from the University of Boston for their mentorship and critical advice throughout this research. I am also grateful to Dr. Sterling Baird from the Acceleration for their mentorship and for facilitating my access to the equipment necessary for my project's completion. A special thanks to Hongchen Wang and Kelvin Chow for their help and advice in operating the Form4 resin 3d Printer and the universal testing machine; this would not have been possible without them.

Appendix 1

```
#Python script for generating the Octet-Truss Lattice
import numpy as np
import trimesh
def generate_octet_truss(grid_size, unit_cell_size, strut_radius):
    """
    Generate an octet-truss lattice as a trimesh object.
    Args:
        grid_size (tuple): Number of unit cells along (x, y, z) directions.
        unit_cell_size (float): Size of each unit cell in mm.
        strut_radius (float): Radius of the struts in mm.
    Returns:
        trimesh.Trimesh: Combined mesh of the octet-truss lattice.
    """
    cylinders = []
    # Create the grid of unit cell centers
    for x in range(grid_size[0]):
        for y in range(grid_size[1]):
            for z in range(grid_size[2]):
                # Origin for the current unit cell
                origin = np.array([x, y, z]) * unit_cell_size
                # Define nodes of the unit cell relative to the origin
                nodes = [
                    origin + np.array([0, 0, 0]),
                    origin + np.array([unit_cell_size, 0, 0]),
                    origin + np.array([0, unit_cell_size, 0]),
                    origin + np.array([0, 0, unit_cell_size]),
                    origin + np.array([unit_cell_size, unit_cell_size, 0]),
                    origin + np.array([unit_cell_size, 0, unit_cell_size]),
                    origin + np.array([0, unit_cell_size, unit_cell_size]),
                    origin + np.array([unit_cell_size, unit_cell_size, unit_cell_size]),
                    origin + np.array([unit_cell_size / 2, unit_cell_size / 2, unit_cell_size / 2]),
```

```
        ]
        # Define edges (connections between nodes)
        edges = [
            (0, 1), (0, 2), (0, 3), (1, 4), (1, 5), (2, 4), (2, 6),
            (3, 5), (3, 6), (4, 7), (5, 7), (6, 7),
            (0, 8), (1, 8), (2, 8), (3, 8), (4, 8), (5, 8), (6, 8), (7, 8)
        ]

        # Create struts (cylinders) for each edge
        for start, end in edges:
            start_node = nodes[start]
            end_node = nodes[end]
            edge_vector = end_node - start_node
            length = np.linalg.norm(edge_vector)

            # Create a cylinder along the edge
            cylinder = trimesh.creation.cylinder(
                radius=strut_radius,
                height=length,
                sections=32
            )

            # Align cylinder to edge vector
            transform = trimesh.geometry.align_vectors([0, 0, 1], edge_vector)
            cylinder.apply_transform(transform)

            # Translate cylinder to the correct position
            midpoint = (start_node + end_node) / 2
            cylinder.apply_translation(midpoint)

            cylinders.append(cylinder)

    # Combine all cylinders into a single mesh
    return trimesh.util.concatenate(cylinders)

def main():
    # Define parameters
    grid_size = (2, 2, 3)       # Number of unit cells in each direction (x, y, z)
    unit_cell_size = 4        # Size of each unit cell (mm)
    strut_radius = 0.3       # Radius of the struts (mm)
    output_file = "lattice_exp14.stl"  # Output STEP file name
    material_density = 1.2       # g/cm³ (example for a plastic-like material)

    print("Generating octet-truss lattice...")
    lattice = generate_octet_truss(grid_size, unit_cell_size, strut_radius)

    # Compute Volume
    volume = lattice.volume  # mm³
    print(f"Total Lattice Volume: {volume:.2f} mm³")
```



```
    # Assign Material Density and Compute Mass
    lattice.density = material_density  # g/cm³
    mass = lattice.mass  # grams
    print(f"Total Lattice Mass: {mass:.2f} g")

    # Export as STEP
    print(f"Exporting lattice to {output_file}...")
    lattice.export(output_file, file_type='stl')
    print("Export complete. You can now import the STEP file into your CAD software.")
if __name__ == "__main__":
    main()
```

Appendix 2

```
import numpy as np
import pandas as pd
from ax.service.ax_client import AxClient, ObjectiveProperties
import matplotlib.pyplot as plt
from mpl_toolkits.mplot3d import Axes3D
obj1_name = "compressive_strength"
obj2_name = "relative_density"
obj3_name = "y_modulus"


def measure_properties(cure_time, cure_temp, wash_time, pre_time, edge_len, strut_dia):
    #Insert matethematical model once the intial experiments are complete.
    y= 0

    #return y


# Define the training data

#training data, all of the parameters for the experiment data
X_train = pd.DataFrame(
    [
        {"cure_time": 42.0, "cure_temp": 35.0, "wash_time": 10.0, "pre_time": 225.0, "edge_len": 4,
"strut_dia": 0.2},
        {"cure_time": 42.0, "cure_temp": 35.0, "wash_time": 10.0, "pre_time": 225.0, "edge_len": 4,
"strut_dia": 0.2},
        {"cure_time": 42.0, "cure_temp": 35.0, "wash_time": 10.0, "pre_time": 225.0, "edge_len": 4,
"strut_dia": 0.2},
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"strut_dia": 0.2},
        {"cure_time": 42.0, "cure_temp": 35.0, "wash_time": 10.0, "pre_time": 225.0, "edge_len": 4,
```

```
"strut_dia": 0.2},
      {"cure_time": 108.0, "cure_temp": 78.0, "wash_time": 3.0, "pre_time": 69.0, "edge_len": 2.21,
"strut_dia": 0.21},
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"strut_dia": 0.380},
      {"cure_time": 32.0, "cure_temp": 71.0, "wash_time": 11.0, "pre_time": 29.0, "edge_len": 3.16,
"strut_dia": 0.380},
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"strut_dia": 0.380},
      {"cure_time": 32.0, "cure_temp": 71.0, "wash_time": 11.0, "pre_time": 29.0, "edge_len": 3.16,
"strut_dia": 0.380},
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"strut_dia": 0.263},
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"strut_dia": 0.377},
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```



```
"strut_dia": 0.371},
      {"cure_time": 149.0, "cure_temp": 47.0, "wash_time": 13.0, "pre_time": 21.0, "edge_len": 2.11,
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"strut_dia": 0.338},
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"strut_dia": 0.338},
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"strut_dia": 0.338},
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"strut_dia": 0.264},
      {"cure_time": 43.0, "cure_temp": 35.0, "wash_time": 2.0, "pre_time": 6.0, "edge_len": 2.40,
"strut_dia": 0.264},
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"strut_dia": 0.264},
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"strut_dia": 0.264},
      {"cure_time": 43.0, "cure_temp": 35.0, "wash_time": 2.0, "pre_time": 6.0, "edge_len": 2.40,
"strut_dia": 0.264},
   ]
)

#The outputs of the experiment data
y_train = [
   {obj1_name: 3.01, obj2_name: 0.0245, obj3_name: 1.10},
   {obj1_name: 1.45, obj2_name: 0.0245, obj3_name: 0.59},
   {obj1_name: 1.44, obj2_name: 0.0245, obj3_name: 1.11},
   {obj1_name: 2.00, obj2_name: 0.0245, obj3_name:0.62},
   {obj1_name: 2.30, obj2_name: 0.0245, obj3_name:0.81 },
   {obj1_name: 90.0, obj2_name: 0.089, obj3_name:33.89},
   {obj1_name: 97.3, obj2_name: 0.089, obj3_name: 32.22},
   {obj1_name: 130.6, obj2_name: 0.089, obj3_name: 27.93},
   {obj1_name: 155.54, obj2_name: 0.089, obj3_name: 35.70},
   {obj1_name: 131.8, obj2_name: 0.089, obj3_name: 27.94},
   {obj1_name: 79.62, obj2_name: 0.156, obj3_name:22.35},
   {obj1_name: 74.11, obj2_name: 0.156, obj3_name:21.17},
   {obj1_name: 76.67, obj2_name: 0.156, obj3_name:20.31},
   {obj1_name: 88.86, obj2_name: 0.156, obj3_name:19.59},
   {obj1_name: 86.65, obj2_name: 0.156, obj3_name:21.62},
   {obj1_name: 214.5, obj2_name: 0.142, obj3_name:214.4},
   {obj1_name: 135.6, obj2_name: 0.142, obj3_name:38.93},
   {obj1_name: 230.94, obj2_name: 0.142, obj3_name:246.29},
   {obj1_name: 299.99, obj2_name: 0.142, obj3_name:106.5},
   {obj1_name: 102.09, obj2_name: 0.16, obj3_name:38.89},
   {obj1_name: 126.07, obj2_name: 0.16, obj3_name:26.10},
   {obj1_name: 126.03, obj2_name: 0.16, obj3_name:25.80},
```

```
    {obj1_name: 126, obj2_name: 0.16, obj3_name:22.7},
    {obj1_name: 126.1, obj2_name: 0.16, obj3_name:24.8},
    {obj1_name: 126.2, obj2_name: 0.16, obj3_name:39.0},
    {obj1_name: 154.56, obj2_name: 0.203, obj3_name:43.27},
    {obj1_name: 163.93, obj2_name: 0.203, obj3_name:39.54},
    {obj1_name: 165.92, obj2_name: 0.203, obj3_name:45.40},
    {obj1_name: 156.95, obj2_name: 0.203, obj3_name:41.08},
    {obj1_name: 165.8, obj2_name: 0.203, obj3_name:45.27},
    {obj1_name: 271.42, obj2_name: 0.271, obj3_name:71.87},
    {obj1_name: 271.61, obj2_name: 0.271, obj3_name:66.71},
    {obj1_name: 271.6, obj2_name: 0.271, obj3_name:69.30},
    {obj1_name: 270.34, obj2_name: 0.271, obj3_name:63.95},
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    {obj1_name: 216.29, obj2_name: 0.271, obj3_name:57.95},
    {obj1_name: 208.7, obj2_name: 0.1603, obj3_name:91.3},
    {obj1_name: 208.6, obj2_name: 0.1603, obj3_name:90.6},
    {obj1_name: 209.3, obj2_name: 0.1603, obj3_name:82.9},
    {obj1_name: 209.3, obj2_name: 0.1603, obj3_name:79.1},
    {obj1_name: 209.3, obj2_name: 0.1603, obj3_name:79.9}
]

# See https://youtu.be/4tnaL9ts6CQ for simple human-in-the-loop BO instructions

# Define the number of training examples
n_train = len(X_train)

#Create the AxClient
ax_client = AxClient()

#Here I am defining the different parameters I am measuring and the outcomes that I wish to optimize
ax_client.create_experiment(
    parameters=[
        {"name": "cure_time", "type": "range", "bounds": [0.0, 300.0]},
        {"name": "cure_temp", "type": "range", "bounds": [35.0, 80.0]},
        {"name": "wash_time", "type": "range", "bounds": [2.0, 30.0]},
        {"name": "pre_time", "type": "range", "bounds": [0.0, 300.0]},
        {"name": "edge_len", "type": "range", "bounds": [1.0, 4.0]},
        {"name": "strut_dia", "type": "range", "bounds": [0.2, 0.5]},
    ],
    objectives={
        obj1_name: ObjectiveProperties(minimize=False, threshold = 200),
        obj2_name: ObjectiveProperties(minimize=True, threshold = 0.15),
        obj3_name: ObjectiveProperties(minimize=False, threshold = 50),
    },
)

# Add existing data to the AxClient
for i in range(n_train):
    #Converts each X_train entry into a dictionary
    parameterization = X_train.iloc[i].to_dict()
```
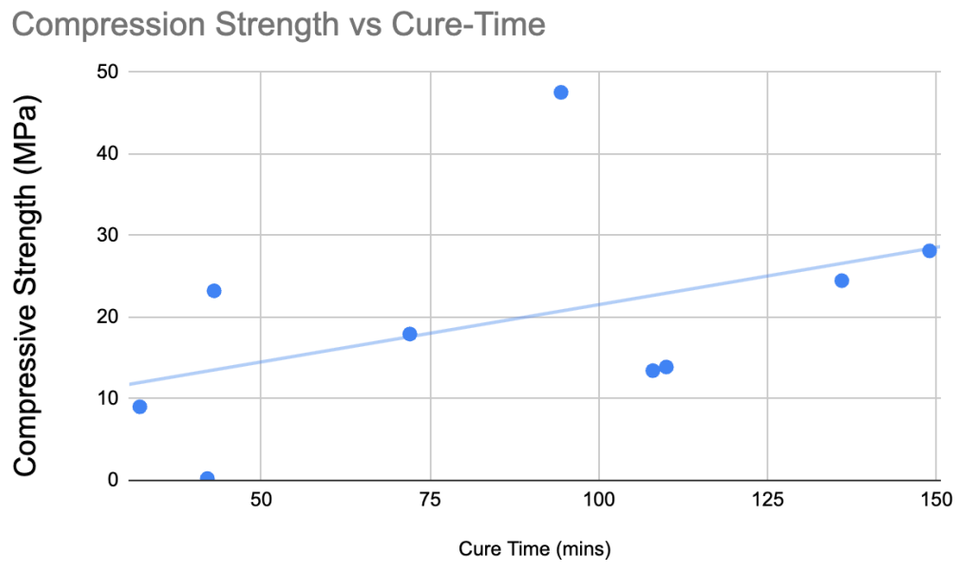
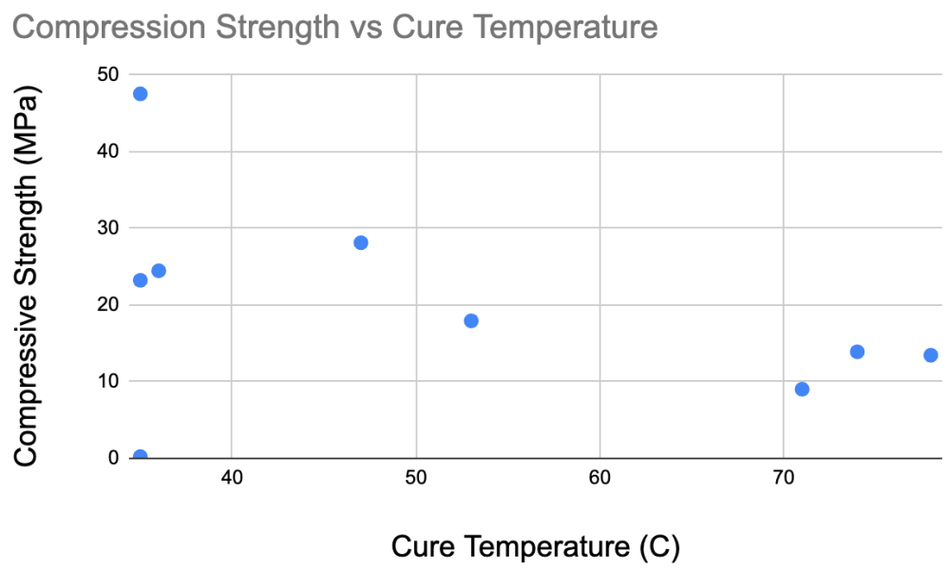



```
    #Adds the Trial to the AxClient
    ax_client.attach_trial(parameterization)
    #Links the output data to the X_Train parameters
    ax_client.complete_trial(trial_index=i, raw_data=y_train[i])

#Number of suggestions per iteration
batch_size = 3
trial_counter = 0


for i in range(19):

    parameterizations, optimization_complete = ax_client.get_next_trials(batch_size)
    for trial_index, parameterization in list(parameterizations.items()):
        # extract parameters
        x1 = parameterization["cure_time"]
        x2 = parameterization["cure_temp"]
        x3 = parameterization["wash_time"]
        x4 = parameterization["pre_time"]
        x5 = parameterization["edge_len"]
        x6 = parameterization["strut_dia"]
        if trial_counter < len(y_train):
            results = y_train[trial_counter]
            ax_client.complete_trial(trial_index=trial_index, raw_data=results)
            trial_counter += 1
# Extract observed trials (including missing values to retain all data)
df_all = ax_client.get_trials_data_frame()

# Ensure that at least one objective value is available
df_all = df_all.dropna(subset=[obj1_name, obj2_name, obj3_name], how='all')

# Debugging: Check if trials contain valid data
print("All Trials DataFrame shape:", df_all.shape)
print("All Trials DataFrame head:\n", df_all.head())

# Get Pareto-optimal points
pareto_results = ax_client.get_pareto_optimal_parameters(use_model_predictions=False)

# Debugging: Check if Pareto results are empty
if not pareto_results:
    print("⚠ Warning: No Pareto-optimal solutions found.")
else:
    print(f"Found {len(pareto_results)} Pareto-optimal points.")

# Convert Pareto results into a DataFrame
pareto_data = [p[1][0] for _, p in pareto_results.items()]  # Extract only objective dictionary
pareto_df = pd.DataFrame(pareto_data)
print("pareto_df:", pareto_df)
# Debugging: Check Pareto DataFrame
print("Columns in pareto_df:", pareto_df.columns)
```

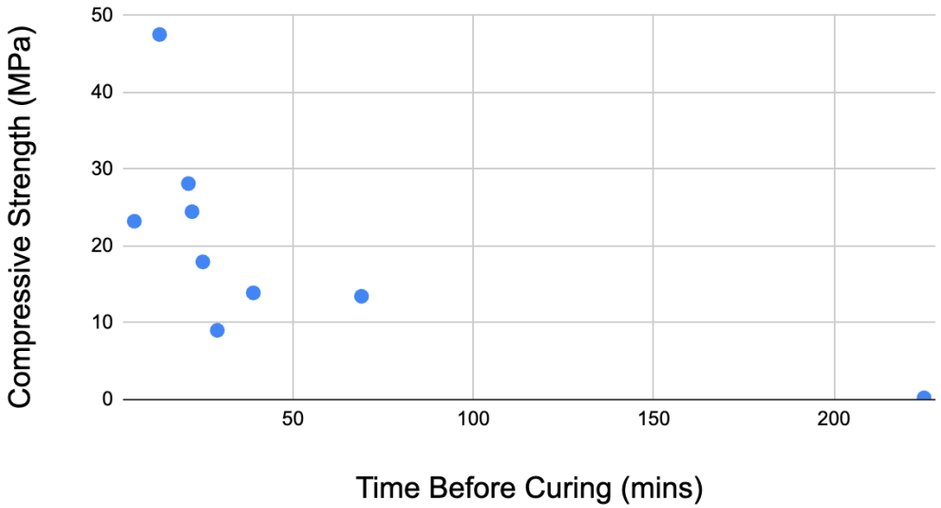
```
print("Pareto DataFrame shape:", pareto_df.shape)

# If Pareto results are empty, stop execution
if pareto_df.empty:
    print("⚠ No Pareto front to plot.")
else:
    # Ensure Pareto DataFrame is sorted for better plotting
    pareto_df = pareto_df.sort_values(by=obj1_name)

    # Extract values for plotting
    jitter = 0.05  # Adjust this value if necessary
    x_all = df_all[obj1_name] + np.random.uniform(-jitter, jitter, size=len(df_all))
    y_all = df_all[obj2_name] + np.random.uniform(-jitter, jitter, size=len(df_all))
    z_all = df_all[obj3_name] + np.random.uniform(-jitter, jitter, size=len(df_all))
    x_pareto = pareto_df[obj1_name]
    y_pareto = pareto_df[obj2_name]
    z_pareto = pareto_df[obj3_name]

    # Create a 3D scatter plot
    fig = plt.figure(figsize=(8, 6))
    ax = fig.add_subplot(111, projection='3d')

    # Plot all observed points
    ax.scatter(x_all, y_all, z_all,
               color="blue", alpha=0.4, label="All Observed Data", marker="o")

    # Plot Pareto-optimal points
    ax.scatter(x_pareto, y_pareto, z_pareto,
               color="red", s=80, label="Pareto-optimal Points", marker="^")

    # Labels and title
    ax.set_xlabel("Compressive Strength")
    ax.set_ylabel("Relative Density")
    ax.set_zlabel("Young's Modulus")
    ax.set_title("3D Scatter of All Trials and Pareto Front")

    ax.legend()
    plt.show()

    plt.scatter(df_all[obj1_name], df_all[obj2_name], alpha=0.5)
    plt.xlabel("Compressive Strength")
    plt.ylabel("Relative Density")
    plt.title("2D Projection: Compressive Strength vs Relative Density")
    plt.show()
```


Appendix 3

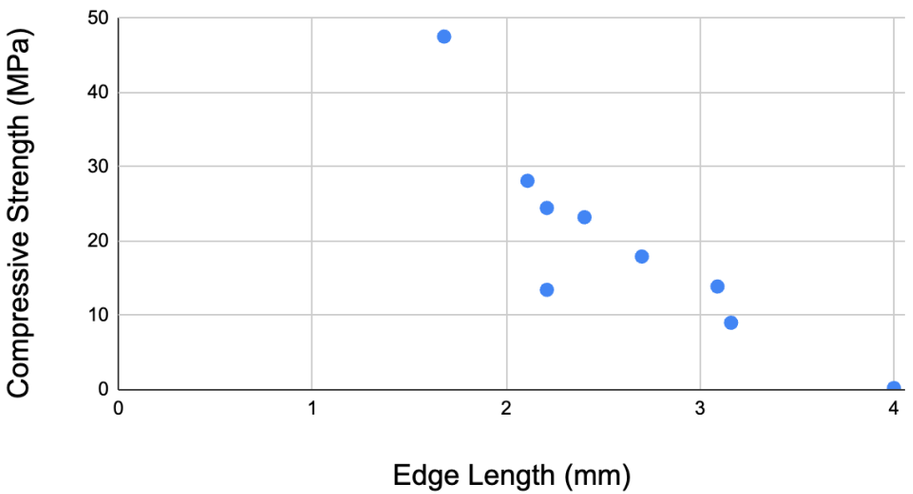




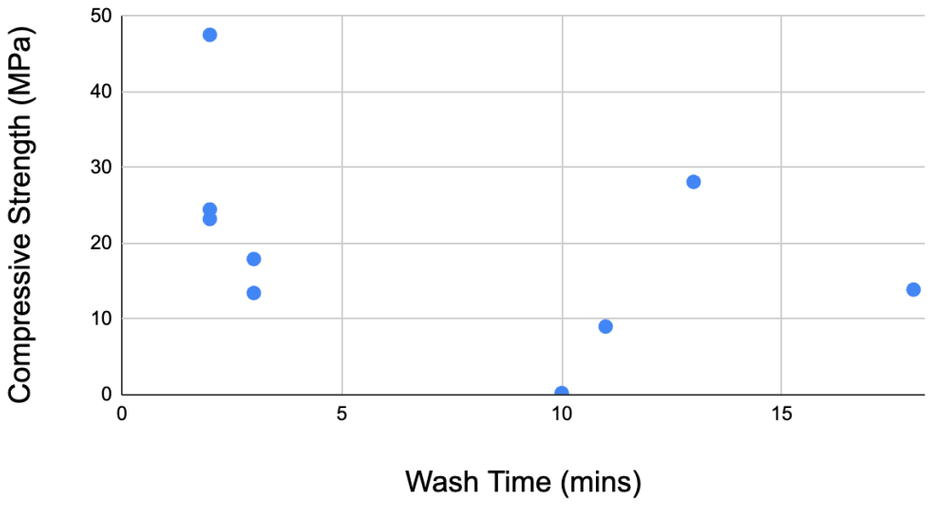
Time before Curing vs Compression Strength



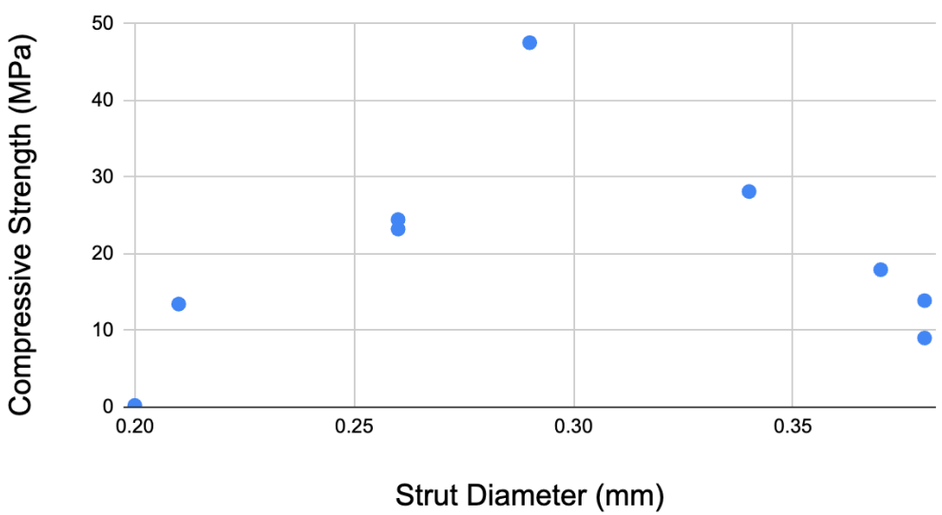
Edge-Length vs Compression Strength

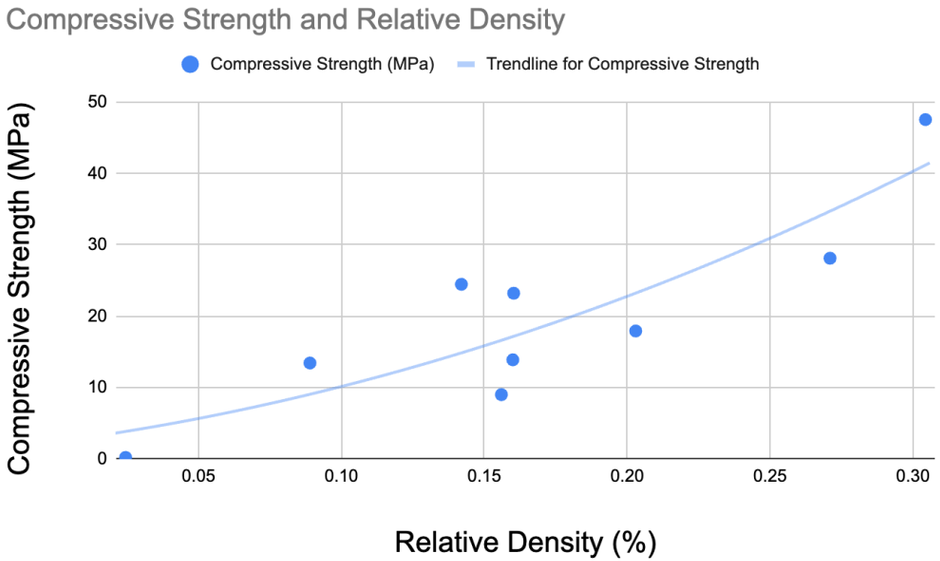


Wash Time vs Compression Strength

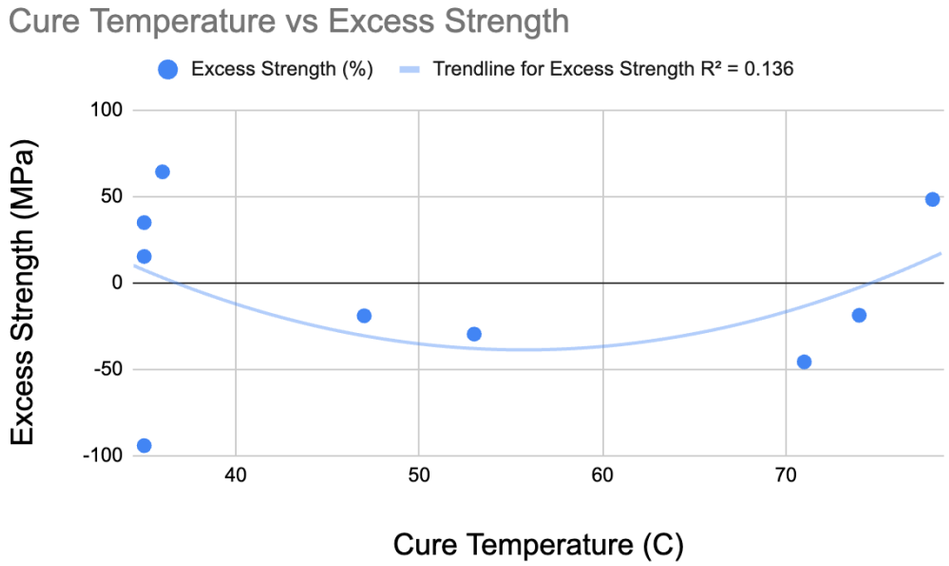


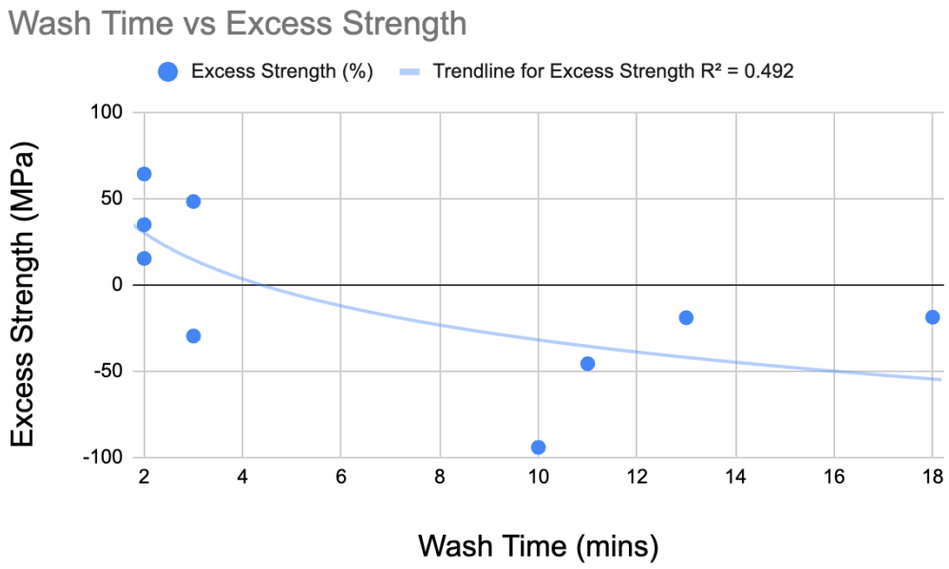
Strut-Diameter vs Compression Strength

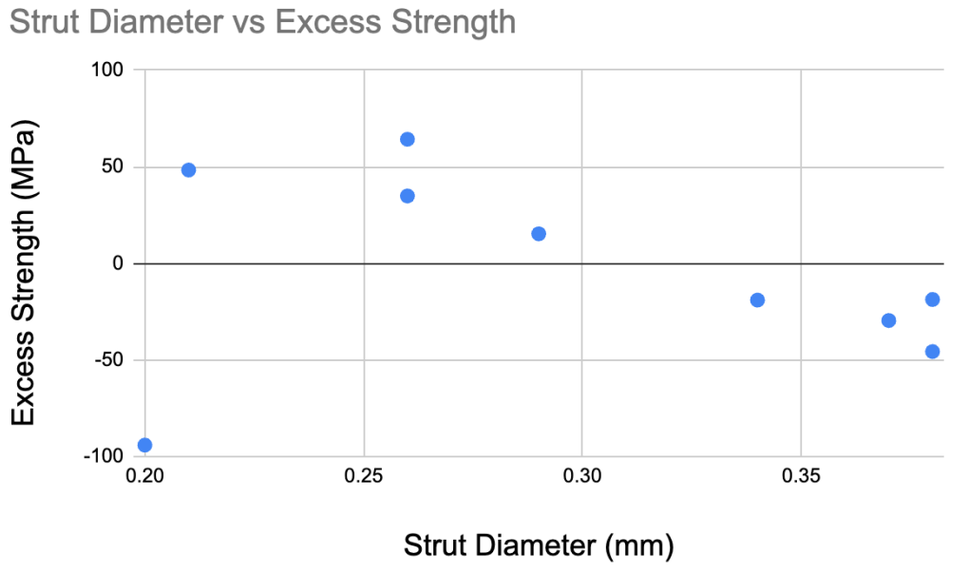


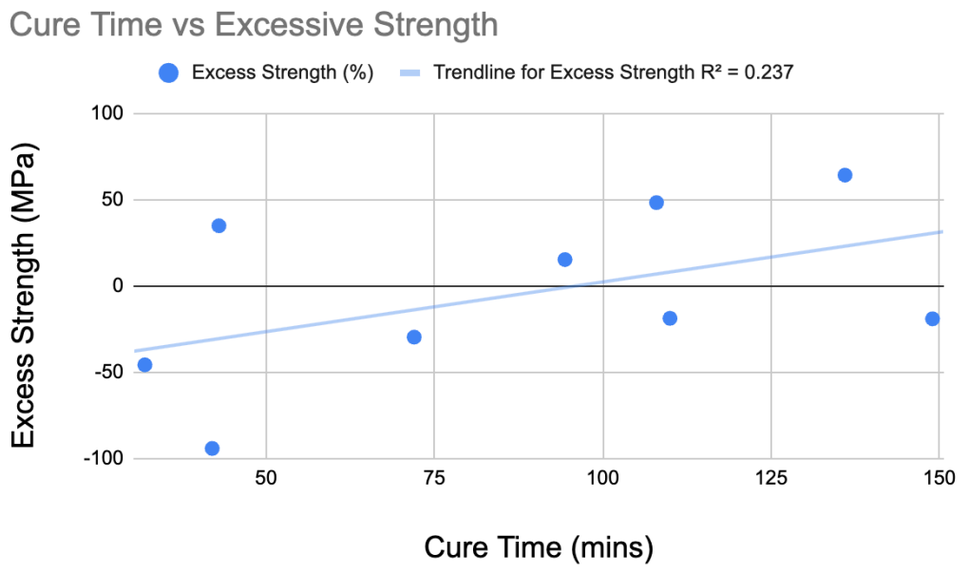

Compressive Strength and Relative Density
Compressive Strength (MPa)
Trendline for Compressive Strength
Compressive Strength (MPa)
50
40
30
20
10
0
0.05
0.10
0.15
0.20
0.25
0.30
Relative Density (%)

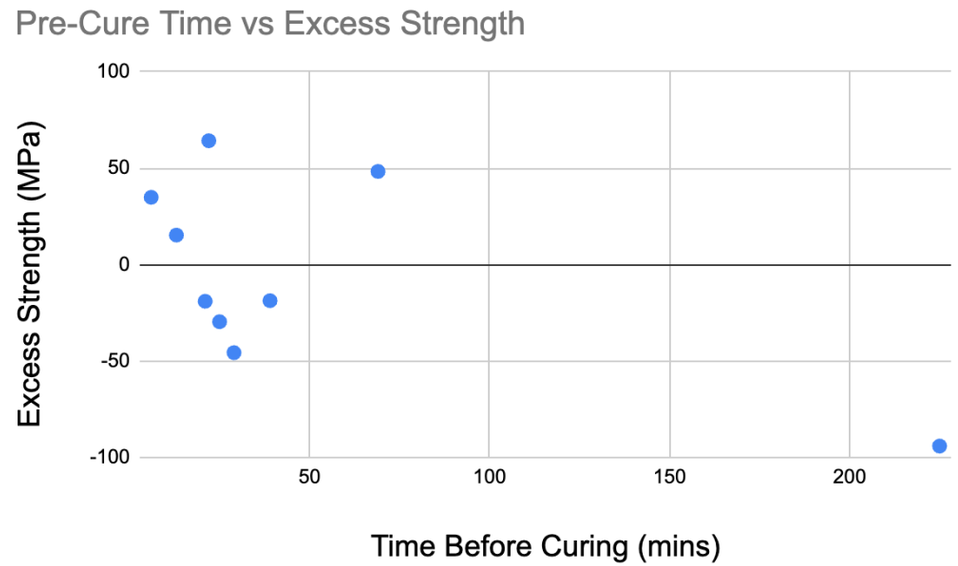
Appendix 4

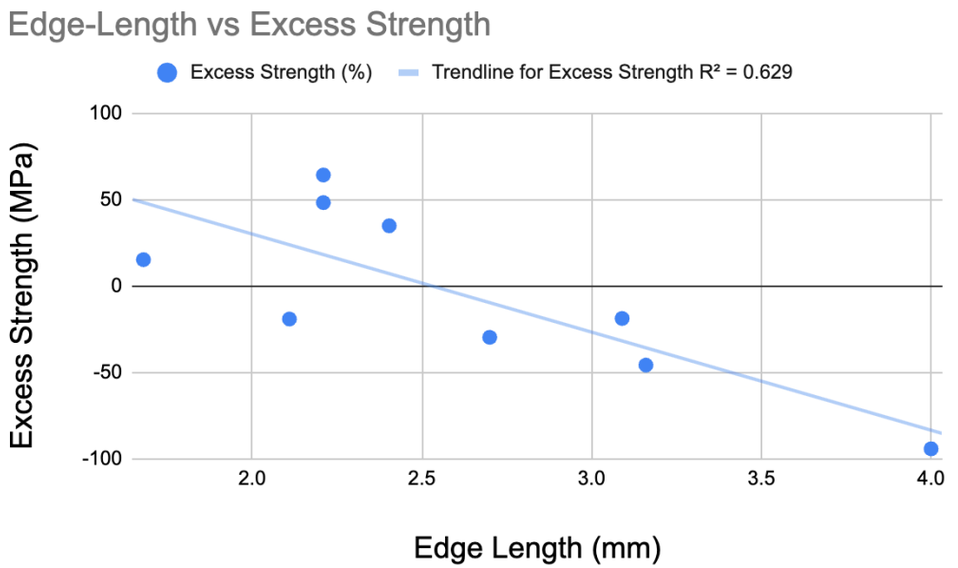

Cure Temperature vs Excess Strength
Excess Strength (%)
Trendline for Excess Strength R² = 0.136
Excess Strength (MPa)
100
50
0
-50
-100
40
50
60
70
Cure Temperature (C)


Wash Time vs Excess Strength
Excess Strength (%)
Trendline for Excess Strength R² = 0.492
Excess Strength (MPa)
100
50
0
-50
-100
2
4
6
8
10
12
14
16
18
Wash Time (mins)


Strut Diameter vs Excess Strength
Excess Strength (MPa)
100
50
0
-50
-100
0.20
0.25
0.30
0.35
Strut Diameter (mm)


Cure Time vs Excessive Strength
Excess Strength (%)
Trendline for Excess Strength R² = 0.237
Excess Strength (MPa)
100
50
0
-50
-100
50
75
100
125
150
Cure Time (mins)


Pre-Cure Time vs Excess Strength
Excess Strength (MPa)
100
50
0
-50
-100
50
100
150
200
Time Before Curing (mins)


Edge-Length vs Excess Strength
Excess Strength (%)
Trendline for Excess Strength R² = 0.629
Excess Strength (MPa)
100
50
0
-50
-100
2.0
2.5
3.0
3.5
4.0
Edge Length (mm)

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COMPRESSIVE STRENGTH OF ADDITIVELY MANUFACTURED RESIN LATTICE STRUCTURES

Optimizing Antibody Concentrations for Immunohistochemistry in Neurotrauma Research

Shriya Singh

Abstract:
Objective/research question: What primary and secondary antibody concentrations optimize the visualization of immunohistochemical (IHC) stains for antigens prevalent in neurotrauma research?
Method: IHC was conducted on six different antigens, with one control. A scoring guide was used to objectively determine which IHC stain was visually optimized. Tested concentrations were chosen based on the manufacturer-recommended concentration and concentrations used in past papers.

Results: The antigens and their respective optimized concentrations are as follows: IBA1, P500S250; GFAP, P1000S500; MAP2, P1000S500; AQP4, P50S500; CD83, P50S500; MBP, P1000S250.
Conclusions: Results suggest a gap in the current secondary antibody literature. Future research should use this study as a reference to facilitate neurotrauma research, limit scientific experimentation on animals, and improve data collection. The goal is to ultimately improve TBI pathology comprehension and find therapeutic treatments to ameliorate patient outcomes.

Keywords: Microglia, astrocytes, traumatic brain injury, immunohistochemistry, antibodies, optimization, staining, neuroinflammation, neurotrauma.

Introduction and background information

Traumatic brain injury (TBI), a result of mechanical forces being applied to the head, displacing the brain within the skull, and disrupting neurological function throughout the brain, affects around 10 million people worldwide.[1-2] Despite the substantial amount of literature on the cellular processes of TBI, the mechanisms that constitute its pathophysiology still need to be further researched.[1] Specifically, understanding the role of acute and chronic neuroinflammation is essential for comprehending how TBI advances over time and how treatments need to be tailored to this progression.

Neuroinflammation involves glial cells, non-neuronal cells in the central nervous system (CNS). Microglia and astrocytes–immune cells specific to the brain–are two of the most researched glial cells in TBI. To study these cells, researchers often use animal models and investigate the prevalence of these cells over time after impact. They inflict a force onto these animals, obtain a tissue sample from their brains, conduct analyses to determine concentrations of glial cells and maps of neural circuits, and repeat these steps.[3] Midline fluid percussion brain injury is one example of an experimental model of diffuse TBI

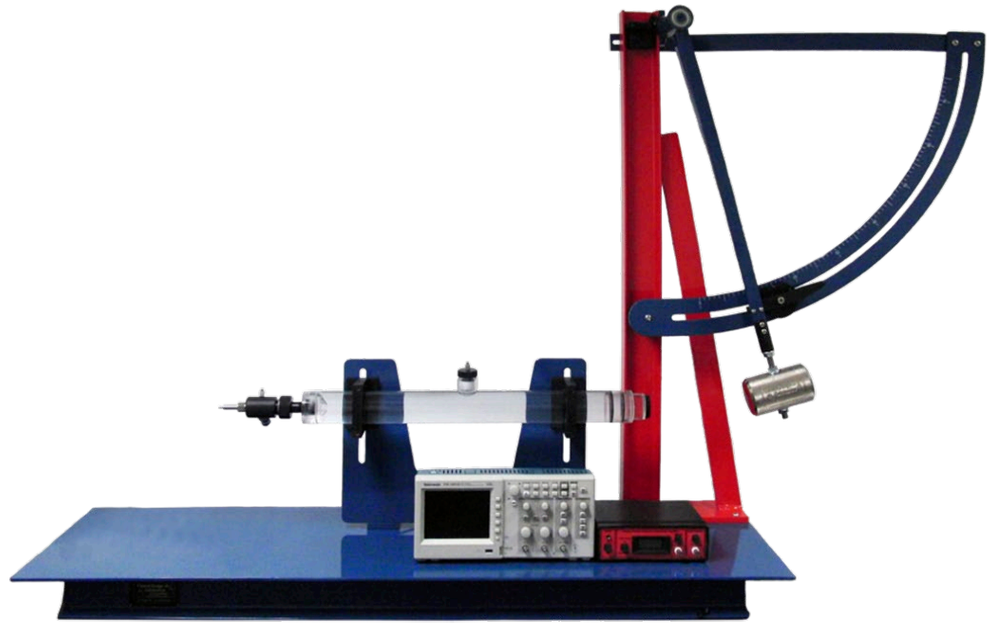

Figure 1. Image of the midline fluid percussion brain injury device.[4]

where a controlled impact of fluid is delivered into the midline of the skulls of rats and mice.4

Injury is induced through a 20-millisecond fluid pulse delivered into the brain through a craniectomy–a surgical procedure that removes part of the skull–and a surgically implanted injury hub. The fluid pulse is generated by the pressure wave created when the weighted end of the pendulum (right) hits the fluid cylinder (middle).[4]

Researchers may conduct analyses at different times after the force is inflicted; in fact, some researchers wait for a specific number of days post injury (DPI) for the onset of specific pathology (e.g., waiting 7 DPI for microglia studies). In some studies' research protocols, animal models perform behavior tasks to better elucidate the relationship between brain trauma and physical actions.

Immunohistochemistry (IHC) is a widely used technique in tissue analysis that uses antibodies–proteins that bind to specific markers on cells–to visualize various components within tissue samples. These markers are called antigens. By using antibodies specific to certain antigens—whether on the surface of cells or within tissues—and attaching a visibility agent to these antibodies, researchers can obtain stains to visualize the location and abundance of specific proteins or cell types. However, optimizing the visualization–that is, maximizing the clarity and usability–of tissue samples remains a challenge, particularly when using new antibodies. Researchers often lack standardized antibody concentrations, leading to trial-and-error testing on non-experimental tissue, which results in unnecessary waste of animal samples. Given the reliance on animal models in TBI research, minimizing scientific animal experimentation is essential.[5] This study aims to establish optimized antibody concentrations for neurotrauma research, providing a reference for future studies. By improving stain clarity and usability, these findings enhance IHC methodologies while promoting more efficient and ethical research practices.

Literature review

The pathophysiology of TBI

Comprehending the pathology of TBI is the first step in understanding how enhancement of the data analysis technique of IHC facilitates research on TBI pathology. A substantial amount of research has been conducted on TBI and its pathophysiology. Giordano & Lifshitz summarize this research, discussing the most common pathophysiology after diffuse TBI.[1-5]

Diffuse TBI occurs when a mechanically-induced force impacts multiple parts of the brain. This can occur when the brain receives an inadequate amount of oxygen (i.e., hypoxic brain injury) or when there is an excess accumulation of fluid in the brain (i.e., cerebral edema). Focal TBI, on the other hand, happens when a mechanically-induced force impacts a localized area of the brain. This can happen during a car accident when the frontal lobe is impacted as the head hits the windshield.

Giordano and Lifshitz[1] and Blennow *et al.*[2] state that the loss of consciousness and a transient extension and/or flexion of the forearms (i.e., the fencing response) are common signs immediately observable after diffuse TBI. However, they may not be observed in all cases of TBI. Common transient symptoms include immediate disorientation, slurred speech, and vomiting. However, it is necessary to note that these symptoms depend on the "parameters of the initiating mechanical force."[1(p14)] In other words, acute symptoms after TBI are defined by characteristics of the mechanism of the injury (i.e., how much force was applied to the brain, where was the impact, how much time did it take, for example).

According to Giza and Hovda, another common pathophysiology of TBI is mechanically induced neuronal membrane disruption which results in an efflux of potassium ions at the cellular level.[1,6] This increase in extracellular potassium levels leads to the depolarization of neurons and the release of glutamate, a prominent excitatory neurotransmitter. The release of glutamate exacerbates the efflux of potassium, which generates a positive feedback loop of excitation directly after impact. As a consequence, if the impact affected the brainstem, the neurons in the lateral vestibular nucleus would become activated, resulting in the fencing response.[1,6] Glucose production in the brain is also affected after impact. In total, Giordano and Lifshitz, supported by Kenzie et al. and Giza and Hovda, claim that the cellular processes in the brain after TBI consist of a "frenzy" of activity to control damage while subsequent pathophysiological activities evolve with the TBI.[1 (p15),6-7]

Other common forms of acute pathophysiology after TBI include diffuse axonal injury (DAI), which disturbs the original neuronal circuitry in the brain; vascular disruption/dysfunction in the form of hematomas; an altered neural circuitry, which affects the way nerves align and connect; and inflammation in the brain.[1,6]

Neuroinflammation, one of the most studied aspects of TBI, is a pathophysiological response to injury in the CNS that removes injurious stimulus and initiates the healing process. It involves the activation of glial cells, specifically astrocytes and microglia, which regulate functional recovery after TBI. Astrocytes form a glial scar–a physical and chemical barrier that forms in the CNS after an injury–around lesions of overtly damaged neurons, although these are more common in focal TBI.[1,8] More importantly, astrocytes promote several neurotrophic factors–chemicals involved in the revival of neurons and the reduction of glutamate, decreasing excitotoxicity.[1,8] Additionally, the activation of microglia, resident innate immune cells in the brain, is triggered immediately after impact. These microglia, according to McGinn *et al.* and Giordano & Lifshitz, undergo morphological and functional changes, and produce substances like chemokines or cytokines to either promote or inhibit inflammation.[1,8-9] When healthy (ramified) microglia, which are very branched and have a small soma (cell body), are activated, they may take two forms: rod microglia, often found in the cortex and hippocampus, are less branched and have an elongated soma; and amoeboid microglia, which are found throughout the brain, are also less branched and have a spherical soma. The exact functions of the different morphologies of microglia require additional research. Delayed development of neurodegenerative pathology may be due to the presence of activated microglia in the brains of patients with TBI years after injury.[1] Hence, it is important to note, while inflammation may be acutely protective, in the long-term, inflammation may exacerbate the injury.[1]

More research is necessary to understand the individual mechanisms that constitute the pathophysiology of TBI.[1] Pathophysiology and the resultant symptoms can be present acutely and chronically; moreover, these pathophysiological processes often occur simultaneously and interdependently, highlighting the complexity of TBI's pathophysiology.[1, 7] Figure 2 further demonstrates the complexity of the pathophysiology of TBI.

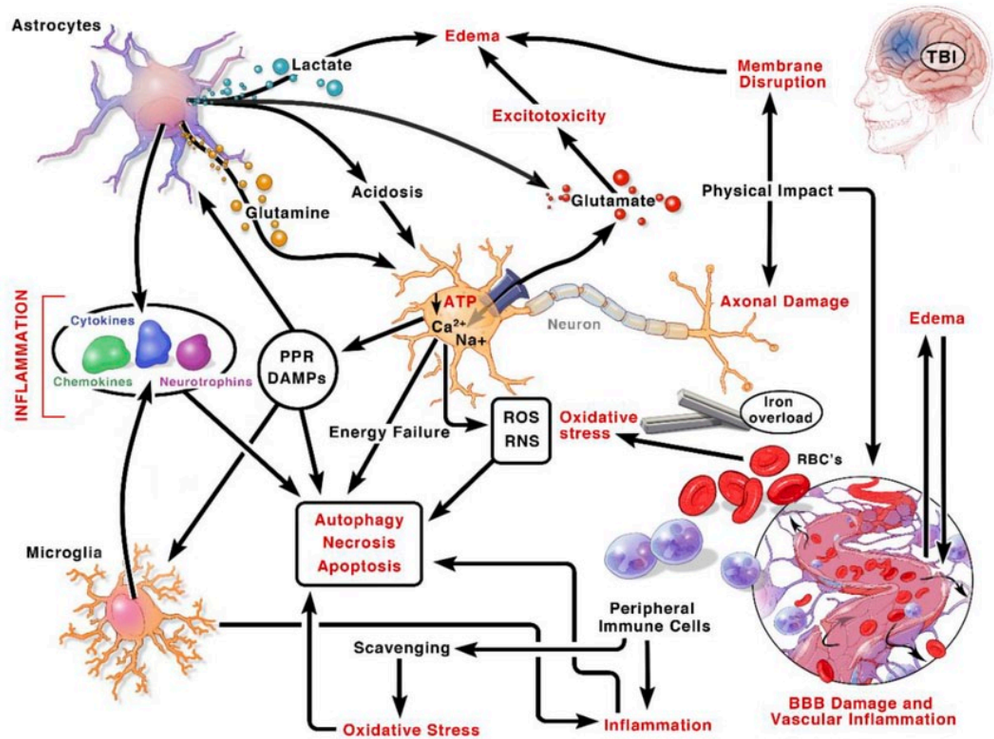

Figure 2. *Map of cellular processes that occur in the brain post-TBI.*[10]

Immunohistochemistry in neurotrauma & neuroinflammation

As mentioned, neuroinflammation has a prominent role in the acute and chronic pathophysiology of TBI. To study neuroinflammation, specifically cells such as microglia and astrocytes, researchers use IHC. Figure 3 illustrates two types of IHC techniques: direct immunoassay, which involves only one primary antibody that binds onto the antigen, and indirect immunoassay, which involves a secondary antibody binding onto the primary antibody. Direct and indirect immunoassays differ in their use of visualization methods: direct immunoassays typically utilize a substrate-enzyme binding process, such as horseradish peroxidase (HRP) with a substrate that emits visible light, while indirect immunoassays may employ fluorescence, where a fluorescent tag is attached to the secondary antibody to visualize the binding.

The diagram compares direct and indirect immunoassay as types of IHC. Direct immunoassay involves the direct binding of a primary antibody, attached to a visualization agent, horseradish peroxidase (HRP) enzyme. Indirect immunoassay involves the additional binding of a secondary antibody, attached to the HRP enzyme, to the primary antibody.

Because this study considers both primary and

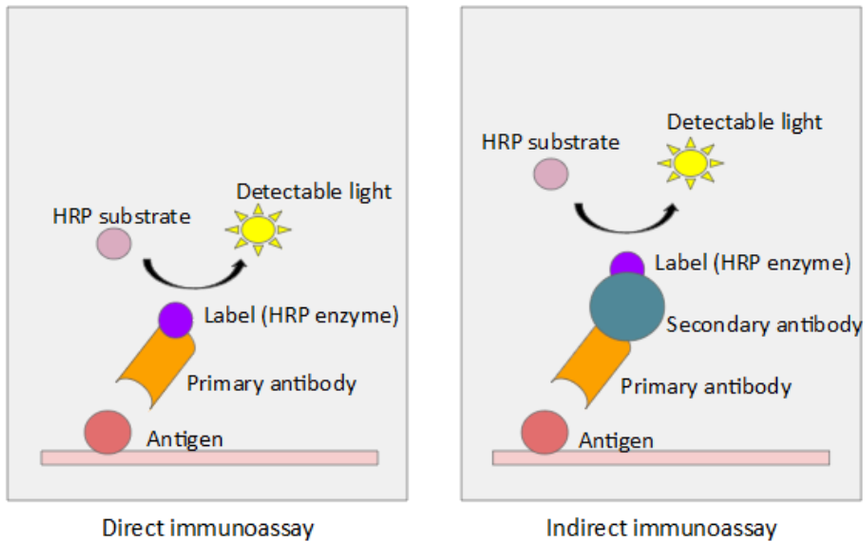

Figure 3. *Comparison of direct and indirect immunoassays in immunohistochemistry (IHC) using the example of horseradish peroxidase (HRP).*[11]

secondary antibodies, it is useful to understand the advantages and challenges of direct and indirect immunoassay. While indirect immunoassay is more complicated as it is difficult to find combinations of primary and secondary antibodies and their respective concentrations that optimize visualization, it can be used for all antigens. It may amplify the signal further, making it easier to visualize stains.[12] Signal amplification occurs through the possibility of a primary antibody being bound by multiple secondary antibodies, intensifying visible light emission.[12] On the contrary, direct immunoassay is easier to investigate, but it can only be used for highly expressed antigens as weakly expressed antigens are not visible without the significant amplification provided only by indirect immunoassay.[12]

IHC protocols involve several steps to ensure tissue sample clarity and reliability, including blocking, fixation, and antigen retrieval. Blocking prevents nonspecific antibody binding (i.e., the binding of the antibody onto molecules other than the targeted antigen), clarifying the image of the tissue sample.[13] Fixation is the process which preserves tissue by stabilizing cellular structures through protein binding.[13] Antigen retrieval then restores the antigenicity–i.e., the ability of an antigen to bind to an antibody–by severing the cross-links among antigens and fixatives.[13] While these steps can be manipulated to affect the visualization of the tissue, this study focuses on the impact of antibody concentrations on tissue visualization because they are one of the most essential yet under documented aspects of IHC.

Scientists have used IHC in the past to study the pathophysiology after TBI. For instance, Neri *et al.* conducted an IHC evaluation of Aquaporin-4 (AQP4)–an antigen found on astrocytes–and its correlation with Cluster of Differentiation 68 (CD68), Ionized calcium-binding adapter molecule 1 (IBA-1), Hypoxia-inducible factor 1-α (HIF-1α), Glial fibrillary acidic protein (GFAP), and Cluster of Differentiation 15 (CD15)–antigens found on glial cells and hypoxic cells–to study neuroinflammation after fatal TBI.[14] This technique determined the stages in time when microglia and astrocytes changed from a ramified to an activated state, whether astrocytes were associated with a beneficial role post-injury, and how these stains correlated with other pathophysiologic processes.[14] Similarly, Tanaka *et al.* investigated neuroinflammation after TBI in progranulin-deficient mice. Progranulin is a growth factor that regulates immune responses; by studying progranulin-deficient mice, researchers determine the role of progranulin in TBI's pathology by understanding what occurs with its absence. Tanaka *et al.* found correlations among different chemicals and cells, such as activated microglia, to conclude that progranulin suppresses excessive inflammatory responses related to active microglia in mice post-TBI.[15] Furthermore, Smith *et al.*, who used IHC to find temporal and spatial changes in the pattern of IBA1 and CD68 staining in rat brains post-TBI, visualized the pathophysiological effects of methamphetamine after severe TBI.[16] Finally, Stankov *et al.*, using a double staining IHC method for CD68 and IBA-1, understood more comprehensively the prevalence of cells like microglia and their morphology after brain contusions.[17] Hence, while fundamentally, Neri *et al.*, Tanaka *et al.*, Smith *et al.*, and Stankov *et al.* conducted different studies, they demonstrate the usefulness of IHC as a technique to understand the pathophysiology of TBI and brain injuries in general.[14-17]

The optimization of the visualization of IHC tissue samples

Optimizing tissue visualization in IHC involves several factors, including antibody concentrations, incubation time, temperature, and antibody selection.[12,18] This study specifically focuses on optimizing primary and secondary antibody concentrations for investigating post-TBI pathophysiology, as antibody concentration is a critical determinant of staining quality. In contrast, factors such as antigen retrieval and blocking serve as supplementary refinements. Additionally, while other aspects of IHC optimization are well-documented, research on antibody concentration optimization remains limited. By optimizing antibody concentrations, this study aims to improve IHC stain visualization, enhancing neuroinflammation and neurotrauma research.

Pre-existing guidelines for researchers conducting IHC are summarized by Magaki *et al.* They claim that the antibody concentration that will give the most clear, visible, and usable staining is determined by multiple dilutions of that concentrated antibody.[18] They recommend starting with the dilution recommended by the manufacturer and testing one dilution above and below it. For instance, if the manufacturer recommends a dilution of 1:400 (one part antibody to 400 parts solution), then testing 1:200, 1:400, and 1:800 dilutions will likely show an optimal visualization in one of the tested dilutions.[18] Because this study analyzed primary and secondary antibodies, it also considered the combinations of each primary antibody with different secondary antibodies, and their respective concentrations when optimizing the visualization of IHC tissue samples.

Gap

While general guidelines on IHC exist, there is a gap in the literature regarding specific antibody concentrations recommended for optimal visualization in neurotrauma. Researchers can find some antibody concentrations by investigating previous papers, but this is tedious and often futile. As a result, they resort to testing various antibody concentrations using the general guidelines outlined above on non-experimental tissue samples, subsequently using animal tissue and antibodies that could have been used for data collection. Researchers additionally tend to stop when their tissue samples meet their immediate needs, potentially overlooking valuable data. Optimizing IHC tissue samples would maximize data extraction from each sample, possibly leading to unexpected findings.

This paper aims to construct a reference for researchers unsure of what antibody concentrations they should use to optimize the visualization of their IHC tissue samples. Specifically, it focuses on antibodies involved in neurotrauma research, a field that heavily utilizes animal models, thereby underscoring the importance of developing methods that minimize redundant scientific animal experimentation.[5]

Method

Lab protocol

This study conducted IHC analyses on specific antibodies most prominent in neurotrauma research following Magaki *et al.*'s recommendation to test the manufacturer-recommended dilution–along with one dilution above and below it–to find the optimal primary and secondary antibody concentrations.[18]

Primary antibodies that bind to a specific antigen

are *anti*-[antigen], and secondary antibodies that bind to particular primary antibodies are *anti*-[primary antibody]. In this study, primary antibodies were raised in a species other than the species of the tissue to prevent nonspecific binding; nonspecific binding would occur as the secondary antibody would have a tendency to bind to the primary antibodies *and* the immunoglobulins–antibodies–naturally present in the tissue. Because rat tissue was used, primary antibodies were raised in a different species (e.g., *rabbit* anti-IBA1 antibodies). Primary antibodies that bind to the following antigens located on neuroinflammatory targets were used; the respective cell/cell regions on which they are mainly found are also provided:

- IBA1: microglia
- AQP4: astrocytes
- GFAP: glial cells
- Microtubule-Associated protein 2 (MAP2): neurons, oligodendrocytes (type of glial cell involved in the production of myelin in the CNS), astrocytes
- Myelin A1 antibody (MBP): myelin sheath (protective and insulating layer around nerve fibers), oligodendrocytes
- CD83: microglia

Primary Antibodies	Primary Antibody Manufacturer	**Citations of Antibody**	**Dilutions from Papers/ Manufacturer**	**Dilutions Tested in This Study**	**IHC Staining References**
Anti-IBA1	FUJIFILM Wako Pure Chemical Corporation[19]	Tanaka et al.[25]	1:100; 1:500 - 1:1000	1:100; 1:250; 1:500; 1:1000	Sroor et al.[32]
Anti-GFAP	Chemicon[20]	Lu et al.[26]	1:1,500; 1:400 - 1:800	1:500; 1:1000; 1:1500	Finney et al.[33]
Anti-MBP	Chemicon[21]	Clark et al., Song et al.[27,28]	1:2000; 1:500	1:200; 1:500; 1:1000	Hickey et al., Song et al., Clark et al.[27,28,34]
Anti-AQP4	Santa Cruz Biotechnology[22]	Han et al.[29]	1:100; 1:50-1:500	1:50; 1:100; 1:500	Tourdias et al., Han et al., Mesentier-Louro [35,29,36]
Anti-CD83	Thermo Fisher Scientific[23]	N/A	1:50-1:200	1:50; 1:100; 1:200	Arbab et al.[37]
Anti-MAP2	BioLegend[24]	Wang et al., Shelton et al.[30,31]	1:500 & 1:1000; 1:200 - 1:1000	1:250, 1:500, 1:1000	Guo et al.[38]

Table 1. *Summary of primary antibodies used in this study: Manufacturers, citations, dilution recommendations, tested dilutions, and IHC staining references for pathology assessment*
Abbreviations*: Anti-IBA1, Anti-Ionized Calcium-Binding Adapter Molecule 1; Anti-GFAP, Anti-Glial Fibrillary Acidic Protein; Anti-MBP, Anti-Myelin Basic Protein; Anti-AQP4, Anti-Aquaporin 4; Anti-CD83, Anti-CD83 Cell Surface Glycoprotein; Anti-MAP2, Anti-Microtubule-Associated Protein 2.*
Note: *Four concentrations are listed for IBA1 because concentrations 1:100, 1:250, and 1:500 were tested for the control batch and 1:100, 1:250, and 1:1000 were tested for the experimental batch.*

This project used secondary antibodies raised in a host species different from the primary antibodies (e.g., horse anti-rabbit for rabbit anti-[antigen]) to ensure proper binding, as antibodies recognize foreign antigens. Two secondary antibodies—horse anti-mouse and horse anti-rabbit—were used, with selection based on species compatibility with the primary antibody. The choice of secondary antibody did not affect the study's primary objective of evaluating antibody concentration effects on IHC tissue sample visualization but rather ensured specific binding to the corresponding primary antibody.

The tissue was fixed/embedded in paraformaldehyde and frozen in optimal cutting temperature (OCT)–a thick liquid at room temperature–because of pre-existing lab conventions. Natural horse serum (NHS) was used as the blocking serum because a blocking serum must originate from the same species in which the secondary antibody was raised, and both secondary antibodies were raised in a horse; proteins in this serum bind to natural immunoglobulins in the tissue, preventing primary and secondary antibodies from binding to non-target proteins. This project utilized a substrate-enzyme binding process–chromogenic staining–with 3,3' -diaminobenzidine (DAB) as the chromogen/substrate; DAB was chosen over fluorescence for its greater stability and longevity. A summary of the protocol, excluding the phosphate-buffered saline (PBS) washes which stabilize the pH and maintain tissue sample integrity after each step, is provided below:

IHC protocol (summary)

1. Removal of slide from the freezer and rehydration with PBS.
2. Antigen retrieval with sodium citrate buffer to unmask epitopes–the part of an antigen to which an antibody attaches itself.
3. Blocking to prevent non-specific binding.
4. Addition of primary antibodies to target the antigen of interest.
5. Addition of secondary antibody to bind to the primary antibody.
6. Blocking of endogenous peroxidases to avoid false-positive results.
7. Addition of Avidin-Biotin Complex (ABC) solution for signal amplification.
8. Application of DAB solution for visualization of antibody binding.
9. Dehydration of the sample and cover slipping using CitraSolv.

The rats in this study underwent diffuse traumatic brain injury (TBI) using the midline fluid percussion injury (mFPI) model, with an impact intensity of 320–340 mV—sufficient to induce TBI pathology without being fatal. Brain tissue was collected at seven and 28 days post-injury (DPI), ensuring that most samples within each antigen cohort originated from the same brain. This approach minimizes variability in pathology prevalence across compared tissue samples.

Secondary Antibodies	**Secondary Antibody Manufacturer**	**Citations of Antibody**	**Dilutions from Papers/ Manufacturer**	**Dilutions Tested in This Study**
Horse anti-mouse	Vector Laboratories[39]	Wen et al.[41]	1:200; 1:150 - 1:750	1:250; 1:500; 1:1000
Horse anti-rabbit	Vector Laboratories[40]	Moreno-Martinez et al.[42]	1:200; 1:150 - 1:750	1:250; 1:500; 1:1000

Table 2. *Summary of secondary antibodies used in this study: Manufacturers, citations, dilution recommendations, and tested dilutions*
Note: *IHC staining references are not included because secondary antibodies are used to enhance staining intensity, whereas primary antibodies bind to the antigen to indicate specific pathology. For the control IBA1, secondary antibody concentrations of 1:100, 1:250, 1:500 were used. After the results, the study shifted the concentration range to 1:250, 1:500, 1:1000 for the experimental antigens. See the "Results" section for further explanation.*

Analysis

Because the definition of a "visually optimized" tissue sample varies between individuals, this study employed an objective scoring system to assess immunohistochemically stained samples. The primary and secondary antibody concentrations determine antigen binding and, consequently, stain visibility. Insufficient concentrations may result in weak staining, while excessive concentrations can obscure tissue details. The goal is to identify the optimal antibody concentrations that yield clear, usable stains for analysis.

Scoring Guide for Optimized Tissue Samples

Score each of the following factors on a scale from 1 to 10:

1. Discernible pathology (DP): Contrast between cells and background, ensuring clear visualization of cellular processes.
2. Background noise (BN): Presence of non-specific staining that may obscure target cells.
3. Uniform staining (US): Consistency of staining across the tissue sample.

Calculate the final score using the following formula:

Final Score = (0.55 × DP) + (0.25 × (10 − BN)) + (0.20 × US)

The highest-scoring sample is considered visually optimized, and its corresponding antibody concentration(s) are identified as optimal for the given tissue and cell type.

A weighted average was taken as opposed to a normal average to prioritize pathology visualization (DP), which directly impacts neurotrauma research, over background noise (BN) and uniform staining (US). Background noise was weighted higher than uniform staining since excessive noise can obscure cellular processes, whereas non-uniform staining primarily affects large-area analyses, which are less common in TBI studies.

Following standard TBI research methodologies regarding DP and BN, this study focused on the primary somatosensory barrel cortex (S1BF) of the rat brain, a region with substantial TBI pathology. This targeted approach minimizes extraneous variables and enhances reproducibility. Nonetheless, including uniform staining in the evaluation allows for applicability to broader TBI pathology research involving larger tissue areas.

IBA1	P100 S100	P100 S250	P100 S500	P250 S100	P250 S250	P250 S500	P500 S100	P500 S250	P500 S500
Background noise	7	6	6	3	2	1.5	3.5	1.5	2
Discernible pathology	4	6	7	7.5	7.5	7.5	6	8	3
Uniform staining	7	6	6	7	7	7	5.5	6.5	7
Final score	4.35	5.5	6.05	7.275	7.525	7.65	6.025	7.825	5.05

Table 3. *Antibody concentration scores for IBA1 (control) Staining*
Abbreviations: *IBA1, Ionized Calcium-Binding Adapter Molecule 1; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.*

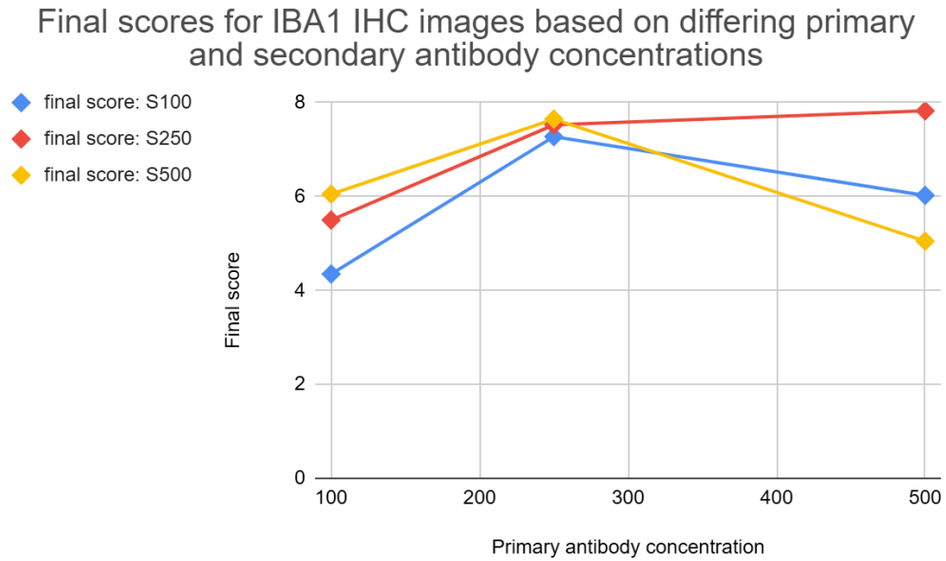

Figure 4. *Final scores for IBA1 (control) IHC images based on differing primary and secondary antibody concentrations.*

Results

IBA1: Control

The laboratory conducting this research specializes in microglia studies and has previously optimized antibody concentrations for the antigen IBA1. To validate the current methodology, this study re-optimized these concentrations. Consistent with prior findings, the optimal primary and secondary antibody concentrations were confirmed as 1:500 and 1:250, respectively. Rabbit anti-IBA1 served as the primary antibody, and horse anti-rabbit served as the secondary antibody. Control IBA1 IHC was performed using 7 DPI rat brain tissue.

Figures 5, 9, 13, 17, 21, 25, and 29 present images selected from different hemispheres to avoid dark spots, folding, tears, and other tissue integrity issues that could affect staining uniformity. These sections, chosen for strong tissue preservation, highlight varying levels of discernible pathology and background noise in IHC samples across different antibody concentrations. Figures 6, 10, 14, 18, 22, 26, and 30 specifically illustrate differences in staining uniformity.

IBA1: Experimental

Previous research performed IHC targeting the antigen IBA1 using secondary antibody concentrations of 1:100, 1:250, and 1:500 (see Figures 4–6) to validate the methodology. To further confirm these findings and maintain consistency with protocols used for other antigens, this study performed IHC targeting IBA1 with secondary antibody concentrations of 1:250, 1:500, and 1:1000, and primary concentrations of 1:250, 1:500, and 1:1000. Primary concentrations were selected around the previously optimized concentration of 1:500. Consistent with earlier results, the optimal primary and secondary concentrations were confirmed as 1:500 and 1:250, respectively. Experimental IHC for IBA1 was conducted using 28 DPI rat brain tissue.

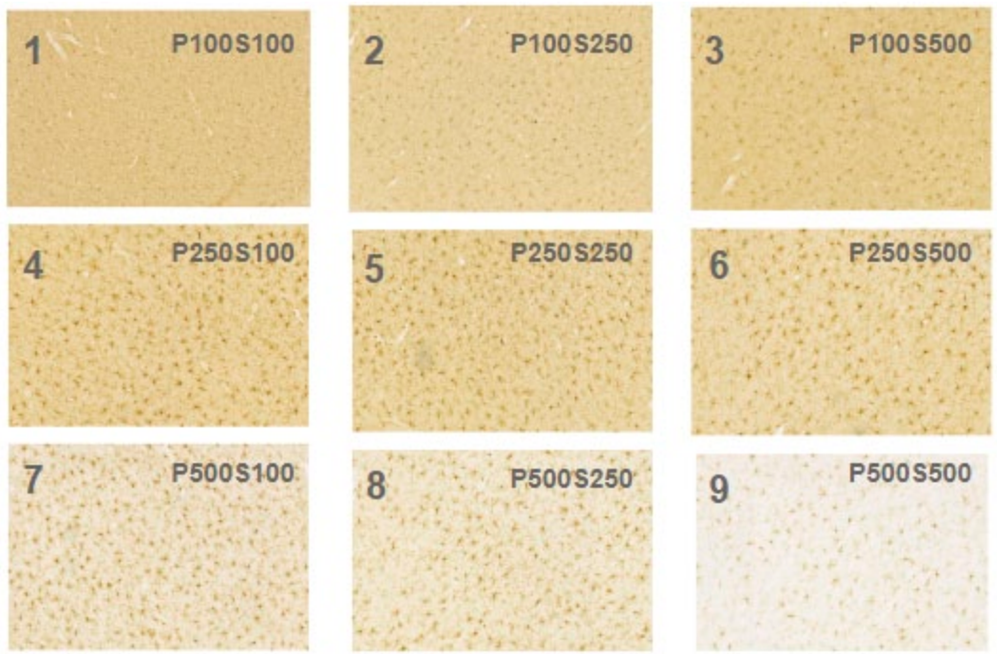

Figure 5. *IHC images targeting the antigen IBA1 (control).* **1**, P100S100, left hemisphere. 2, P100S250, right hemisphere. 3, P100S500, right hemisphere. 4, P250S100, left hemisphere. 5, P250S250, left hemisphere. 6, P250S500, right hemisphere. 7, P500S100, left hemisphere. 8, P500S250, right hemisphere. 9, P500S500, right hemisphere. All images are of the S1BF region magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

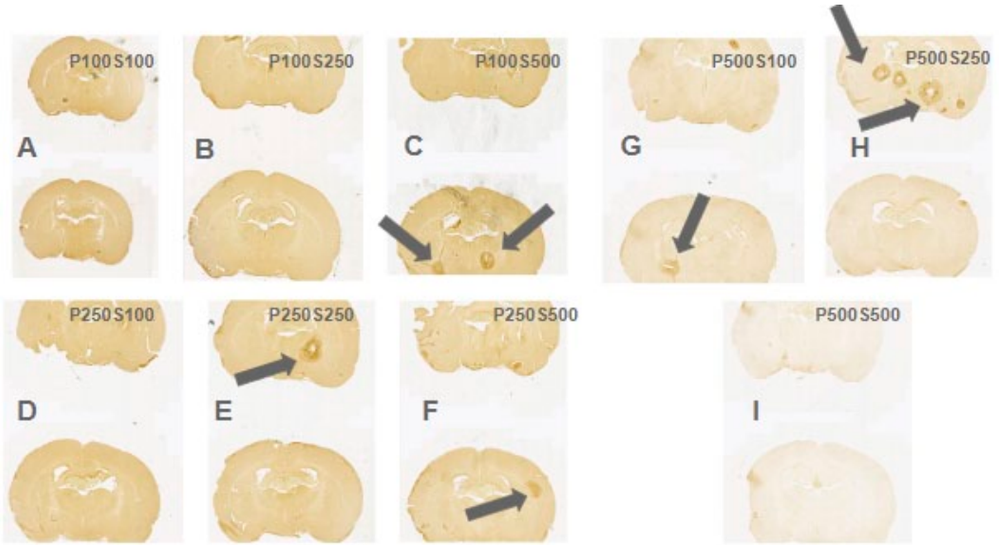

Figure 6. *Macroscopic pictures of IBA1 (control) tissue samples that demonstrate varying levels of uniform staining.* **A**, P100S100, US score = 7. **B**, P100S250, US score = 6. **C**, P100S500, US score = 6. **D**, P250S100, US score = 7. **E**, P250S250, US score = 7. **F**, P250S500, US score = 7. **G**, P500S100, US score = 5.5. **H**, P500S250, US score = 6.5. **I**, P500S500, US score = 7. Arrows indicate dark spots, which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

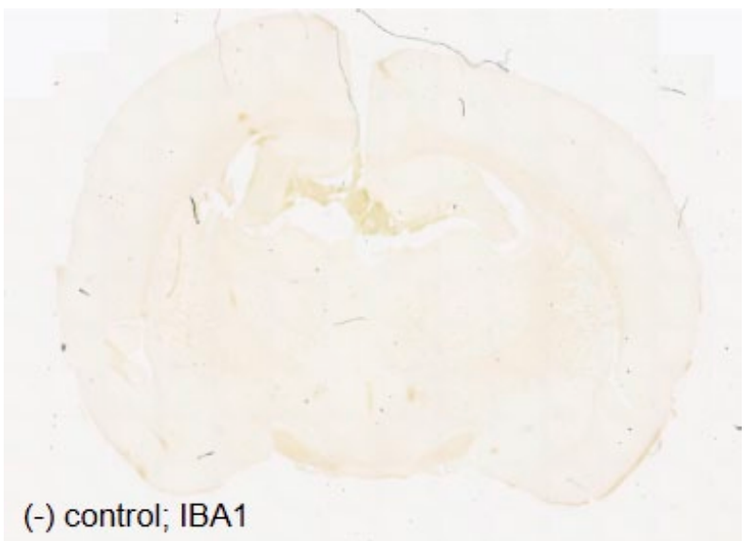

Figure 7. ***IBA1 – Negative control for comparison to the IHC images. The primary antibody is not applied, but the lowest concentration of the secondary antibody (Horse anti-rabbit; 1:500) is.***

IBA1	P250 S250	P250 S500	P250 S1000	**P500 S250**	P500 S500	P500 S1000	P1000 S250	P1000 S500	P1000 S1000
Background noise	5	7.5	8	4	6	7.5	3	3.5	3
Discernible pathology	6	5.5	5.5	6.5	6	5.5	5.5	6	6
Uniform staining	7	6	5	6.5	8	7	7	7	6
Final score	5.95	4.85	4.525	**6.375**	5.9	5.05	6.175	6.325	6.25

Table 4. *Antibody concentration scores for IBA1 (experimental) Staining*
Abbreviations: *IBA1, Ionized Calcium-Binding Adapter Molecule 1; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.*

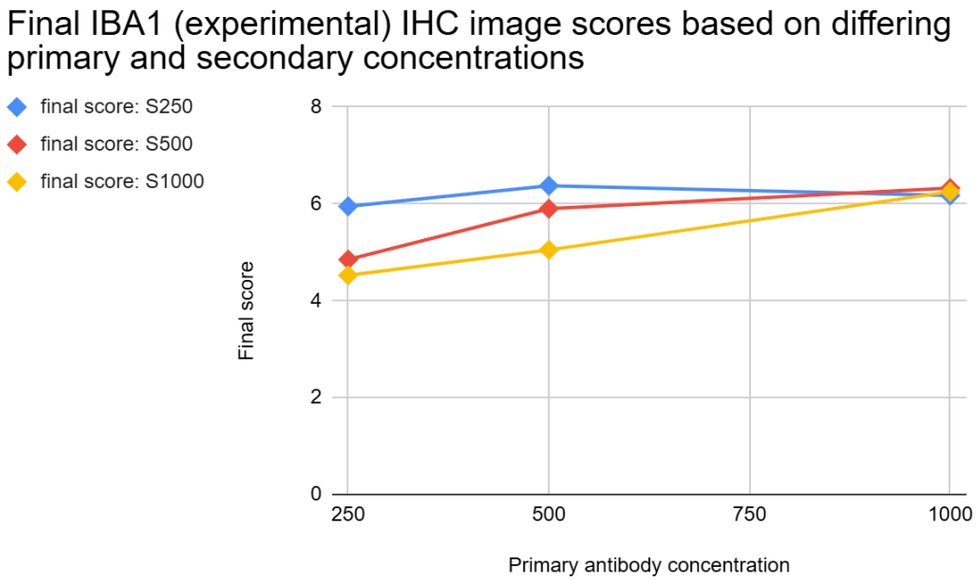

Figure 8. *Final scores for IBA1 (experimental) IHC images based on differing primary and secondary antibody concentrations.*

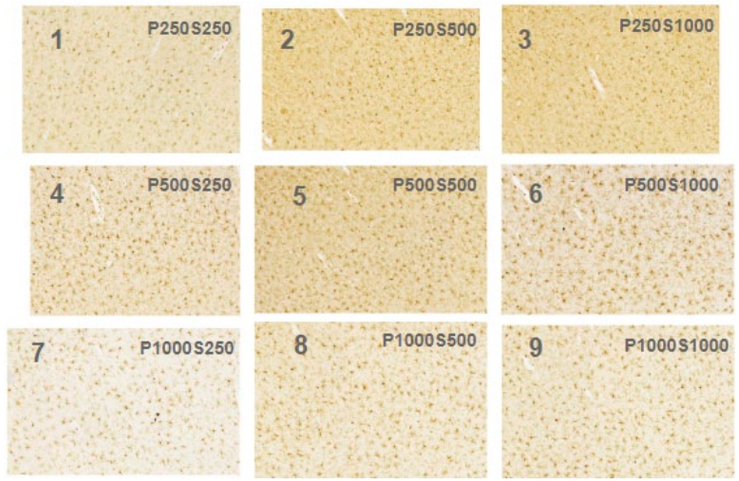

Figure 9. *IHC images targeting the antigen IBA1 (experimental).* **1**, P250S250, right hemisphere. **2**, P250S500, left hemisphere. **3**, P250S1000, left hemisphere. **4**, P500S250, left hemisphere. **5**, P500S500, left hemisphere. **6**, P500S1000, left hemisphere. **7**, P1000S250, left hemisphere. **8**, P1000S500, left hemisphere. **9**, P1000S1000, left hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

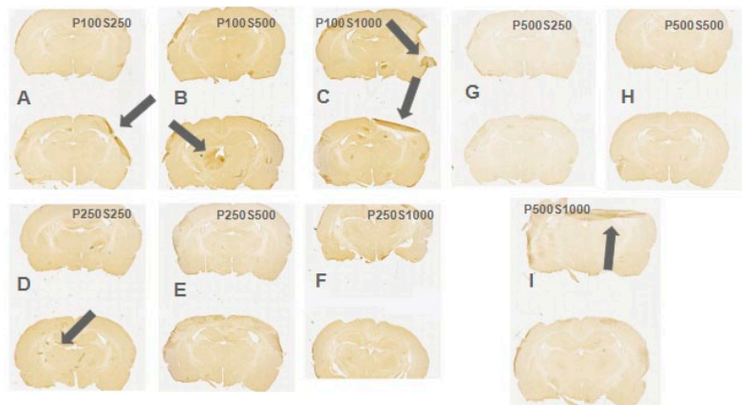

Figure 10. *Macroscopic pictures of IBA1 (experimental) tissue samples that demonstrate varying levels of uniform staining.* **A**, P250S250, US score = 7. **B**, P250S500, US score = 6. **C**, P250S1000, US score = 5. **D**, P500S250, US score = 6.5. **E**, P500S500, US score = 8. **F**, P500S1000, US score = 7. **G**, P1000S250, US score = 7. **H**, P1000S500, US score = 7. **I**, P1000S1000, US score = 6. Arrows indicate folding (**A, C, I**), fragmentation (**D, C**), and dark spots (**B**) which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

Figure 11. *IBA1 (experimental) – Negative control for comparison to the IHC images*. The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-rabbit; 1:1000) is.

GFAP

This study identified primary and secondary antibody concentrations of 1:1000 and 1:500, respectively, as optimal for visualizing the antigen GFAP. Mouse anti-GFAP served as the primary antibody, and horse anti-mouse served as the secondary antibody. GFAP IHC was performed using 7 DPI rat brain tissue.

GFAP	P500 S250	P500 S500	P500 S1000	P1000 S250	**P1000 S500**	P1000 S1000	P1500 S250	P1500 S500	P500 S1000
Background Noise	4	5	4	5	4.5	5.5	3.5	4	3
Discernible pathology	5	6	5	4	7	6	4	6.5	2
Uniform staining	4	4	7	7	4	4	4	4	7
Final score	5.05	5.35	5.15	4.85	**6.025**	5.225	4.625	5.875	4.25

Table 5. *Antibody concentration scores for GFAP staining*
Abbreviations: *GFAP, Glial Fibrillary Acidic Protein; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P1000S500 indicates a primary antibody concentration of 1:1000 and a secondary antibody concentration of 1:500.*

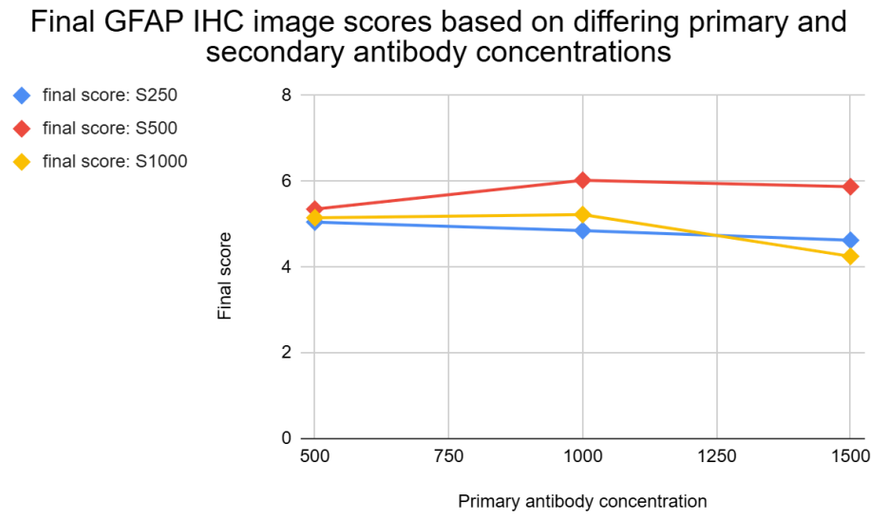

Figure 12. *Final scores for GFAP IHC images based on differing primary and secondary antibody concentrations.*

Figure 13. *IHC images targeting the antigen GFAP.* **1**, P500S250, right hemisphere. **2**, P500S500, right hemisphere. **3**, P500S1000, right hemisphere. **4**, P1000S250, left hemisphere. **5**, P1000S500, right hemisphere. **6**, P1000S1000, right hemisphere. **7**, P1500S250, left hemisphere. **8**, P1500S500, left hemisphere. **9**, P1500S1000, left hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

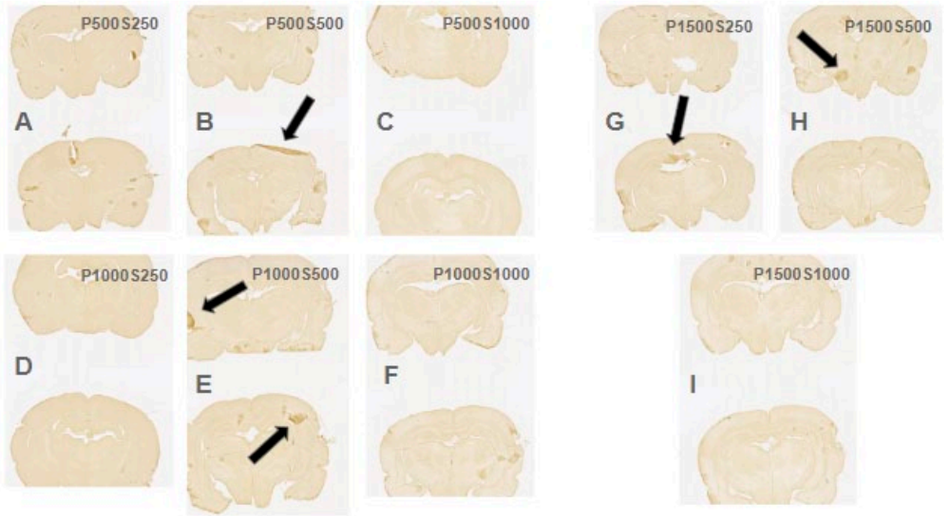

Figure 14. *Macroscopic pictures of GFAP tissue samples that demonstrate varying levels of uniform staining.* **A**, P500S250, US score = 4. **B**, P500S500, US score = 4. **C**, P500S1000, US score = 7. **D**, P1000S250, US score = 7. **E**, P1000S500, US score = 4. **F**, P1000S1000, US score = 4. **G**, P1500S250, US score = 4. **H**, P1500S500, US score = 4. **I**, P1500S1000, US score = 7. Arrows indicate dark spots (**E, G, H**) and folding (**B**), which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

Figure 15. *GFAP – Negative control for comparison to the IHC images.* The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-rabbit; 1:1000) is.

MAP2	P250 S250	P250 S500	P250 S1000	P500 S250	P500 S500	P500 S1000	P1000 S250	**P1000 S500**	P1000 S1000
Background Noise	3	4	2.5	5	4.5	5	3.5	4	2.5
Discernible pathology	3.5	6	3	6	6.5	5.5	6.5	7	3
Uniform staining	6.5	6.5	7	5	7	6.5	6.5	6	7
Final score	4.975	6.1	4.925	5.55	6.35	5.575	6.5	**6.55**	4.925

Table 6. *Antibody concentration scores for MAP2 staining*
Abbreviations: *MAP2, Microtubule-Associated Protein 2; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P1000S500 indicates a primary antibody concentration of 1:1000 and a secondary antibody concentration of 1:500.*

MAP2

A primary antibody concentration of 1:1000 and a secondary antibody concentration of 1:500 produced optimal staining for the MAP2 antigen. Mouse anti-MAP2 served as the primary antibody, and horse anti-mouse served as the secondary antibody. IHC was performed for MAP2 using 7 DPI rat brain tissue.

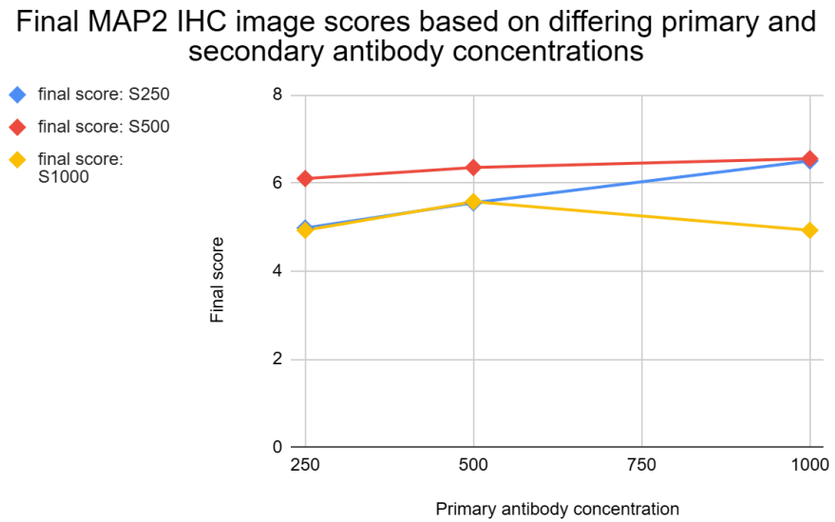

Figure 16. *Final scores for MAP2 IHC images based on differing primary and secondary antibody concentrations.*

Figure 17. *IHC images targeting the antigen MAP2.* **1**, P250S250, right hemisphere. **2**, P250S500, left hemisphere. **3**, P250S1000, left hemisphere. **4**, P500S250, left hemisphere. **5**, P500S500, right hemisphere. **6**, P500S1000, right hemisphere. **7**, P1000S250, right hemisphere. **8**, P1000S500, left hemisphere. **9**, P1000S1000, left hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

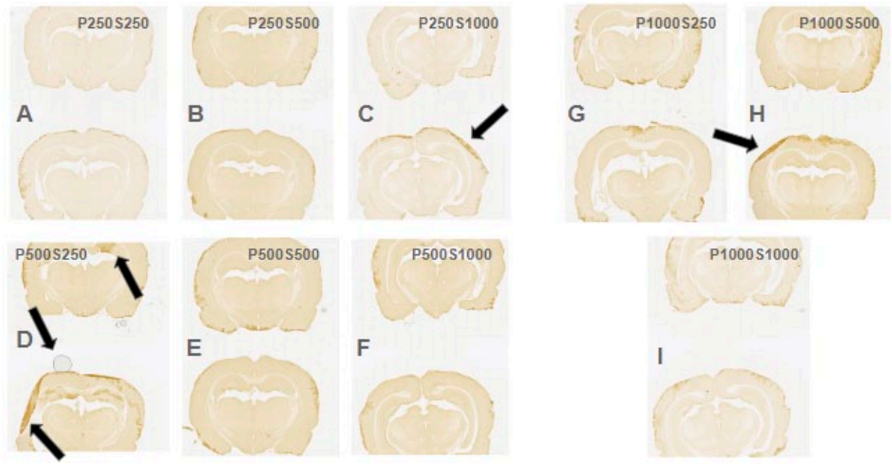

Figure 18. *Macroscopic pictures of MAP2 tissue samples that demonstrate varying levels of uniform staining.* **A**, P250S250, US score = 6.5. **B**, P250S500, US score = 6.5. **C**, P250S1000, US score = 7. **D**, P500S250, US score = 5. **E**, P500S500, US score = 7. **F**, P500S1000, US score = 6.5. **G**, P1000S250, US score = 6.5. **H**, P1000S500, US score = 6. **I**, P1000S1000, US score = 7. Arrows indicate dark spots (**D**), folding (**D, C, H**), and air bubbles (**D**), which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

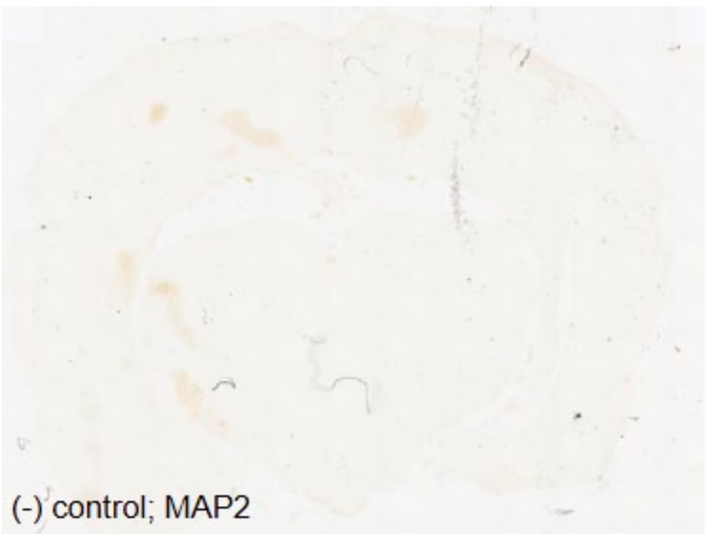

Figure 19. *MAP2 – Negative control for comparison to the IHC images*. The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-mouse; 1:1000) is.

AQP4

This study found that a primary antibody concentration of 1:50 and a secondary antibody concentration of 1:500 produced optimal staining for the AQP4 antigen. Mouse anti-AQP4 served as the primary antibody, and horse anti-mouse served as the secondary antibody. AQP4 IHC was performed on 7 DPI rat brain tissue using secondary concentrations of 1:250 and 1:500, and on 28 DPI tissue using a secondary concentration of 1:1000.

AQP4	P50 S250	**P50 S500**	P50 S1000	P100 S250	P100 S500	P100 S1000	P500 S250	P500 S500	P500 S1000
Background Noise	5	5	3	3.5	1.5	2	1.5	1.5	1
Discernible pathology	5	5.5	3	3.5	1.5	2	1.5	1	1
Uniform staining	4.5	7	7	7.5	8	6.5	8	8	8
Final score	4.9	**5.675**	4.8	4.628125	4.55	4.4	4.55	4.275	4.4

Table 7. *Optimized antibody concentrations for AQP4 staining*
Abbreviations: *AQP4, Aquaporin-4; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P50S500 indicates a primary antibody concentration of 1:50 and a secondary antibody concentration of 1:500.*

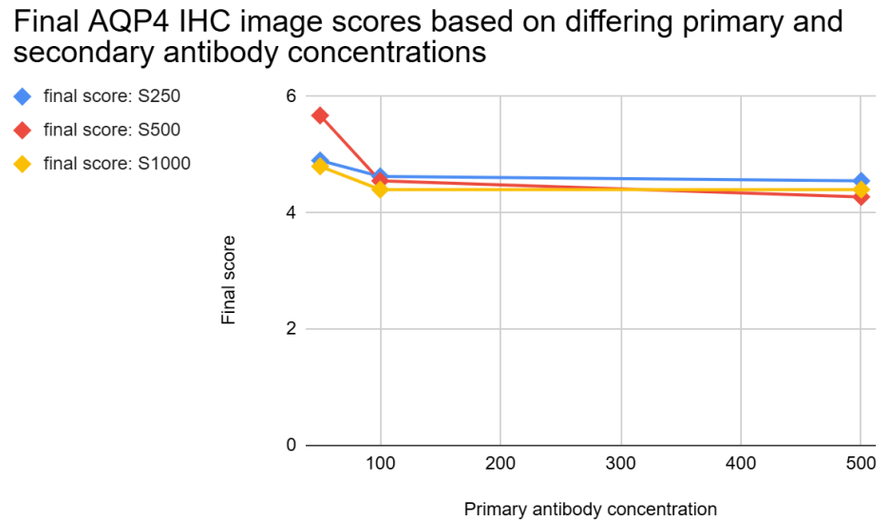

Figure 20. *Final scores for AQP4 IHC images based on differing primary and secondary antibody concentrations.*

Figure 21. *IHC images targeting the antigen AQP4.* **1**, P50S250, right hemisphere. **2**, P50S500, left hemisphere. **3**, P50S1000, left hemisphere. **4**, P100S250, left hemisphere. **5**, P100S500, left hemisphere. **6**, P100S1000, right hemisphere. **7**, P500S250, right hemisphere. **8**, P500S500, right hemisphere. **9**, P500S1000, left hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

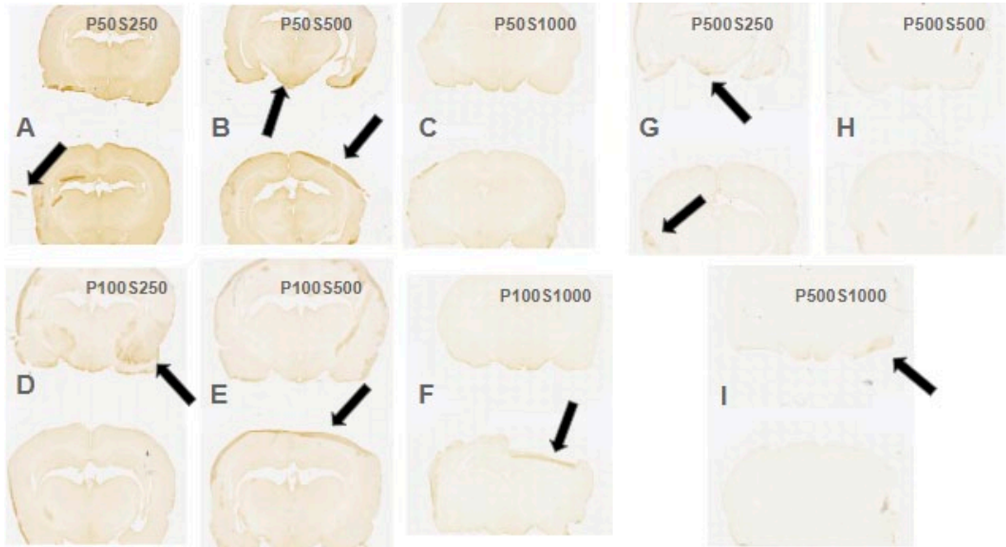

Figure 22. *Macroscopic pictures of AQP4 tissue samples that demonstrate varying levels of uniform staining.* **A**, P50S250, US score = 4.5. **B**, P50S500, US score = 7. **C**, P50S1000, US score = 7. **D**, P100S250, US score = 7.5. **E**, P100S500, US score = 8. **F**, P100S1000, US score = 6.5. **G**, P500S250, US score = 8. **H**, P500S500, US score = 8. **I**, P500S1000, US score = 8. Arrows indicate dark spots (**B, D, I, G**), folding (**E, F**), and fragmentation (**A**), which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

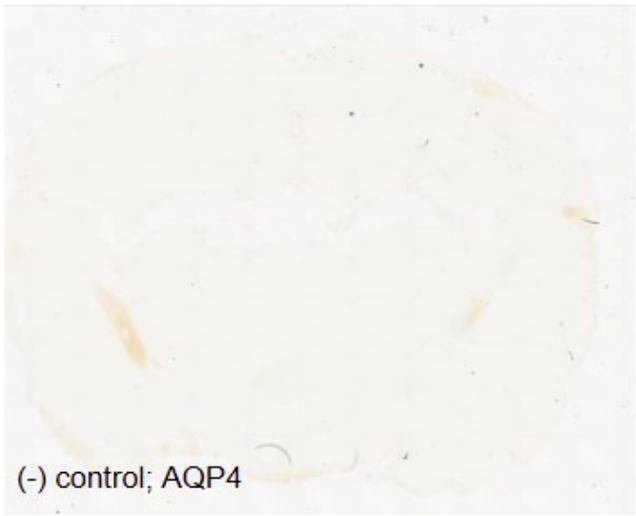

Figure 23. *AQP4 – Negative control for comparison to the IHC images.* The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-mouse; 1:1000) is.

CD83	P50 S250	**P50 S500**	P50 S1000	P100 S250	P100 S500	P100 S1000	P200 S250	P200 S500	P200 S1000
Background Noise	7	5	2.5	7	2.5	5.5	5	5	2
Discernible pathology	6	7	2.5	6	3	6.5	6.75	6	4
Uniform staining	7	6	6	8	8	7	3	3	1
Final score	5.45	**6.3**	4.45	4.45	5.125	6.1	5.5625	5.15	4.4

Table 8. *Antibody concentration scores for CD83 staining*
Abbreviations: *CD83, Cluster of Differentiation 83; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P50S500 indicates a primary antibody concentration of 1:50 and a secondary antibody concentration of 1:500.*

CD83

A primary antibody concentration of 1:50 and a secondary antibody concentration of 1:500 produced optimal staining for the CD83 antigen. Rabbit anti-CD83 served as the primary antibody, and horse anti-rabbit served as the secondary antibody. 7 DPI tissue samples were used to target the CD83 antigen.

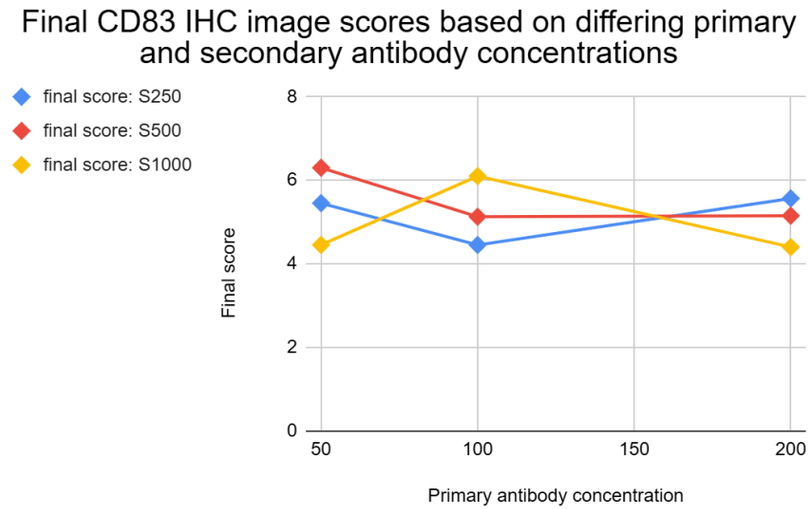

Figure 24. *Final scores for CD83 IHC images based on differing primary and secondary antibody concentrations.*

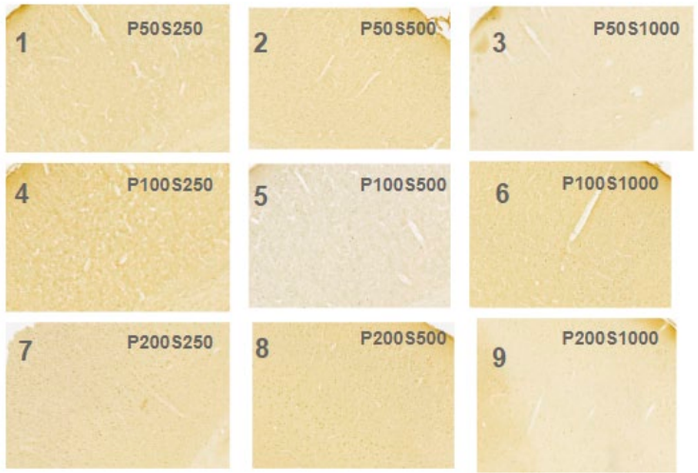

Figure 25. *IHC images targeting the antigen CD83.* **1**, P50S250, left hemisphere. **2**, P50S500, right hemisphere. **3**, P50S1000, left hemisphere. **4**, P100S250, left hemisphere. **5**, P100S500, left hemisphere. **6**, P100S1000, right hemisphere. **7**, P200S250, left hemisphere. **8**, P200S500, right hemisphere. **9**, P200S1000, right hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

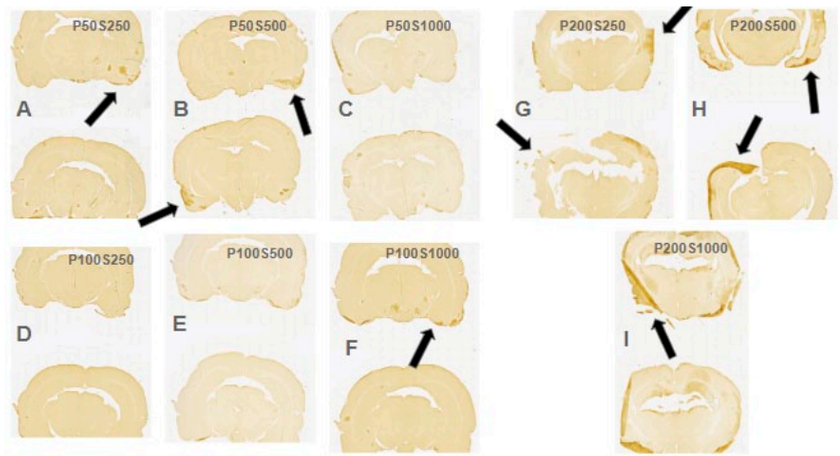

Figure 26. *Macroscopic pictures of CD83 tissue samples that demonstrate varying levels of uniform staining.* **A**, P50S250, US score = 7. **B**, P50S500, US score = 6. **C**, P50S1000, US score = 6. **D**, P100S250, US score = 8. **E**, P100S500, US score = 8. **F**, P100S1000, US score = 7. **G**, P200S250, US score = 3. **H**, P200S500, US score = 3. **I**, P200S1000, US score = 1. Arrows indicate dark spots (**A, B, F, G, H**), folding (**H, I**), and fragmentation (**G, I**), which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

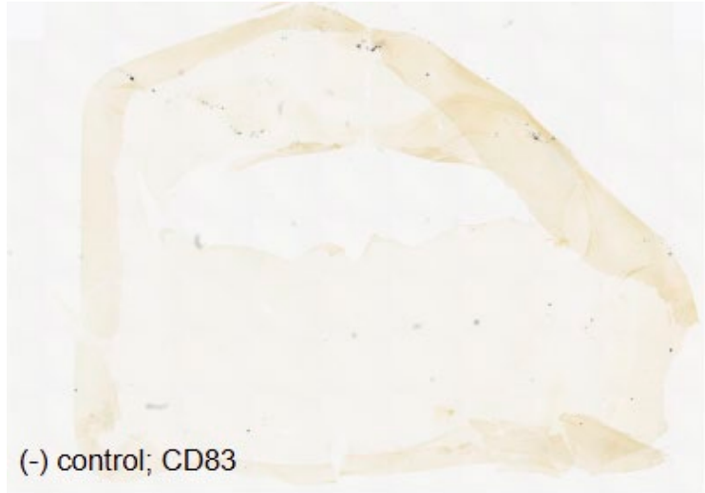

Figure 27. *CD83 – Negative control for comparison to the IHC images.* The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-rabbit; 1:1000) is.

MBP

This study found that a primary antibody concentration of 1:1000 and a secondary antibody concentration of 1:250 produced optimal staining for the MBP antigen. Rabbit anti-MBP served as the primary antibody, and horse anti-rabbit served as the secondary antibody. 28 DPI tissue samples were used to target the MBP antigen.

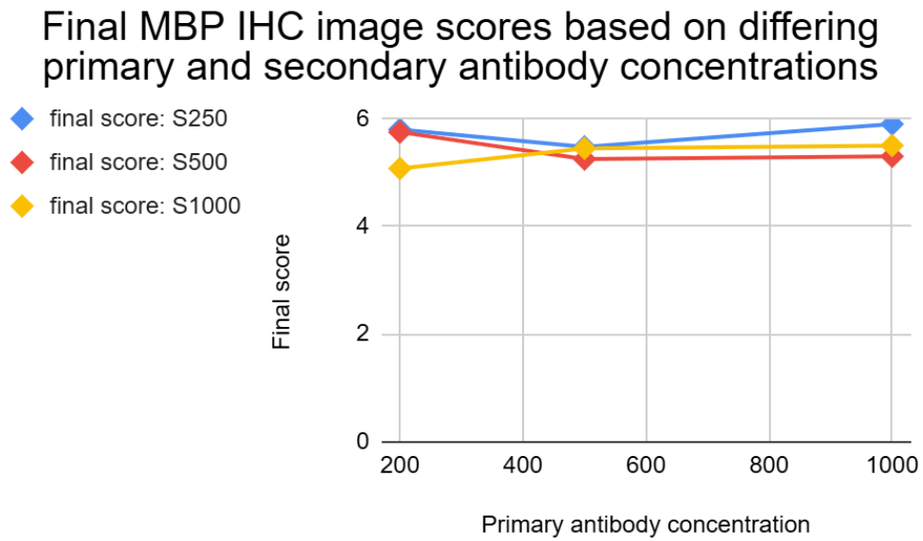

Figure 28. *Final scores for MBP IHC images based on differing primary and secondary antibody concentrations.*

MBP	P200 S250	P200 S500	P200 S1000	P500 S250	P500 S500	P500 S1000	**P1000 S250**	P1000 S500	P1000 S1000
Background Noise	4.5	5	5	6	4	4	5	3	3
Discernible pathology	5.5	6	5.5	6.5	5	5	7	5	5
Uniform staining	7	6	4	4.5	5	6	4	4	5
Final score	5.8	5.75	5.075	5.475	5.25	5.45	**5.9**	5.3	5.5

Table 9. *Antibody concentration scores for MBP staining*
Abbreviations: *MBP, Myelin Basic Protein; P, primary antibody; S, secondary antibody.*
Note: *The highest final score and its corresponding antibody concentrations are bolded. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P1000S250 indicates a primary antibody concentration of 1:1000 and a secondary antibody concentration of 1:250.*

Figure 29. *IHC images targeting the antigen MBP.* **1**, P200S250, left hemisphere. **2**, P200S500, right hemisphere. **3**, P200S1000, left hemisphere. **4**, P500S250, left hemisphere. **5**, P500S500, right hemisphere. **6**, P500S1000, right hemisphere. **7**, P1000S250, right hemisphere. **8**, P1000S500, left hemisphere. **9**, P1000S1000, right hemisphere. All images are of the S1BF region and magnified 13-14x. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

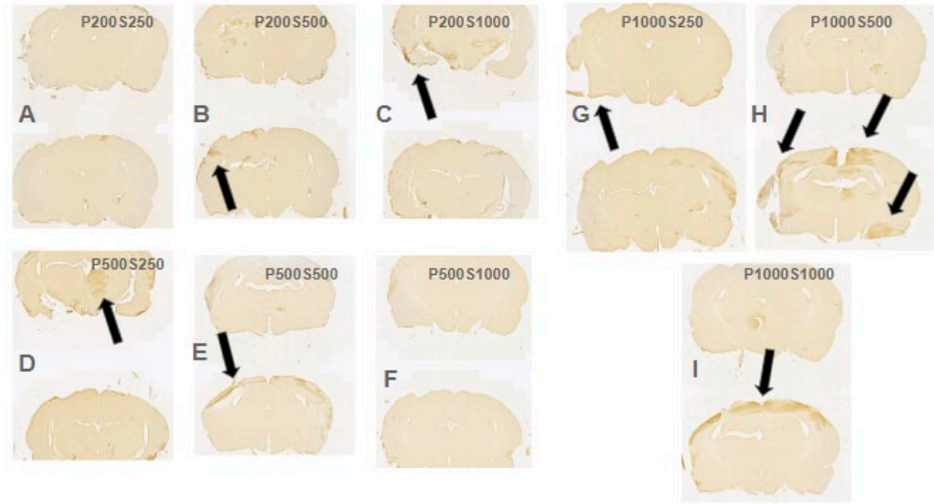

Figure 30. *Macroscopic pictures of MBP tissue samples that demonstrate varying levels of uniform staining.* **A**, 200S250, US score = 7. **B**, P200S500, US score = 6. **C**, P200S1000, US score = 4. **D**, P500S250, US score = 4.5. **E**, P500S500, US score = 5. **F**, P500S1000, US score = 6. **G**, P1000S250, US score = 4. **H**, P1000S500, US score = 4. **I**, P1000S1000, US score = 5. Arrows indicate dark spots (**D, B, C, H, I**), folding (**E, I**), and fragmentation (**G, H**), which worsen the uniform staining score of the respective image. Antibody concentrations are denoted as the ratio of one part antibody to parts of solution; e.g., P500S250 indicates a primary antibody concentration of 1:500 and a secondary antibody concentration of 1:250.

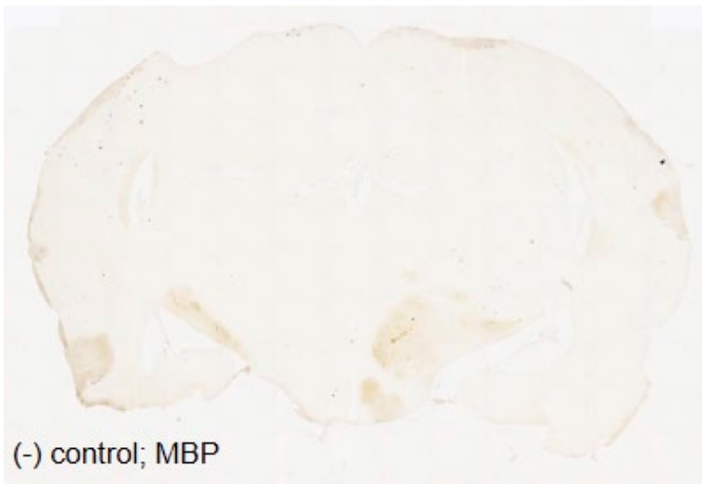

Figure 31. *MBP – Negative control for comparison to the IHC images*. The primary antibody is not applied, but the lowest concentration of the secondary antibody (horse anti-rabbit; 1:1000) is.

Discussion

Findings

Most stains followed a consistent trend: decreasing secondary antibody concentrations led to reduced staining intensity, which often correlated with more uniform staining, minimal background noise, and reduced discernible pathology. This outcome is expected, as lower secondary antibody concentrations reduce binding to the primary antibody, thereby diminishing signal intensity.

Several stains appeared visually similar—specifically, GFAP P100S500 (1:100 primary, 1:500 secondary) compared to GFAP P1500S500, and MAP2 P1000S250 compared to MAP2 P1000S500. To differentiate between these, previous studies were consulted to identify the specific pathological features that should be visible.[33,38]

This study prioritized the visibility of pathology. While other researchers may prefer MAP2 P1000S250 for applications requiring broader anatomical coverage due to its uniformity and low background, MAP2 P1000S500 received higher scores here due to its clearer pathological detail, which is particularly relevant for traumatic brain injury (TBI) research and its alignment with IHC images from prior literature.[38] Researchers should define their imaging goals—whether structural clarity or pathological detail—before selecting antibody concentrations.

While most stains followed the expected pattern, an unexpected result was observed with CD83 at a primary concentration of 1:100. As the secondary antibody concentration decreased, staining intensity initially decreased but then unexpectedly increased from P100S500 to P100S1000 (Figure 7). Similarly, the experimental IBA1 IHC batch deviated from the expected pattern. Although this could be due to extraneous variables, it offers new insight into secondary antibody concentrations and staining intensity. Future studies should replicate these experiments to determine whether these anomalies result from antibody concentration manipulation or other factors.

Optimal primary and secondary antibody concentrations showed no clear pattern across antigens. Various combinations of primary and secondary antibody concentrations yielded optimal results for each antigen, likely due the binding efficacy of different antibody types to distinct antigens. Except for CD83 and AQP4, the optimal primary antibody concentration for each antigen was either the lowest or moderate concentration tested. This aligns with the observation that increasing primary antibody concentration often increases background staining.

The deviation in CD83 may be due to the range of concentrations tested. Since no previous paper used the exact CD83 antibody product used in this research, the manufacturer-recommended concentration was used, which may have led to suboptimal visual results. Future research should test a broader concentration range to confirm this. AQP4 findings suggest that more concentrated antibody concentrations may provide visual optimization, challenging current literature on AQP4 antibody concentrations and providing new insights.

Antigen	Tissue samples	Primary antibody and optimized concentration	Secondary antibody and optimized concentration
IBA1 (C)	7 DPI	Rabbit anti-IBA1; 1:500	Horse anti-rabbit; 1:250
IBA1 (EXP)	28 DPI	Rabbit anti-IBA1; 1:500	Horse anti-rabbit; 1:250
GFAP	7 DPI	Mouse anti-GFAP; 1:1000	Horse anti-mouse; 1:500
MAP2	7 DPI	Mouse anti-MAP2; 1:1000	Horse anti-mouse; 1:500
AQP4	7 DPI and 28 DPI	Mouse anti-AQP4; 1:50	Horse anti-mouse; 1:500
CD83	7 DPI	Rabbit anti-CD83; 1:50	Horse anti-rabbit; 1:500
MBP	28 DPI	Rabbit anti-MBP; 1:1000	Horse anti-rabbit; 1:250

Table 10. *Optimized primary and secondary antibody concentrations for various antigens*

Abbreviations: C, control. EXP, experimental.

Secondary antibody concentrations of 1:1000 were not optimal, likely due to insufficient staining intensity. Initial IBA1 testing showed that secondary antibody concentrations above 1:100 resulted in excessive background noise, leading to a revised range of 1:250–1:1000 for experimental antigens. As the data show, optimal secondary antibody concentrations vary by antigen, though they were consistently 1:250 or 1:500 in this study. Future studies should explore a broader range of secondary antibodies, as current literature primarily uses 1:200, contrary to this study's findings, and variability across antigens should be considered. A wider range of primary antibody concentrations should also be tested to determine if an optimal concentration exists outside those explored in this study.

Scoring in this study was antigen-specific; scores should not be compared across different antigens. For example, while the optimized MBP image received a score of 5.9 and MAP2 received 6.55, this does not indicate that MAP2 optimization was superior.

Limitations

This study aimed to objectively determine optimal antibody concentrations but has several limitations.

Scoring Subjectivity: While quantitative scoring was used, some subjectivity remains. Cell counting methods could improve objectivity, but resource limitations prevented their inclusion in this study. Future research should incorporate these techniques.

Variability in IHC Protocols: Tissue folding and fragmentation occasionally occurred, likely due to processing variability or chance errors. Extraneous factors, such as hydrophobic barrier (PAP) pen efficacy, temperature fluctuations, and pH imbalances, may have influenced staining outcomes. Replicating protocols is recommended to determine whether optimized results are due to concentration changes or external variables.

Limited Antigen Selection: Due to resource constraints, this study focused on a subset of antigens relevant to neurotrauma research. Expanding antigen selection in future studies could benefit a wider research audience, potentially extending beyond neurotrauma research.

IHC Staining Technique: This study used DAB staining for its stability and ease of storage. However, fluorescence IHC staining is also widely used in neurotrauma research for its ability to provide high resolution images and detect multiple proteins simultaneously. Future studies should determine optimal antibody concentrations for fluorescence IHC.

Lack of Secondary Antibody Literature: Current literature primarily recommends a standard 1:200 concentration for secondary antibodies, regardless of primary concentration or technique. This study provides new insight, showing that optimal secondary concentrations vary by antigen, emphasizing the need for further research across antigens and staining methods.

Primary Antibody Range: While primary antibody concentrations have more extensive literature, variability in optimal concentrations suggests that broader testing is warranted, especially for antibodies like CD83 and AQP4, where the optimal concentration may be outside the tested range.

Tissue Consistency: Resource constraints prevented using the same DPI tissue across all antigens. AQP4 IHC required tissue obtained at different post-injury time points. Both 7 DPI and 28 DPI were selected because pathology is present at both stages with minimal differences, which likely had negligible impact on research outcomes.[43] However, slight variations may exist, and future studies should standardize tissue selection across antigens to minimize extraneous variables and better understand antigen-specific staining patterns and cellular interactions post-injury. It is important to note that this study focused on optimizing staining for each antigen, rather than multiple injury time points. The primary limitation is the inability to use multiple timepoints of injury for *each* antigen, with a focus on pathology at specific time points instead. ***Implications & Future Directions***

This study has important implications for neurotrauma research. By identifying optimal antibody concentrations for key antigens, it enhances IHC data analysis while promoting the ethical principle of reducing animal use. Improved IHC methodologies maximize data extraction from fewer samples, minimizing unnecessary scientific animal experimentation.

Additionally, this study offers novel insight into immunohistochemistry, particularly regarding secondary antibody dilutions, CD83 and AQP4 concentrations, and the relationship between secondary concentrations and staining intensity. Researchers are encouraged to further investigate these areas. Future directions include:

- Incorporating quantitative analysis methods to improve objectivity.
- Investigating mediating factors between antibody concentrations and image quality.
- Optimizing additional aspects of IHC, such as antigen retrieval and blocking procedures.
- Modifying the scoring guide to prioritize different image features and appeal to a broader research audience.
- Expanding applications to TBI pathology studies, including cross-analysis with immunocytochemistry, MRI, and Western blot.

Despite the substantial prevalence of TBI in sports and the military, its pathology remains unexplored. Incremental advancements in research are critical for deepening the understanding of TBI, developing therapeutic treatments, and improving patient outcomes. This study aims to contribute to that broader effort, emphasizing both the scientific and societal significance of advancing TBI research.

Ethics Statement

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Arizona College of Medicine and followed the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering and the quantity of animals used.

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Appendix

This appendix contains a general IHC protocol template and an example completed protocol for IBA1 to demonstrate the specifics of the IHC protocols used by this study.

Appendix A: IHC protocol template for each antigen

Date: | Basic [antigen] Protocol with Antigen Retrieval & Blocking

Project: optimization

Notes:

Primary antibody: [Name of primary antibody]

Secondary antibody: [Name of secondary antibody]

Day 1 ( )

Remove slides from -80° freezer and put in -20° freezer → 15 min

Remove slides from -20° freezer at RT→ 15 min

Place slides on a slide warmer at 50° → 60 min

Rehydration:

1X PBS (3 x 5 min)

Antigen Retrieval:

Place slides in sodium citrate buffer (pH 6.0)

Microwave: Power 6 for 3 min, then Power 3 for 10 min

Note: Place two dishes into microwave, other dish can be filled with water

Let slides cool for 60 min (or cool to the touch)

1X PBS wash (3 x 5 min)

PAP Pen

Block:

10% NHS serum, 0.3% Triton-100 in 1X PBS, 300 ul/slide = incubate for 60 min

NHS → ([total volume of liquid])(0.10) = [microliters of NHS used]

Triton → ([total volume of liquid])(0.003) = [microliters of Triton used]

PBS → total ul - (NHS + Triton) = [microliters of PBS used]

1X PBS wash (3 x 5 min)

Primary antibody:

NOTE: negative control gets no primary; continue with blocking solution or PBS overnight

Optimization of primary in 1% NHS serum, 0.1% Triton-100 in 1X PBS, 300 ul/slide → RT overnight

	Primary antibody	NHS	Triton	PBS
S dilution				
M dilution				
L dilution				

DAY 2 ( )

1X PBS wash with 0.1% Tween (3 x 5 min)

Secondary Antibody:

Optimized secondary in 4% N__S serum, 0.4% Triton-100 in 1XPBS → 60 min (300 ul/slide)

NOTE: put the negative control secondary for in the largest dilution category

	Secondary antibody	NHS	Triton	PBS
S dilution				
M dilution				
L dilution				

1X PBS wash with 0.1% Tween (3 x 5 min)

Block endogenous peroxidases

Place slides in 9 ml of H202 in 200 ml 1X PBS → 20 minutes (300 ul/slide)

Watch for bubbles. If none appear, H202 is bad

Make ABC solution

Needs to sit in the fridge for 30 minutes before application

Place 2 drops A + 2 drops B in a falcon tube containing 5 ml 1X PBS

1X PBS wash with 0.1% Tween (3 x 5 min)

Apply ABC, 300 ul/slide → 30 min

1X PBS wash with 0.1% Tween (3 x 5 min)

DAB

Make DAB solution according to the instructions on the box → 300 ul/slide

Look at about 2-4 minutes for each slide, wait for them to get dark → time dependent

DON'T FORGET: all trash must go into a falcon tube filled with 10 ml of bleach. All pipette tips and Kimwipes, etc. need to be placed into this falcon tube waste. Spray DAB with bleach when done.

Wash in tap water (5-10 min)

The ions in the tap water stop the DAB from processing

Dehydrate slides in EtOH to dehydrate the tissue

70% EtOH → 10 dips X2

90% EtOH → 10 dips X2

100 EtOH → 10 dips X2

Citrasolv #1 & Citrasolv #2 → 10 min each

Permount coverslip and leave in the hood to dry overnight

Clean with Xylene and long Q-tips the next day

Appendix B: Example completed IBA1 IHC protocol

Date: | Basic IBA1 Protocol with Antigen Retrieval & Blocking

Project: optimization

Notes:

Primary antibody: Rabbit anti-IBA1

Secondary antibody: Horse anti-rabbit

Day 1 ( )

Remove slides from -80° freezer and put in -20° freezer → 15 min

Remove slides from -20° freezer at RT→ 15 min

Place slides on a slide warmer at 50° → 60 min

Rehydration:

1X PBS (3 x 5 min)

Antigen Retrieval:

Place slides in sodium citrate buffer (pH 6.0)

Microwave: Power 6 for 3 min, then Power 3 for 10 min

Note: Place two dishes into microwave, other dish can be filled with water

Let slides cool for 60 min (or cool to the touch)

1X PBS wash (3 x 5 min)

PAP Pen

Block:

10% NHS serum, 0.3% Triton-100 in 1X PBS, 300 ul/slide = incubate for 60 min

NHS → (3000)(0.10) = 300 ul

Triton → (3000)(0.003) = 9 ul

PBS → total ul - (NHS + Triton) = 3000 - 309 = 2691 ul

1X PBS wash (3 x 5 min)

Primary antibody:

NOTE: negative control gets no primary; continue with blocking solution or PBS overnight

Optimization of primary in 1% NHS serum, 0.1% Triton-100 in 1X PBS, 300 ul/slide → RT overnight

	Primary antibody	NHS	Triton	PBS
1:250	3.6 ul	9 ul	0.9 ul	886.5 ul
1:500	1.8 ul	9 ul	0.9 ul	888.3 ul
1:1000	0.9 ul	9 ul	0.9 ul	889.2 ul

DAY 2 ( )

1X PBS wash with 0.1% Tween (3 x 5 min)

Secondary Antibody:

Optimized secondary in 4% NHS serum, 0.4% Triton-100 in 1X PBS → 60 min (300 ul/slide)

NOTE: put the negative control secondary for in the largest dilution category

	Secondary antibody	NHS	Triton	PBS
1:250	3.6 ul	36 ul	3.6 ul	856.8 ul
1:500	1.8 ul	36 ul	3.6 ul	858.6 ul
1:1000	1.2 ul	48 ul	4.8 ul	1146 ul

1X PBS wash with 0.1% Tween (3 x 5 min)

Block endogenous peroxidases

Place slides in 9 ml of H202 in 200 ml 1X PBS → 20 minutes (300 ul/slide)

Watch for bubbles. If none appear, H202 is bad

Make ABC solution

Needs to sit in the fridge for 30 minutes before application

Place 2 drops A + 2 drops B in a falcon tube containing 5 ml 1X PBS

1X PBS wash with 0.1% Tween (3 x 5 min)

Apply ABC, 300 ul/slide → 30 min

1X PBS wash with 0.1% Tween (3 x 5 min)

DAB

Make DAB solution according to the instructions on the box → 300 ul/slide

Look at about 2-4 minutes for each slide, wait for them to get dark → time dependent

DON'T FORGET: all trash must go into a falcon tube filled with 10 ml of bleach. All pipette tips and Kimwipes, etc. need to be placed into this falcon tube waste. Spray DAB with bleach when done.

Wash in tap water (5-10 min)

The ions in the tap water stop the DAB from processing

Dehydrate slides in EtOH to dehydrate the tissue

70% EtOH → 10 dips X2

90% EtOH → 10 dips X2

100 EtOH → 10 dips X2

Citrasolv #1 & Citrasolv #2 → 10 min each

Permount coverslip and leave in the hood to dry overnight

Clean with Xylene and long Q-tips the next day

The Influence of Films on Romantic Expectations of Young LGBTQ+ Men

Anthony Bilello

Abstract: This paper analyzes the influence that films featuring LGBTQ+ relationships have in fostering the romantic expectations of young LGBTQ+ men aged 15-20. The films Bohemian Rhapsody (2018) and Rent (2005) were analyzed based on how LGBTQ+ relationships were characterized, finding dramatic or unrealistic representation of physical and emotional expectations in addition to expectations of conflict resolution. This data informed a survey asking LGBTQ+ and straight men about their expectations of romance, romantic partners, and themselves in a relationship. The survey displayed a strong difference in expectations, specifically in terms of physical expectations.

Keywords: LGBTQ+ romantic expectations, romantic beliefs, films, young LGBTQ+ men

Introduction

The foundation of the research conducted in this report is to determine how films may influence the romantic expectations of LGBTQ+ young adult (15-20 years old) men compared to young straight men. This is due to the lack of representation of the LGBTQ+ community in films throughout the history of cinema as researcher Nick Bamford notes (Bamford, 2016), in addition to the lack of research on how men's romantic expectations are shaped by films, as much of the pre-existing theories and research on the topic are focused primarily on women, such as the theory of "princess culture", a theory regarding the influence of films on young girls (Koontz, 2017). By combining these topics, this research aimed to determine whether films may influence romantic expectations on a group seldom researched previously.

Literature Review

Introduction

This research focused on how films influence romantic expectations in young LGBTQ+ men compared to straight men. The two primary areas of focus were how romantic expectations in general are shaped by films and the relationship of the film industry to the LGBTQ+ community.

Body of Knowledge

Romantic expectations in film

Much research presently exists on how media, including movies, can impact viewers and their ideas, opinions, perceptions, and thoughts. One theory, known as the "Cultivation Theory", presented by researchers from the International Journal of Research in Psychology, explains that watching media can instill unrealistic beliefs and influence viewers' outlook on the world (Banaag et al. 2014). This theory became the basis for various studies focusing on how movies impact the viewers' ideas, such as studies conducted by Kubrak (2020), Krans (2023), and Segrin (2002) aiming to identify the theory's validity. Kubrak and Segrin did this by issuing surveys to participants, while Krans conducted a meta-analysis. (Kubrak, 2020; Krans, 2023; Segrin, 2002). Both aimed to evaluate extreme beliefs about romance coupled with film watching. The results displayed that viewers were more likely to mimic beliefs of films they watched, demonstrating the

validity of the "Cultivation Theory" (Banaag, 2014). This preliminary research is further expanded by another theory which identifies how media can influence viewers. This theory, presented by the *Journal of Social and Personal Relationships* and titled the theory of "princess culture", credits romance movies for girls as depicting highly fictitious relationships and makes the young viewers believe that all romantic relationships will be perfect (Koontz, 2017). Like the "Cultivation Theory" (Banaag, 2014), this theory provides another angle analyzing how films influence opinions and romantic expectations (Banaag, 2014) (Koontz, 2017). The theory is expanded upon by results of studies which examine similar film topics portraying unrealistic romance. Evidence of the theory's validity is displayed by researchers Galloway (2015), Ray (2022), and Welch (2021), as their studies aimed at researching how romance films specifically might create unrealistic expectations of romantic partners as the theory hypothesizes. Each study surveyed participants, looking at beliefs about romance and how audience opinions may be influenced by the films they consumed (Galloway, 2015; Ray, 2022; Welch, 2021). They provided evidence supporting the theory of "princess culture" by establishing a correlation between greater expectations for love and the consumption of films, with even greater emphasis on female participants as they were the majority (Koontz, 2017). These findings indicate the validity of the theory of "princess culture" displaying the link between film viewing and unrealistic expectations of romance, while also highlighting gaps in the research as most participants, as well as the focus of the theory, are women. Both ideas, backed up by research, display the influence films can have on viewers, specifically romantic beliefs. Furthermore, romantic expectations are not only influenced by films but also by gender. Relevant to this topic is research conducted by Harris (2004) and Punyanunt-Carter (2006), who conducted similar research on the role of gender alongside films in forming romantic expectations by administering surveys asking both male and female participants how romance movies influenced their romantic beliefs. Similar conclusions arose with a clear difference in expectations between genders, with men having unrealistic expectations of sex and women with unrealistic expectations of romance itself. These results reveal another way romantic expectations are influenced by films, similar to the previous theories and studies such as the previously mentioned researchers Galloway (2015) or Welch (2021).

LGBTQ+ Representation

In addition to how films influence romantic expectations, another relevant topic of review is how films and audiences view the LGBTQ+ community. Researchers have evaluated portrayals of the LGBTQ+ community throughout cinema history, such as researcher Nick Bamford of Bournemouth University who conducted a meta-analysis of various films containing LGBTGQ+ characters. His research showed that while the quantity of LGBTQ+ portrayals have increased, the quality is lacking, with "watered-down" portrayals (Bamford, 2016). Likewise, an analysis by researcher Makkena Lambert on the history of LGBTQ+ representation in horror movies specifically contains similar results to Bamford's (2016) study, noting that the few depictions of LGBTQ+ characters were often stereotypical (Lambert, 2023). Researchers Brown (2021), Guskos (2023), and Rachmah (2019) evaluated portrayals of films featuring characters who are either explicitly LGBTQ+ or have certain "mannerisms" labeled as "queer". The results provide evidence of highly unrealistic and stereotypical depictions of the LGBTQ+ community (Brown, 2021; Guskos, 2023; Rachmah, 2019). Despite this agreement, researcher Onika Umayam disagrees. Umayam's analysis is similar to others in design; however, it indicates that LGBTQ+ representation in films is capable of representing the LGBTQ+ community more positively than the previous researchers implied (Umayam, 2024). This disagreement highlights potential gaps in the research, as the film Umayam studies, *Love Simon*, is focused on romance (Umayam, 2024), while the objects of the previous studies were films promoted to a general audience (Brown, 2021; Guskos, 2023; Rachmah, 2019; Umayam, 2024). General audiences have also been subject to research on how they view the LGBTQ+ community, such as analyses conducted by Thompson (2021) and McInroy (2017), asserting that general audiences exposed to harmful stereotypes have unrealistic beliefs of the LGBTQ+ community. Unlike the last set of studies, researchers Madžarević (2018) and McKee's (2000) findings indicate that films can promote the LGBTQ+ community positively (Madžarević, 2018; McKee, 2000). Madžarević's study on audiences revealed that they

reacted positively to the LGBTQ+ community due to the movie they were assigned to watch. McKee's study evaluated how gay men identified with LGBTQ+ characters in films and helping them accept themselves. Both McKee (2000) and Madžarević's (2018) studies help display the power films have influencing viewers in a positive way. The previous studies provide ample research on the LGBTQ+ community, the film industry, and audiences. One topic barely addressed is the community's relationship to romance and how it may be shaped by the films they watch.

Call to Research

Research on films and their relationship to expectations of romance is extensive; however, research gaps are present. Firstly, research often focuses mostly on women. For example, the theory of "princess culture" is aimed at identifying reasons behind high expectations in women only. Men are not the only group neglected by research on romantic expectations and film, and fewer sources discuss the LGBTQ+ community. In the previous studies, the LGBTQ+ population was often never addressed or surveyed. Additionally, upon collecting the previous research on films and their influence on the LGBTQ+ community and their perception, rarely did researchers discuss implications of films influencing the LGBTQ+ community's relationships with each other. Most studies and analyses focused solely on the community and their relationship/acceptance by others, which leaves much to be desired in terms of research on romantic relationships in terms of the LGBTQ+ community and films. This is where this research is relevant, by aiming to fill this gap of young gay men and how romantic expectations are shaped by the films they watch. The research question posed based on this gap is: how do movies featuring an LGBTQ+ romance plotline influence the expectations of romance in LGBTQ+ men aged 15-20?

Methods

Film Analysis

In first part of the study, I analyzed two films which both portray romantic relationships among LGBTQ+ characters, those two films being *Rent* (Columbus, 2005) and *Bohemian Rhapsody* (Singer, 2018). Both films feature romantic relationships between explicitly LGBTQ+ men. In *Bohemian Rhapsody*, the protagonist is a characterized version of real-life singer Freddie Mercury, a singer known for his feminine and flamboyant mannerisms (Singer, 2018). He finds himself in various relationships throughout the course of the film; however, the two which were the focus of this analysis were his romantic involvements with Paul Prenter and Jim Hutton (Singer, 2018). The relationship with Paul Prenter is presented as a failed and unfulfilling relationship; however, his future partner Jim is presented as much more loving (Singer, 2018). Of the variety of characters in *Rent,* the characters of Tom Collins and Angel Dumott Schunard, an LGBTQ+ couple who are battling positive diagnoses of HIV/AIDS, were the focus of this analysis (Columbus, 2005). Tom Collins is presented as an intelligent and quiet gay man, and his boyfriend Angel is presented as a crossdressing feminine gay man who is outgoing and sociable (Columbus, 2005). I recorded each instance of interactions between the characters and looked particularly to see if any traits and actions aligned with each other in both movies. In addition, I analyzed the way each character is presented both in their physical appearance and their mannerisms in an effort to categorize the data as well as to outline what aspects of the characterization are heavily focused on when portraying a relationship between two people who are LGBTQ+ in film. Upon the conclusion of each individual film analysis, I compiled all of the data into categories based on what appeared to be the main focuses to portray in an LGBTQ+ character and their relationships, these categories being physical appearance, personality, and conflict/ conflict resolution, which as further explained in the results section were the main characteristics driving LGBTQ+ romantic relationships in accordance with the films' portrayal.

Survey

With the film analysis completed, I compiled the data into the distinct categories of physical appearance, personality, and conflict/conflict resolution. I also had a list of common traits and behaviors such as the common tendency for LGBTQ+ men to be presented as feminine, as with Freddie Mercury and Angel (Singer, 2018; Columbus, 2005). Both data categories influenced the questions I asked in my survey. The survey was formatted with the Romantic Beliefs Scale which was a 7-point Likert Scale allowing participants to agree or disagree with a given statement, thus intending to evaluate the extent to which these films might influence viewers. Each statement was designed to emulate the specific traits focused on by the films reviewed in the analysis, for example one statement reads "I expect my romantic partner to dress more feminine" because in both *Bohemian Rhapsody* (2018) and *Rent* (2005), at least one LGBTQ+ character is portrayed as dressing more feminine, and this is a major part of their romantic characterization, thus potentially influencing their romantic expectations. To answer this statement, participants must choose between numbers 1-7, 1 stating "strongly disagree", and 7 stating "strongly agree" with 2-6 offering more mixed opinions in between 1 and 7. If a participant did not wish to answer or note their opinion on a statement, they were allowed to skip it. Furthermore, the statements were all grouped into three separate categories, these being the same ones I determined earlier in the film analysis: physical appearance, personality, and conflict/conflict resolution. The survey also begins by asking questions related to demographic information, including participant and parental consent (if the participant is under the age of 18), preferred gender identity, whether or not the participant was born with their preferred gender identity, age range, sexual orientation, and current relationship status. Another demographical question I asked was "have you watched at least three films within the last 20 days?" There was also a pair of questions asking participants "what is the most important value in a romantic partner?" and the second question asks participants to rank various romantic traits such as empathy, intelligence, and physical appearance from most to least important. The survey was open to all men, not just LGBTQ+ men, allowing for any difference in romantic expectations to be compared and noted. Furthermore, another requirement for participants in this survey was to be between the ages of 15-20. I distributed the survey using various methods including advertising my survey to students in a local school's Gay Straight Alliance club; the club's members consisted of various people who fit my demographic. Furthermore, I issued my survey online in various communities such as LGBTQ+ support groups and student groups of 15–20-year-olds. To ensure my results contained the most varied responses, utilizing online communities allowed more participants fitting my demographic to find my survey.

Data Collection

After my data was collected, I conducted a T-Test to compare the results of the LGBTQ+ participants to the straight/heterosexual participants. The T-Test was conducted using the JASP program.

Method Alignment

Romantic Beliefs Scale

The structure of the survey uses a Likert scale, specifically the "Romantic Beliefs Scale", a seven-point scale where participants select a choice representing their opinions based on a given statement. I used the scale as participants can easily agree or disagree with the statements and provided them with ample choices allowing for the most accurate answers from participants based on each statement. The "Romantic Beliefs Scale" was used in the survey by Galloway, Engstrom, and Ermers-Sommer (Galloway et al.,, 2015), asking about romantic expectations, and the use of this scale helped the researchers gather accurate answers based on statements of "high expectations", similar to the survey I conducted. Furthermore, the reason I conducted a survey is because the "Romantic Beliefs Scale" – which, as discussed, is the best way to assess romantic beliefs in participants -- is designed for a survey like Galloway's survey (Galloway et al., 2015). One question also allowed participants to rank romantic traits such as intelligence, empathy, and physical appearance from the most to least important which provides further information about each participant's romantic values in a more concise way.

Film Analyses

The statements, as well as the categories of physical, emotional, and conflict resolution expectations are based on the films I examined: 2018's *Bohemian Rhapsody* (Singer 2018) and 2005's *Rent* (Columbus, 2005). I chose these two films since both films por-

tray various LGBTQ+ characters and relationships: for example, in *Bohemian Rhapsody* the portrayal of Freddie Mercury and his relationships (Singer, 2018), and in *Rent* with various LGBTQ+ characters such as Angel and Tom (Columbus, 2005). I also chose these two films as they would have likely been viewed by my target demographic of 15–20-year-olds, since *Rent* (having been released in 2005) would have existed for many years while not being so old that young adults to avoid it, and *Bohemian Rhapsody* having released in 2018 is likely to have been viewed by young adults upon or soon after release. I examined romantic interactions between the LGBTQ+ characters to note dramatized and/or heavily focused on aspects of the characters, like the emphasis on the physical appearance of characters in both films or how conflicts are resolved. The analysis itself was conducted similarly to Rachmah's study on the 2017 film *Beauty and the Beast* in which she analyzed scenes featuring the character Le Fou to assess the extent to which his portrayal was "queer coded" and how it harms the LGBTQ+ community's representation (Rachmah, 2019). However, my study is focused more on romantic interactions found in the researched films. Rachmah's study guided my own analysis on the films since she focuses on characterization as well as how characters interact with others (Rachmah, 2019), and to assess dramatized romantic elements among LGBTQ+ characters I also needed to look at characterization above all else. I examined this to inform my survey on what categories of expectations to focus on and informing several statements such as those regarding dressing/acting feminine as both films feature crossdressing.

Data Collection

I used various measures to ensure my data was organized, including a variety of questions which serve to ensure participants are within my target demographics of LGBTQ+ men aged 15-20, including questions related to their preferred gender identity and their age. The only exception was that for those who identify as straight men, I used their data as a control group to compare their results to the LGBTQ+ participants. I used the T-Test I used to compare the responses from LGBTQ+ men the straight men to show distinct romantic expectations unique to the LGBTQ+ men, thus displaying any heightened romantic expectations among the LGBTQ+ participants.

Participants

As mentioned previously in the literature review, there is a lack of representation of LGBTQ+ men in addition to stereotypes. My research on how films featuring romantic elements influence LGBTQ+ men and their romantic expectations is crucial in determining how being either absent or mocked in films can impact romantic expectations differently in LGBTQ+ men than straight people and women. Furthermore, the participants being 15-20 years old is important as young adults are entering society, and it is important to study their interaction with the world as they will soon be influencing society on a larger scale. I also wished to assess participants and their familiarity with films, so I asked whether they watched at least 3 films within the last twenty days. Originally, I was aiming to look at LGBTQ+ men from Nassau County, NY since it is a diverse county located in between bustling New York City and rural Suffolk County which lends to a sampling of various ideas and cultures. I thought this would lend the research to contain little cultural-based bias due to the wide variety of cultures present in Nassau County. While originally, I believed this would work well, I quickly noticed redundancy since participants who answered outside of my location answered with little difference in data.

Results

Content Analysis of Bohemian Rhapsody

The characters of note in this analysis are Freddie Mercury, Mary Austin (Freddie's first love), Paul Prenter (first male love interest), and Jim Hutton (second male love interest). The first romantic scene is between Freddie and Mary, where she encourages his experimentation with feminine clothing (Timestamp: 0:09:57-0:11:16). This scene is relevant in this analysis since while it does not feature two LGBTQ+ men in a relationship, it displays Freddie's tendency to dress in "feminine" clothing. Soon after, Freddie is introduced to his second love interest, Paul Prenter, who acts as

Queen's manager. (0:22:12-0:22:50). Paul's appearance is "masculine", wearing unrevealing outfits, short hair, and a mustache. In the next noteworthy scene, a male truck driver eyes Freddie and enters the bathroom. Freddie can be seen looking at the door and thinking, foreshadowing the truth of his sexuality (0:28:56-0:29:38). Later, Freddie plays a song as Paul proceeds to kiss him to Freddie's rejection (0:35:04-0:37:19). This scene displays the romantic feelings Paul presses onto Freddie. The next scene takes place after Mary and Freddie break up due to Freddie's sexuality. Freddie holds a party where he dons a flamboyant outfit and flirts with other men including Paul (1:00:00-1:03:11). After the party, Freddie meets Jim Hutton, his caterer. Freddie touches him inappropriately for which he apologizes, and they kiss after flirtation (1:03:11-1:05:47). After an unrelated drama, Freddie is alone with Paul who withholds calls from Freddie's friends on the grounds that he is "too busy with work" (1:27:58-1:33:34). After Freddie confronts Paul, he threatens to release stories of Freddie's exploits to blackmail him (1:33:47-1:36:06). The final noteworthy scene is Freddie's reconnection with Jim Hutton, where, while they hold hands, he meets his family (1:50:17-1:52:45).

Content Analysis of Rent:

This analysis is based on romantic interactions between Tom Collins and Angel Dumott Schunard: two gay men with AIDS. The first scene of this relationship sees Angel treat Collins after he gets mugged, bonding over both having AIDS (0:14:19-0:15:31). Next, Angel performs the song "Today 4 You" for Collins and his friends, all while wearing "feminine" clothing like a wig, leggings, and high heels, which is a running character trait (0:27:07-0:29:45). Next, Collins and Angel attends an AIDS support group; Angel is still dressed "feminine" while Collins is dressed in "ordinary clothes" (0:38:54-0:41:45). Soon after, Angel and Tom have a duet, "I'll Cover You", where they act intimate by hugging, holding hands, and kissing (0:58:50-1:01:20). Many of their further interactions take place during unrelated scenes such as them hugging during Maureen's protest (1:02:55-1:10:14), kissing during "Viva La Vie Boheme" (1:16:37-1:16:40), and dressing as James Bond and Pussy Galore for New Years (1:26:19-1:29:10). The next scenes are shown during the song "Without You", where Angel is held by Collins on the train and in the hospital (1:43:14-1:45:25). Angel then succumbs to AIDS by the end. The final noteworthy scene after Angel's funeral has Collin's friends sing that they want a "taste of what Angel had" referring to Angel and Collins' love (1:50:58-1:53:28).

Analysis

Content Analysis

Upon the conclusion of the content analysis, several key factors of LGBTQ+ romances emerged, those being how they are represented physically, emotionally, and through conflict. Starting with physical traits, both films heavily emphasize on the appearance of the men. In each relationship there was always one man presented as dressing "feminine" (i.e. Freddie Mercury and Angel), and one dressing traditionally "masculine" (i.e. Paul, Jim, or Collins). The fact that both films address this fact exemplifies how films characterize male LGBTQ+ relationships which may influence how viewers expect a relationship between queer men to behave physically. Furthermore, the emotional traits displayed by each film align similarly with the physical traits. For characters who dress more "feminine", they are coupled with extroverted and outgoing personalities such as Freddie and Angel who often do not care what others think, while characters like Jim and Paul are much more reserved and introverted, as they don't talk nearly as often. The only outlier is Collins, who also contributes to conversations and is not interested in what others think of them. Finally, conflict/conflict resolution is also shown to be important to the relationships, although the presentation is different. In *Bohemian Rhapsody,* conflict is internal such as with Jim being offended by Freddie grabbing him without consent (1:03:11-1:05:47), or Paul keeping information from Freddie (1:27:15-1:33:34). In *Rent,* conflict is external as Angel and Collins must grapple with AIDS and Angel's slow death throughout the film. Overall, the three categories of physical, emotional, and conflict resolution expectation served as the main ways romance between LGBTQ+ men were depicted which informed the next half of my research.

Survey

First, I collected 110 responses from participants, though 30 were discarded due to not fitting the age or gender requirement. The remaining responses were inputted into the statistical analysis software JASP, which assisted with analyzing my data through a series of four T-Tests, three based on my three Likert Scales on "physical expectations", "emotional expectations", and "expectations of conflict resolution", in addition to a fourth test on the question asking participants to rank six given traits of a partner from most to least important to them.

Physical Expectations

Results for the physical expectations portion of the survey can be referred to in Figure 1 seen below.

The results suggest that statements based on the results of the content analysis regarding physical traits and expectations of romantic partners are different between LGBTQ+ and straight men in terms of clothing-based expectations. All four significant statements revolve around how the participants believed to be expected to dress by their partner or how they expect their partner to dress. Specifically, LGBTQ+ men are more expected to dress feminine while expecting their partners to dress masculine, while the opposite is true for straight men. While the specifics are different, physical expectations overall are not the most important trait to both groups as the final two statements' p-values were high.

Independent Samples T-Test ▾

Independent Samples T-Test

	W	df	p
I expect my romantic partner to dress more feminine	184.500		< .001
I expect my romantic partner to dress more masculine.	1225.000		< .001
I feel as if I am expected to dress more feminine by my romantic partner.	924.500		< .001
I feel as if I am expected to dress more masculine by my romantic partner.	392.500		0.003
Physical appearance in general is my favorite trait about my partner.	706.000		0.658
My physical appearance in general is my partner's favorite trait about me.	717.000		0.306

Note. Mann-Whitney U test.

Descriptives ▾

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
I expect my romantic partner to dress more feminine	LGBTQ+	41	2.829	1.595	0.249	0.564
	Straight	37	5.297	1.331	0.219	0.251
I expect my romantic partner to dress more masculine.	LGBTQ+	41	4.390	1.759	0.275	0.401
	Straight	37	2.459	1.325	0.218	0.539
I feel as if I am expected to dress more feminine by my romantic partner.	LGBTQ+	37	2.838	1.708	0.281	0.602
	Straight	34	1.441	0.927	0.159	0.644
I feel as if I am expected to dress more masculine by my romantic partner.	LGBTQ+	37	3.838	1.573	0.259	0.410
	Straight	35	4.886	1.728	0.292	0.354
Physical appearance in general is my favorite trait about my partner.	LGBTQ+	37	3.324	1.617	0.266	0.486
	Straight	36	3.167	1.732	0.289	0.547
My physical appearance in general is my partner's favorite trait about me.	LGBTQ+	36	3.500	1.665	0.277	0.476
	Straight	35	3.029	1.636	0.276	0.540

Figure 1: A T-Test was used to compare physical romantic expectations in the LGBTQ+ participants and straight participants. The LGBTQ+ group (M=2.892, SD=1.595) performed significantly lower than the straight participants (M=5.297, SD=1.331) in Statement 1. The LGBTQ+ group (M=4.390, SD=1.759) performed significantly higher than the straight participants (M=2.459, SD=1.325) in Statement 2. The LGBTQ+ group (M=2.838, SD=1.708) performed significantly higher than the straight participants (M=1.441, SD=0.927) in Statement 3. The LGBTQ+ group (M=3.383, SD=1.573) performed significantly lower than the straight group (M=4.886, SD=1.728) in Statement 4. The other two statements were not significant in terms of differences between how LGBTQ+ participants and straight participants responded.

Emotional Expectations

Results for the emotional expectations portion of the survey can be seen in Figure 2 seen below.

The results suggest that emotional expectations based on the findings of the content analysis are not nearly as significant on average compared to physical expectations, since only one of the statements is significant. LGBTQ+ participants did not agree with the statement that a romantic partner should change their personality to better fit the participant's taste as much as the straight participants did; however, the small difference means suggest that neither group agreed with that statement immensely.

Expectations of Conflict Resolution

Results for the expectations of conflict resolution portion of the survey can be seen in Figure 3 seen below.

These results show that statements based on expectations of conflict resolution are the most consistent

Independent Samples T-Test ▾

Independent Samples T-Test

	W	df	p
I expect my romantic partner to give up everything for me.	692.000		0.387
My romantic partner exhibits traits I recognize from characters in movies.	714.500		0.308
I expect my romantic partner to change their personality to better fit my taste	538.000		0.021
I expect my romantic partner to be perfect in every way.	777.000		0.817
I would prefer my romantic partner to be quiet and reserved.	602.000		0.116
I would prefer my romantic partner to be outgoing and extroverted.	872.500		0.230

Note. Mann-Whitney U test.

Descriptives ▾

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
I expect my romantic partner to give up everything for me.	LGBTQ+	42	2.167	1.267	0.196	0.585
	Straight	37	2.514	1.539	0.253	0.612
My romantic partner exhibits traits I recognize from characters in movies.	LGBTQ+	37	4.351	1.230	0.202	0.283
	Straight	34	4.000	1.279	0.219	0.320
I expect my romantic partner to change their personality to better fit my taste	LGBTQ+	42	1.786	1.116	0.172	0.625
	Straight	36	2.444	1.443	0.241	0.590
I expect my romantic partner to be perfect in every way.	LGBTQ+	42	1.738	1.014	0.156	0.583
	Straight	36	1.972	1.630	0.272	0.826
I would prefer my romantic partner to be quiet and reserved.	LGBTQ+	42	3.190	1.435	0.221	0.450
	Straight	36	3.722	1.560	0.260	0.419
I would prefer my romantic partner to be outgoing and extroverted.	LGBTQ+	42	4.690	1.456	0.225	0.311
	Straight	36	4.361	1.246	0.208	0.286

Figure 2: A T-Test was used to compare emotional expectations for relationships in the LGBTQ+ group and the straight group. For the first two statements there were no significant differences between LGBTQ+ and straight responses. The third statement saw the LGBTQ+ group (M=1.786, SD=1.116) perform significantly lower than the straight participants (M=2.444, SD=1.443). The final three statements did not yield any significant results.

Independent Samples T-Test

Independent Samples T-Test

	W	df	p
If after a conflict I say sorry, that should be the end of the problem.	728.000		0.946
I value truth and honesty, even if it hurts me.	822.000		0.346
If those around do not accept my partner, that will create conflict between my partner and I.	749.500		0.883
If something bad happens to me, I expect my partner to be completely by my side through it all.	732.000		0.979
If my needs are not met by my partner, I have the right to leave the relationship.	753.000		0.854
To truly resolve a conflict, compromises must be made between me and my partner.	792.000		0.539
Communication is the most important factor in solving conflicts for me.	886.000		0.084

Note. Mann-Whitney U test.

Descriptives

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
If after a conflict I say sorry, that should be the end of the problem.	LGBTQ+	42	3.524	1.469	0.227	0.417
	Straight	35	3.571	1.650	0.279	0.462
I value truth and honesty, even if it hurts me.	LGBTQ+	42	6.214	0.871	0.134	0.140
	Straight	35	6.000	0.970	0.164	0.162
If those around do not accept my partner, that will create conflict between my partner and I.	LGBTQ+	42	3.048	1.724	0.266	0.566
	Straight	35	2.914	1.541	0.260	0.529
If something bad happens to me, I expect my partner to be completely by my side through it all.	LGBTQ+	42	5.095	1.322	0.204	0.259
	Straight	35	5.086	1.422	0.240	0.280
If my needs are not met by my partner, I have the right to leave the relationship.	LGBTQ+	42	5.429	1.434	0.221	0.264
	Straight	35	5.343	1.562	0.262	0.290
To truly resolve a conflict, compromises must be made between me and my partner.	LGBTQ+	42	6.167	0.762	0.118	0.124
	Straight	35	5.743	1.578	0.267	0.275
Communication is the most important factor in solving conflicts for me.	LGBTQ+	42	6.571	0.630	0.097	0.096
	Straight	35	6.114	1.157	0.196	0.189

Figure 3: A T-Test was used to compare expectations of conflict resolution between LGBTQ+ respondents and straight respondents. The first six statements did not yield any significant differences in responses between LGBTQ+ and straight participants. The final statement was slightly significant as LGBTQ+ participants (M=6.571, SD=0.630) performed somewhat significantly higher than straight participants (M=6.114, SD=1.157).

comparisons of both groups and their answers. This is because the results show no significant data. The only statement close to significance is that communication is the most important factor in solving conflicts, although the p-value being .084 renders only slight significance. Since there were no outright significant results, conflict resolution expectations seem to be consistent for the most part between LGBTQ+ and straight men.

Ranking of Traits

Results for the rankings of romantic traits in partners portion of the survey can be seen in Figure 4 seen below.

The results of the ranking of romantic traits for partners from most to least important showed no significance between LGBTQ+ and straight participants. These results suggest no significant difference in basic romantic expectations between both groups. One interesting note, this question is the only one that I did not base on my content analysis, so it suggests that when not based on films, romantic expectations remain the same; however, when film traits are added inherent differences appear.

Survey Analysis

The survey results, which are based on the traits exhibited by the characters in the content analysis, provided insight into differences between how traits shown in films can influence the LGBTQ+ male community compared to straight men. The survey displayed that questions relating to how participants expect their partners and themselves to dress in terms

Independent Samples T-Test

Independent Samples T-Test

	W	df	p
Trust	578.500		0.114
Respect	737.500		0.869
Empathy	592.000		0.173
Communication	763.500		0.661
Physical	833.500		0.228
Intelligence	774.500		0.576

Note. Mann-Whitney U test.

Descriptives

Group Descriptives

	Group	N	Mean	SD	SE	Coefficient of variation
Trust	LGBTQ+	39	1.872	1.341	0.215	0.717
	Straight	37	2.243	1.278	0.210	0.570
Respect	LGBTQ+	39	3.333	1.364	0.218	0.409
	Straight	37	3.243	1.362	0.224	0.420
Empathy	LGBTQ+	39	3.359	1.630	0.261	0.485
	Straight	37	3.892	1.760	0.289	0.452
Communication	LGBTQ+	39	3.308	1.559	0.250	0.471
	Straight	37	3.162	1.642	0.270	0.519
Physical	LGBTQ+	39	4.795	1.436	0.230	0.299
	Straight	37	4.378	1.622	0.267	0.370
Intelligence	LGBTQ+	39	4.333	1.383	0.221	0.319
	Straight	37	4.027	1.756	0.289	0.436

Figure 4: A T-Test was used to compare the average rankings of six traits of a romantic partner in between LGBTQ+ participants and straight participants. The results for all six average rankings had no significant differences between either group of respondents.

of dressing "masculine" and "feminine" displayed the most significant differences. These results display the unique differences among both sets of participants. Furthermore, the fact that these statements were based on the exaggerated displays of the physical appearances of film characters in the content analysis seemed to confirm my initial hypothesis that films featuring romance between LGBTQ+ men would influence the romantic expectations of LGBTQ+ men. While physical expectations were largely significant, the expectations of emotion and conflict resolution had less significant responses. This shows that while there was some significance in responses, overall, physical expectations saw the most significant results. Furthermore, the survey results display that there were no differences between the ways straight and LGBTQ+ participants rank different romantic traits, which supports my hypothesis since as mentioned earlier the ranking question was not informed by the results of the content analysis. The fact that no differences were seen there shows that when character traits from films are taken out of the equation, there are no significant results.

Limitations

The biggest limitation of my research is what can be reasonably evaluated through the survey as opposed to an interview. The interview conducted by Koontz where she evaluated romantic expectations in women based on princess movies saw her finding detailed results regarding these women and their specific expectations. Since my research did not find specific and personalized results like Koontz's interview, the conclusions on romantic expectations in LGBTQ+

men may be limited. For example, one statement in my survey which reads "my romantic partner exhibits traits I recognize in movies" yielded non-significant results; however, without details like what traits or what movies, only so much could be reasonably concluded.

Implications

The results displaying heightened physical expectations for LGBTQ+ men contain broader implications for the film industry. Film companies and producers should be encouraged to contain fewer stereotypical physical depictions of LGBTQ+ men, and the expectations of viewers would likely become much more realistic since the highest difference in regard to viewer expectations comes from physical expectations and apparel. Furthermore, this could benefit LGBTQ+ actors. If companies heed this advice, LGBTQ+ actors would be allowed to dress how they want rather than conforming to any preconceived expectations of how LGBTQ+ people should dress. Finally, this research implies that the LGBTQ+ male viewers of films would benefit from being aware of the influence that films featuring gay male relationships can play on their romantic expectations, which would go a long way in benefitting the lives and relationships of young LGBTQ+ men.

Conclusion

The results of the research conducted on LGBTQ+ men and how films influence their romantic expectations showing a relation to some higher expectations provide similar insight on how films influence their viewers, specifically highlighting higher physical expectations. The results displaying these greater physical expectations are most similar to the findings of researchers such as Galloway (2015), Ray, (2022) and Welch (2021) as they all displayed the fact that films can influence the opinions of viewers. However, this research studied various film tropes relating to how LGBTQ+ people are characterized, and how this may influence LGBTQ+ men compared to straight men. By doing this, the conclusions add further insight for the LGBTQ+ male community and their relationship to films. The results suggest extremely characterized physical traits common in films featuring LGBTQ+ characters have some influence on how members of this community view romantic relationships compared to their straight counterparts.

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Natural Pesticides: Assessing the Influence of Salinity Stress and Mitigation via *Bacillus subtilis* Application on the Development of Lamiaceae Botanicals and their Impact on the Natural Predator *Hippodamia convergens*

Kevin Tritschler

Abstract: Botanicals are plant extracts created for pest deterrence. While this potential has been thoroughly verified, prior research has seldom investigated how their suitability is impacted by abiotic stressors, nor how they may impact non-target species exposed to them. This research investigates how salinity stress, and mitigation via *B. subtilis* application, impacts this suitability. It was hypothesized that higher salinity would lead to stronger pest deterrence, based on prior literature correlating salinity stress with increased monoterpenoid production. The results showed trivial ($\eta < 0.1$, $p < .001$) impacts to plant growth under higher salinity levels; the application of *Bacillus subtilis* had significant species-dependent influences. Regarding the deterrence of the natural predator *Hippodamia convergens*, the results found no discernable sublethal trends regarding salinity, and a correlation with a slower time of death. The commercial pesticide λ-cyhalothrin showed similar lethality compared to the botanical treatments, raising concerns regarding botanicals' toxicity to non-target organisms.

Keywords: Botanicals, Pesticide, Salinity Stress, *H. convergens*, Lamiaceae

Introduction

Botanicals are bioactive extracts of compounds from plants, involving an alcohol or water-based solvent, which are used specifically for insect-deterring purposes. Such extracts are verified via screenings of the individual constituent components (Acheuk et al., 2022), and have been promoted as a potential alternative to pesticides because they remain similarly effective without the reported health risks. In particular, the widespread usage of conventional pesticides has left large concentrations of residuals behind in soils, waterways, and exposed biomass (Aktar et al., 2009); these residuals hold the toxicity of their initial pesticides and are often ingested by other organisms. In using monoterpenoids from plants in place of these chemicals, it is hoped that this long-term toxicity problem can be mitigated.

While multiple plant families may be used for botanical synthesis, the Lamiaceae family is a promising choice because its terpene composition is particularly effective against arthropod pest species (Ebadollahi et al., 2020; Conti et al., 2010). But while there is strong evidence that Lamiaceae-based extracts may be effective against several species of pests, this role may be influenced by several factors related to the plants' initial growth; this includes watering level, salinity, temperature, light, and soil composition (Németh-Zámbori et al., 2016; Assaf et al., 2022; Mansinhos et al., 2024), among others. Research to connect these environmental influences to changes in the efficacy of botanicals remains limited (Wyckhuys et al., 2023), particularly in reference to natural predators that may be used in Integrated Pest Management (IPM) system schema and thus are likely to be exposed to botanicals as a non-target species. This research aims to investigate how the efficacy of botanicals from three species in the Lamiaceae family – *Lavandula x intermedia*, *Ocimum basilicum*, and *Thymus vulgaris* – are influenced by the conditions of salinity stress and bacterial supplementation, and how these changes impact the vitality of the natural predator *Hippodamia convergens* when it is exposed to such botanicals.

Literature Review

Botanicals have been well established as a potential alternative to pesticides in prior literature, as several monoterpenoids found in plants have been proven to serve roles in deterring pests (Weaver, 2000). These monoterpenoids, among other components, are typically extracted by steeping cut biomass in a solvent of water, alcohol, or some combination thereof depending on the desired compounds; flavonoids, for instance, are best extracted via 70% isopropyl solvents (Belwal et al., 2018). These extracts, once steeped, are then boiled to remove the solvent, and, similarly to commercial pesticides, are diluted in water before application.

While a plethora of plant families may be considered for extract preparation, a few families hold particular interest in prior investigations. Linalool, for example, is a monoterpene used by plants as protection from oxidative stressors (Elisabetsky 2002), principally found in the families Lamiaceae (mint), Lauraceae (laurel), and Rutaceae (citrus) (Howe, 2020). For the Lamiaceae family in particular, this is one of a variety of compounds that hold the potential to deter insects (Yann Guitton et al., 2018); indeed, Lamiaceae family extracts have been proven to deter several insect genera, such as the *Callosobruchus* beetles (El Abdali et al., 2022), *Tribolium* beetles (Clemente et al., 2003), *Crematogaster* ants (Günter et al. 2008; Shim et al. 2000), and the larvae of mosquitoes (Chokechai-jaroenporn et al., 1994). In addition to their practical effectiveness, the cost effectiveness of botanicals has been testified across most of Sub-Saharan Africa, as an option readily available to farmers who may use them (Shai et al., 2023).

The current gap in the literature lies not within the practical effectiveness of botanicals, but in the conditions in which their source plants are grown; such factors have not yet been directly compared to changes in the effectiveness of botanicals. In regards to salinity stress - a common abiotic stressor, exacerbated by irrigation and deforestation (Yadav et al., 2011) - the Lamiaceae family is considered more resistant than several other plant families (Wu et al., 2016; Stefanakis et al., 2024; Avasiloaiei et al., 2023); even so, moderate salinity stress (defined as approximately 50-100 mM in prior literature) was shown to induce chlorosis and stem necrosis within the family. Such stress was also shown to decrease certain components in the plant oils such as α-terpineol, eugenol, and potassium ions (Talebi, 2018; Mahdi Z. & Sharam S, 2011). However, the botanical-relevant monoterpenoids were shown to increase in concentration under this same condition (Farsaraei et al. 2020; Assaf et al, 2022; Dehghani Bidgoli et al., 2019). As stated by Mansinhos et al. (2024), this suggests that the presence of salinity stress and similar abiotic stressors may promote oil production among the Lamiaceae; though there is not enough replicable data to verify any significant impact on the botanicals' principal role (Isman & Grieneisen, 2014).

Bacillus subtilis application is an attested option for mitigating external damages to plants (Awan Z. & Shoaib, 2019), including salinity stress (Ilangumaran & Smith, 2017); some prior literature indicates that it may reverse any changes caused to the plants (Khatami et al., 2023). The three species used in this study - *L.* x *intermedia*, *O. basilicum*, and *T. vulgaris* - have been proven to be effective candidates for botanical synthesis (Naveen et al., 2021; Lazarević et al., 2020). However, due to stark differences in the composition of their oils, they are expected to respond differently to these treatments. Darrag et al. (2021), for instance, has shown *T. vulgaris* to have greater volatile diversity than *O. basilicum*, giving it higher lethality against the red pine weevil. It's hypothesized that this higher diversity may make it more sensitive to change from salinity stress.

XA-Y$_{\#\#}$-Z

X indicates the Species discussed.
(L = *L.* x *intermedia*, B = *O. basilicum*, T = *T. vulgaris*)
A indicates which of 2 pots in a treatment and species combination are being discussed.
(ex. LA-C50 and LB-C50 are the same treatment & species, but different pots)
Y indicates if the treatment applied *B. subtilis* or not.
(C = not applied, B = applied)
indicates the salinity, in mM, of the water treatment.
(ex. "-C50" indicates the water's salinity was 50mM)
Z indicates which stems are being discussed.
(Represented by roman numerals; only used for height & leaf measurements)

Figure 1. This naming system was used to distinguish the pots and their extracts from each other. It was not used in prior literature.

Methods

This study spanned from May 11th to October 29th, 2024, from setting up the location for growing the plants to the last analysis test conducted. A dating system, in which days since the administration of the first saline water treatment occurred, was used to monitor the workflow of the experiment. Thus, "Day 0" hereafter refers to May 19th, 2024, with subsequent numbers counting the days after this timestamp.

A naming system was used for each experimental unit, as described in Figure 1, to keep track of the treatments at all stages of research.

Plant Growth and Treatment Administration

All three plant species were obtained from a local plant nursery in Oceanside, New York. The three species of plants were divided into 36 pots, 12 for each species. The pots were arranged in 3x2 rectangles of 6 each, with each rectangle corresponding to a different treatment combination. Figure 2 demonstrates the layout in a visual diagram.

The greenhouse was monitored for 6-7 hours every seven days, from Day -7 until Day 42, to measure the stem height and leaf quantity of each pot, as well as any observed wilting.

The salinity and *B. subtilis* treatments were administered in 150 ml of water. Three salinity concentrations – 0 mM, 50 mM, and 100 mM of sodium - were

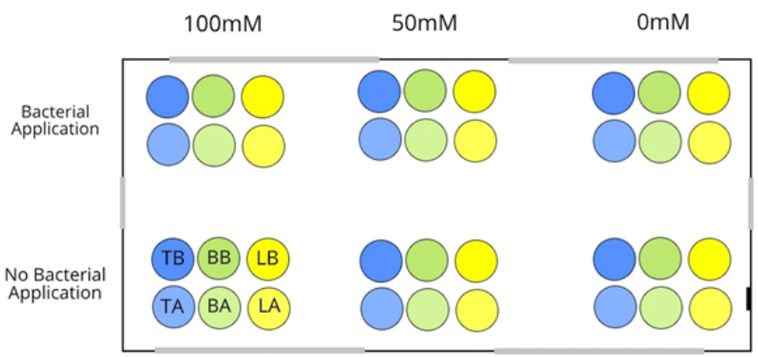


Figure 2. Layout of the greenhouse in which the plants were grown. The gray rectangles on the sides represent the openable flaps used for the greenhouse's four doors and two windows. The black rectangle in the lower right corner represents the location of the thermometer used in this study, held by a hook-and-loop fastener.

used, distributed as shown in Figure 2. For the *B. subtilis* treatments, 0.5g of *B. subtilis* powder was added per half a liter of water, following packaging instructions; 3g was added to each relevant pot initially (Awan & Shoaib, 2019). The bacterial application occurred biweekly, while the saline application occurred weekly, per the recommendations of similar *B. subtilis* powders.[1]

Figure 3. A demonstration of how the TA-C_0 extract was prepared.

Extract Preparation

On the final day of the growing period (Day 42), the plants were transferred to an indoor unit, harvested, and assessed in multiple phases. The soil containing residues of *B. subtilis* was stored in a large sealable bag. The stems of the plants were carefully cut from the roots, and the total biomass (roots, stems, and leaves) was measured for each pot. The total shoot mass was then calculated for all 36 pots, and except for *T. vulgaris*, the final weights of each stem were measured.

Once a pot's biomass data was obtained, 2 g of biomass from each pot was separated following 9 large cuts to each pot's shoot biomass. Extracts were prepared using 78.6 g (approximately 91.6 ml)[2] of a 70% Isopropyl solvent distributed to 36 jars, using the proportions of 1g of plant biomass per 50 ml of solvent established by Abbed et al (2019). Each jar was labeled with a pot's name; each corresponding pot's 2 g of shoot biomass was then transferred into its designated jar. Extracts were then sealed with a lid, shaken thrice, gyrated for 3 seconds, and left to sit for 7 days. Extract samples of 10 ml each were then taken and placed into three mugs simultaneously in a pot of hot water for 60 minutes; when the water reached 82.6℃ (the boiling point of Isopropyl), heat was applied in cycles of 15-25 seconds every 60 seconds, in order to keep the extract temperature between 80-90℃ and the pot's water between 85-100℃. Once these extracts were sufficiently reduced, they were recorded in a visible light spectrophotometer (calibrated under the 70% isopropyl solvent as a baseline) to obtain their absorbance spectrum profiles.

Repellency Tests

Supplies for a harborage test were ordered after reviewing prior methods for pest repellency tests (Krüger, Knobelspiess, & Schmolz, 2017; Krüger, Schmolz, & Vander Pan, 2021), and arrived on Day 78. Via a similar assessment on Day 80, *H. convergens* was chosen for this experiment, due to its relevance for IPM systems as a natural predator and minimal risk of invasion.

Two separate repellency tests were conducted for *H. convergens*, being the aforementioned harborage method (Day 115-116), and single-dose individual acute toxicity (IAT) tests (Days 116-117). The harborage tests had six groups of *H. convergens* species transferred via a funnel into a crystallizing dish, giving each trial group five minutes to adapt to the container. Photos were taken every five minutes, with observed behaviors and relative locations documented for each group. Each group used half of the extracts prepared for one of the three Lamiaceae species in this experiment; transitions between trial groups involved cleaning the dish with a sterile wipe, the application

1 *Bacillus subtilis* Powder Fertilizer 900g – Organic Soil Booster, Enhances Nutrient Uptake & Root Development. (2024).

2 At the time of experimentation, it was assumed that the density of the 70% isopropyl solvent used was 78.6g per 100ml of isopropyl, so 78.6g of such was used per extract. Post-experimentation, it was found that the assumed density was of isopropyl itself; the bulk product purchased for this experiment typically has a density closer to 85.8g per 100ml instead.

of beeswax to seven filter paper discs, labeling and adhesion of said discs, and administration of the ladybugs, with the dish then sealed using a metal sifter and six rubber bands. Overall, each test had nine locations to plot location points from, being the six treatment discs, the wall, the ceiling, and the center of the dish.

For the IAT tests, three ladybugs were moved from their satchel via tweezers, and placed into 59 ml portion cups. From there, 1 ml of an extract was administered into each cup, with a stopwatch recording the time of death for each ladybug relative to when the extracts were first applied. Each treatment and species combination thus had six experimental unit data points for acute toxicity. To compare the extracts' lethality time, four different control substances were also tested; these controls were 1 ml of reverse-osmosis filtered water, 70% isopropyl solvent, a sample field pesticide (0.16% λ-cyhalothrin), and the test extract made for the boiling procedure.

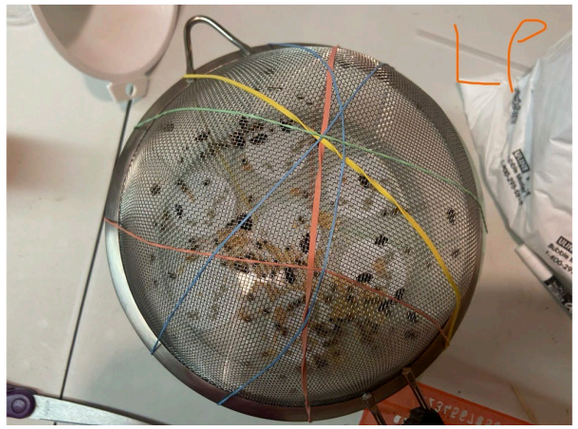


Figure 4. Aerial view of the harborage method set-up, during the first of two trials for L. x intermedia.

Figure 5. The state of all 36 pots on Day 42, prior to harvesting. From left to right: *T. vulgaris*, *O. basilicum*, *L x intermedia*. From closest to farthest row: C_0, C_{50}, C_{100}, B_0, B_{50}, B_{100}.

Results

Plant Growth Characteristics

The 50-day growing period used in this study went as planned, with no major disruptions that hindered the project's main goals. The greenhouse used in this study, and the external variation in weather around it, were not heavily monitored or controlled. This ensured that all non-experimental variables remained realistic; except for Days 33 & 34, CO_2 levels, temperature, and sunlight reflected those observed in the experiment's environment (Long Island, New York).

Stem Height & Leaf Count

Noticeable height increases were seen between the first three watering sessions, which leveled off from Day 14 onwards. The overall ranking of each treatment combination did not change substantially; the treatment with the largest plants at Day -7, tended to have the largest at Day 42 (Figure 6a-6b). In both species, C_{100} and C_0 grew the most, followed by the three *B. subtilis* treatments, then followed by C_{50}. Of these, only the placement of C_{50} involved a change in ranking over time.

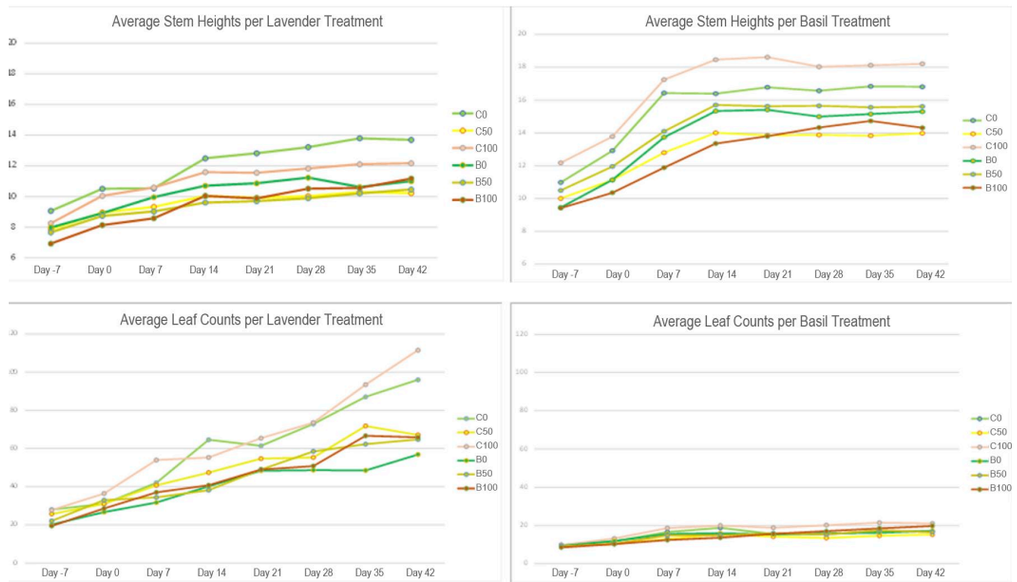


Figure 6a-6d. The consistent scales for these line graphs emphasize the difference between *O. basilicum* and *L x intermedia*, regarding their average stem heights and leaf abundance.

Likely due to the rapid onset of wilting sometime after Day 14, and the timespan in which the growing period occurred, none of the plants developed flower buds. Any stems that did not wilt still developed leaves consistently (Figure 6c-6d). The differences between these rankings became more pronounced over time, suggesting that any treatment effects gradually amplified.

An ANCOVA was performed to account for the factors of salinity, *B. subtilis* application, and species, with regard to the covariates of time and leaf count. The results showed a significance of $p < 0.001$ for all factors individually, and of $p < 0.05$ for species vs salinity or *B. subtilis* application. However, all comparisons made had trivial effect size ($\eta < 0.1$), except for the factor of species. This may be due to the length of the growing period of this study, or the onset of wilting among the plants.

Games-Howell

Games-Howell Post Hoc Comparisons - Species

Comparison	Mean Difference	SE	t	df	p_{tukey}
Lavandula x intermedis - Ocimum basilicum	−4.196	0.223	−18.858	879.642	< .001

Note. Results based on uncorrected means.

Games-Howell Post Hoc Comparisons - Salinity (mM)

Comparison	Mean Difference	SE	t	df	p_{tukey}
0 - 50	1.152	0.299	3.850	703.951	< .001
0 - 100	0.164	0.324	0.506	685.873	0.868
50 - 100	−0.988	0.318	−3.104	675.556	0.006

Note. Results based on uncorrected means.

Games-Howell Post Hoc Comparisons - Used B. subtilis?

Comparison	Mean Difference	SE	t	df	p_{tukey}
No - Yes	1.455	0.257	5.660	977.974	< .001

Note. Results based on uncorrected means.

Figure 7. The Games-Howell post hoc pictured was performed on the variables of species, salinity, and *B. subtilis* application. The comparisons between the same treatment across different species is avoided here, in order to assess the impacts of the two other variables.

Simple Main Effects - Salinity (mM) ▼

Level of Species	Level of Day #	Sum of Squares	df	Mean Square	F	p
Lavandula x intermedis	-7	9.497	2	4.749	0.477	0.621
	0	7.308	2	3.654	0.367	0.693
	7	11.463	2	5.732	0.576	0.562
	14	34.067	2	17.034	1.712	0.181
	21	45.036	2	22.518	2.263	0.105
	28	54.362	2	27.181	2.732	0.066
	35	43.741	2	21.870	2.198	0.112
	42	48.445	2	24.222	2.434	0.088
Ocimum basilicum	-7	4.377	2	2.189	0.220	0.803
	0	3.407	2	1.703	0.171	0.843
	7	31.784	2	15.892	1.597	0.203
	14	16.944	2	8.472	0.851	0.427
	21	33.559	2	16.779	1.686	0.186
	28	27.386	2	13.693	1.376	0.253
	35	38.827	2	19.413	1.951	0.143
	42	30.771	2	15.385	1.546	0.214

Simple Main Effects - Used B. subtilis? ▼

Level of Species	Level of Day #	Sum of Squares	df	Mean Square	F	p
Lavandula x intermedis	-7	11.345	1	11.345	1.140	0.286
	0	22.486	1	22.486	2.260	0.133
	7	14.044	1	14.044	1.411	0.235
	14	25.399	1	25.399	2.552	0.110
	21	27.262	1	27.262	2.740	0.098
	28	21.602	1	21.602	2.171	0.141
	35	40.881	1	40.881	4.108	0.043
	42	22.025	1	22.025	2.213	0.137
Ocimum basilicum	-7	25.236	1	25.236	2.536	0.112
	0	32.603	1	32.603	3.276	0.071
	7	78.422	1	78.422	7.881	0.005
	14	29.536	1	29.536	2.968	0.085
	21	30.567	1	30.567	3.072	0.080
	28	19.108	1	19.108	1.920	0.166
	35	17.518	1	17.518	1.761	0.185
	42	22.489	1	22.489	2.260	0.133

Figure 8a & 8b. The results of a Simple Main Effects test on the ANCOVA data are pictured. The influence of salinity and *B. subtilis* application on stem height and leaf count appeared to have the most influence about midway through the growing period, but were of questionable significance.

As expected, the equality of variances test was violated, as this experiment involved height and leaf measurements of different species. A Games-Howell post-hoc test revealed that on average, *O. basilicum* was about 4.196cm larger than *L. x intermedia* with significance of $p < 0.001$ (Figure 7). Regarding salinity, a non-monotonic relationship was observed; no significant difference was observed between the 0 mM and 100 mM treatments when separated by species, as both were significantly taller than the 50 mM treatments ($p < 0.001$, $p < 0.01$, respectively). This was also the case when post-hoc tests were run on *B. subtilis* application, with respect to salinity and time; significance of $p < 0.05$ was reached for 0 mM and 100 mM, but not for 50 mM.

The post hoc test for *B. subtilis* application revealed that the untreated plants had a significantly ($p < 0.001$) higher average stem height than the treated plants. This does not mean that the treatment inhibited plant growth, however; the rankings for each plant treatment roughly stayed the same over time, suggesting this was influenced by the initial plants selected. Of all six treatments, the B_{100} group experienced the least observable wilt.

A separate ANCOVA test, with time as a factor instead of a covariate, was performed in order to assess the significance of salinity and *B. subtilis* application over time (Figure 8a, 8b). The results were as follows: for salinity, increasing significance and variability were observed within the two species over time, leveling off after Day 14 at a p-value above the significance threshold ($p > 0.05$). For *B. subtilis* application, the p-value initially decreased from the start, but later increased, and hovered at a nonsignificant ($p > 0.05$) p-value during the later time periods. It became significant on a few days, but the oscillations suggest this was likely due to observational errors.

Biomass

Regarding the influences on the plants' shoot mass in this study, the variables of salinity and *B. subtilis* application (and comparisons thereof) all had significance of $p < 0.05$; salinity had a small effect size ($\omega \leq 0.06$). The trends across salinity in regard to *B. subtilis* application were unprecedented (Figure 9). *L.* x *intermedia* initially grew less under *B. subtilis* treatment between 50 mM and 0 mM, but had significant growth advantage at 100 mM ($p < 0.01$); *O. basilicum* had an overall increase in growth with time, which again was only significant ($p < 0.001$) at 100 mM, where the saline group fared better; and *T. vulgaris* had no significant difference in growth, displaying an oscillating pattern when *B. subtilis* was compared, with large standard deviations for each point. This study did not account for the change in biomass over time , in order to avoid prematurely terminating any of the plants.

Extract Preparation

The 10ml samples had an average corresponding mass of 8.5019 grams (SD = 0.0731), giving a density of about 0.8502 g/ml. This is close to the density of 70% isopropyl sold as a supply[3], so it is not unprecedented. However, once all extracts were heated together, there was substantial variation in the volume remaining (Figure 10), with an average final mass of 3.62486g (57.36% reduction; SD = 0.94912). As no

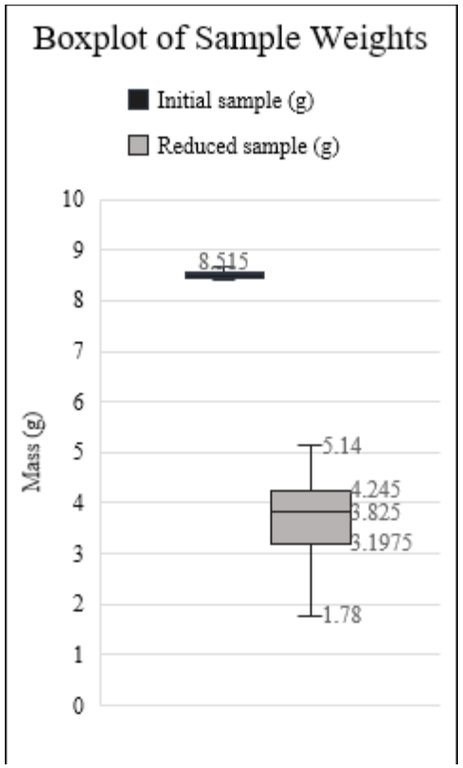


Figure 10. The median value of the initial sample, and the five quartile markers for the reduced sample, are labeled above.

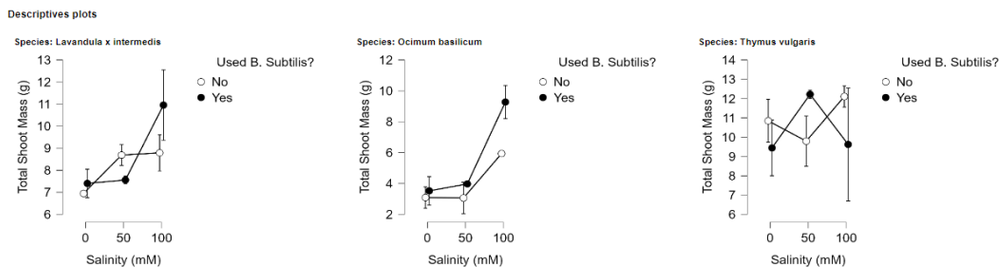


Figure 9 The shift seen under 100mM of salinity for *O. basilicum* may have been influenced by two stems under the BA-B_{100} treatment lasting significantly longer without wilting. Overall, however, this is a highly unprecedented finding, as most prior literature suggests that salinity stress poses a detriment to overall plant growth.

3 Isopropyl Alcohol 70/30 (Ipa 70%) 1 Gallon. (2023). Retrieved November 6, 2024, from Lab Pro Inc

ANCOVA - Absorbance

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η^2_p
Used B. Subtilis?	76.410	1	76.410	694.661	< .001	0.026	0.083
Salinity (mM) ✳ Used B. Subtilis?	57.621	2	28.811	261.926	< .001	0.020	0.064
Wavelength (nm)	1611.568	1	1611.568	14651.235	< .001	0.551	0.655
Salinity (mM)	219.310	2	109.655	996.905	< .001	0.075	0.205
Sample Volume (ml)	22.457	1	22.457	204.164	< .001	0.008	0.026
Reduction from 10ml	14.397	1	14.397	130.885	< .001	0.005	0.017
Unit Shoot Biomass (g)	10.191	1	10.191	92.652	< .001	0.003	0.012
Unit Shoot Biomass (% of total biomass)	64.276	1	64.276	584.349	< .001	0.022	0.070
Residuals	848.834	7717	0.110				

Note. Type III Sum of Squares

Descriptives ▼

Descriptives - Absorbance

Salinity (mM)	Used B. Subtilis?	N	Mean	SD	SE	Coefficient of Variation
0	No	1288	0.919	0.852	0.024	0.928
	Yes	1288	0.751	0.682	0.019	0.908
100	No	1288	1.171	0.907	0.025	0.775
	Yes	1288	0.572	0.595	0.017	1.040
50	No	1288	0.645	0.606	0.017	0.939
	Yes	1288	0.882	0.580	0.016	0.658

Figure 11. The ANCOVA test performed following the spectrometry analysis is pictured.

spillage was seen during the boiling process or transfer from the pot, this was likely due to uneven heating among the mugs used. The deviances in final volume were logged as a potential variable for the IAT test analyses.

Spectrometry

The spectrometry graphs for all 36 extracts showed an overall pattern with high absorbance in the purple band (380-450 nm) of the graph, low absorbance in the band for green and yellow (500-630 nm), and another peak of high absorbance in the visible red band (630-700 nm). An ANCOVA test was performed, assessing the factors of salinity, *B. subtilis* application, sample volume, and the biomass used for each (Figure 11). Most factors had a significant effect on the absorbance value, but only the light's wavelength had a strong effect size; all other factors' effect sizes were trivial ($\eta < 0.1$). This indicated that all extracts followed a similar absorbance spectrum pattern (hue), but that said pattern shifted on the y-axis (tone) depending on each extracts' individual factors.

Repellency Tests

The *H. convergens* samples were ordered on Day 78, but due to significant delays in shipment, the harborage and IAT tests occurred on Days 115-117. The specimens were unharmed and survived all three days in the satchel they were delivered in.

Sublethal Exposure: Harborage Tests

The way in which the counts were performed for the harborage tests meant that the observed total may have changed over time; to account for this, the proportions ((count*100)/total) of the observed total ladybugs in one area were used as the dependent variable. The covariant of time, and the factors of species, salinity, and *B. subtilis*, were used for a linear regression model. The results showed a weak correlation ($r = 0.335$) for the model produced, which accounted for 8.7% of the variance in the percentage of ladybugs in a given area over time (Figure 12a). The model is significantly better ($p < .001$) than the null hypothesis; however, only the factor of *B. subtilis* application held

Model Summary - % of Ladybugs in Area

Model	R	R^2	Adjusted R^2	RMSE	Durbin-Watson Autocorrelation	Durbin-Watson Statistic	Durbin-Watson p
H_0	0.000	0.000	0.000	6.317	0.057	1.883	0.387
H_1	0.335	0.112	0.087	6.038	−0.049	2.096	0.669

ANOVA

Model		Sum of Squares	df	Mean Square	F	p
H_1	Regression	961.027	6	160.171	4.394	< .001
	Residual	7619.204	209	36.456		
	Total	8580.231	215			

Note. The intercept model is omitted, as no meaningful information can be shown.

ANCOVA - % of Ladybugs in Area

Cases	Sum of Squares	df	Mean Square	F	p	η^2	ω^2
Species	71.933	2	35.966	1.854	0.159	0.008	0.004
Salinity (mM)	113.897	2	56.948	2.936	0.055	0.013	0.009
Used B. Subtilis?	685.145	1	685.145	35.317	< .001	0.080	0.077
Species ✳ Used B. Subtilis?	406.365	2	203.182	10.473	< .001	0.047	0.043
Salinity (mM) ✳ Used B. Subtilis?	932.429	2	466.215	24.032	< .001	0.109	0.104
Species ✳ Salinity (mM) ✳ Used B. Subtilis?	622.083	4	155.521	8.017	< .001	0.073	0.063
Timestamp (minutes)	90.052	1	90.052	4.642	0.032	0.010	0.008
Species ✳ Salinity (mM)	1836.590	4	459.148	23.668	< .001	0.214	0.205
Residuals	3821.737	197	19.400				

Note. Type III Sum of Squares

Figure 12a & 12b. The results for the linear regression model, and the ANCOVA test, are pictured. While the test for autocorrelation failed here, indicating no pattern present among the residuals, it is unclear if the model used here is the most appropriate for the chosen harborage method.

significance ($p < .001$) in explaining any changes in position over time (Figure 12b).

To investigate the impacts of each factor in further detail, an ANCOVA was performed, with the same layout of factors and covariates (Figure 12b). This test showed that while the factors of species and salinity had insignificant individual effects, all three tested factors had significance ($p < 0.001$) when tested between each other, pairwise or aggregated. Species and *B. subtilis* had trivial effect sizes ($\eta < 0.1$) when compared together; salinity had a small to moderate effect size ($0.1 < \eta < 0.25$), in comparison to the other two factors. Notably, the factor of time only had a trivial effect ($\eta < 0.1$) on the position proportions over time, though this may be due to the way in which the observations were taken.

As the equality of variances test was violated ($p < 0.001$), a Games-Howell post hoc test was performed on the combined influence of all three factors (Figure 13a). The insignificance of the factors of species and salinity in influencing the proportions over time was confirmed. It was shown that the 50 mM treatment had the lowest proportion over all six groups; based on the correlation plots, this parabolic trend seemed to be most pronounced among the *O. basilicum* extracts. Application of *B. subtilis* led to significantly ($p < 0.001$) better repellency, with different patterns present for each species of plant tested (Figure 13b).

A critical finding of these harborage tests was a significant decrease in the quantity of ladybugs attempting to climb the walls of the crystalizing dish. The exact observations and proportions over time suggest that this was first due to congregation towards the ceiling of the container, and then from the 5-minute

Games-Howell ▼

Games-Howell Post Hoc Comparisons - Species

Comparison	Mean Difference	SE	t	df	P_{tukey}
Lavandula x intermedis - Ocimum basilicum	−1.393	1.126	−1.238	140.956	0.433
Lavandula x intermedis - Thymus vulgaris	−0.488	0.988	−0.494	137.258	0.874
Ocimum basilicum - Thymus vulgaris	0.905	1.042	0.868	132.509	0.661

Note. Results based on uncorrected means.

Games-Howell Post Hoc Comparisons - Salinity (mM) ▼

Comparison	Mean Difference	SE	t	df	P_{tukey}
0 - 50	0.620	1.068	0.580	141.953	0.831
0 - 100	−1.134	1.037	−1.094	141.746	0.520
50 - 100	−1.754	1.047	−1.675	141.483	0.218

Note. Results based on uncorrected means.

Games-Howell Post Hoc Comparisons - Used B. Subtilis?

Comparison	Mean Difference	SE	t	df	P_{tukey}
No - Yes	3.562	0.827	4.309	210.005	< .001***

*** p < .001

Note. Results based on uncorrected means.

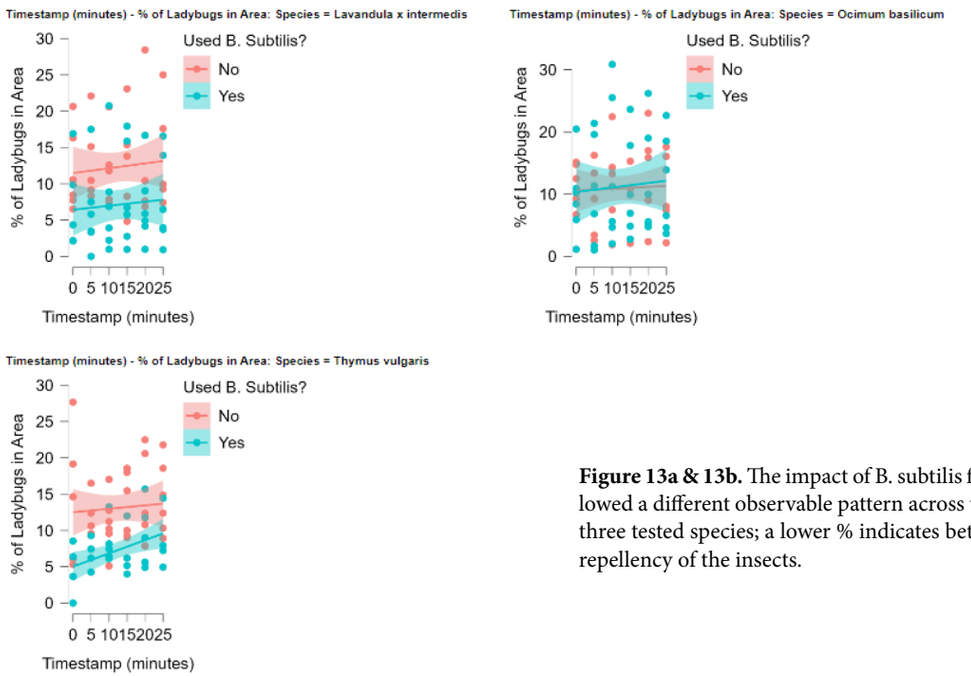


Figure 13a & 13b. The impact of B. subtilis followed a different observable pattern across the three tested species; a lower % indicates better repellency of the insects.

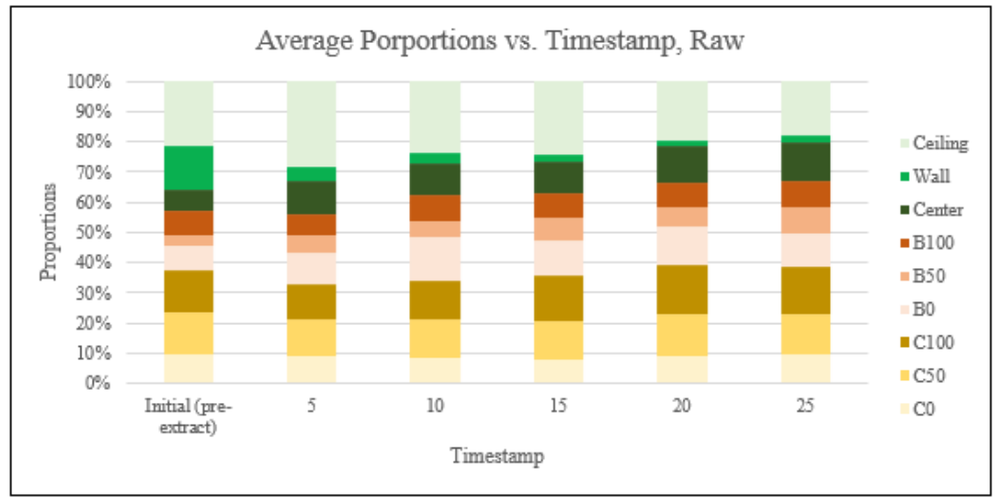


Figure 14. A stacked bar chart was made on the average of all six harborage trials. The recording for the "Initial" stage occurred about 15 seconds before each of the six extracts in a group were administered.

timestamp onwards, due to difficulty staying on the mesh ceiling (Figure 14). This suggests that if these extracts were applied to plants directly, exposed insects may have a harder time remaining latched on to the plants' surfaces under the concentration used for this experiment.

Lethal Exposure: IAT Tests

The IAT tests calculated how long it took *H. convergens* to cease all movement in a cross-legged position for more than 3 seconds. While some experimental units did resume movement following this event, their recorded time of death was only overturned if they were able to flip themselves over, to distinguish alive specimens from simple reflexive movements. The volume of the portion cups used was 59 ml, and with 1 ml (0.85019 g) of extract being added to each, resulted in a concentration of ~0.01441 g/cm^3. The beetles in this experiment were in direct contact with the fluid if they were not climbing the walls of each cup.

An ANCOVA test was performed for the time of death of all tested ladybugs over the conditions of species, *B. subtilis* application, and salinity (mM). The JASP analyses showed that for *T. vulgaris,* application of *B. subtilis* resulted in a less variable, overall faster time of death, while for *O. basilicum* and *L x intermedia*, the opposite trend was observed (Figure 15). The difference between the two groups across each plant species noticeably increased, on average, as the salinity concentration increased ($p < .05$, excluding *L* x *intermedia*). The equal variances test was not violated ($p = 0.166$) for this part of the experiment.

A correlation test was performed for the final shoot weight of the plants, the sample's final volume, and the time of death for all tested ladybugs, in order to support the initial hypothesis that more productive growth would lead to a weaker deterrence (Figure 16). A correlation of $r = 0.205$ was found, indicating a weak positive relationship between the time of death and the plant's shoot biomass, which dropped to $r = 0.124$ when salinity was a partial out. Similarly, the correlation between the sample volume and the time of death had a correlation of $r = -0.122$ when salinity was reduced to a partial out, with a similar patten when the data was split between all three tests species. Along with the data suggesting a trivial influence of biomass on extract coloration, this suggests that the relationship between plant growth and its extracts' efficacy as a botanical was much weaker than previously believed.

ANCOVA - Time of Death (seconds)

Cases	Sum of Squares	df	Mean Square	F	p	η^2	η_p^2	ω^2
Species	5675.345	2	2837.672	0.134	0.874	0.002	0.003	0.000
Used B. Subtilis?	20610.262	1	20610.262	0.975	0.326	0.008	0.010	0.000
Salinity (mM)	188643.000	1	188643.000	8.928	0.004	0.074	0.082	0.065
Species ✻ Used B. Subtilis?	209200.168	2	104600.084	4.951	0.009	0.082	0.090	0.065
Residuals	$2.113\times10^{+6}$	100	21128.430					

Note. Type III Sum of Squares

Descriptives

Descriptives - Time of Death (seconds)

Species	Used B. Subtilis?	N	Mean	SD	SE	Coefficient of Variation
Lavandula x intermedis	No	17	206.128	102.790	24.930	0.499
	Yes	18	292.317	198.167	46.708	0.678
Ocimum basilicum	No	18	188.762	88.201	20.789	0.467
	Yes	18	285.611	221.484	52.204	0.775
Thymus vulgaris	No	18	282.155	156.791	36.956	0.556
	Yes	18	185.447	68.913	16.243	0.372

Figure 15. The influence of B. subtilis application on the time of death was negative for T. vulgaris, but positive for L. x intermedia and O. basilicum.

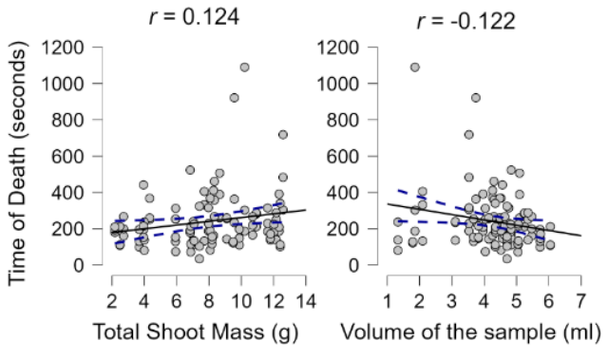
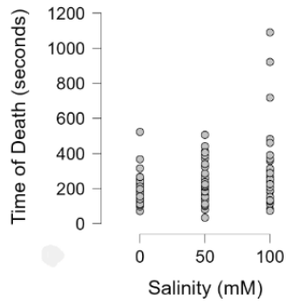


Figure 16. These correlation plots had the salinity of the treatment as a partial out, and lacked significance ($p > .05$).

Finally, salinity stress was shown to have a significant ($p < 0.05$) relationship with time of death (Figure 17); however, contrary to the hypothesis, it was found to be related to a slower time of death ($r = 0.223$). This is highly unprecedented, as prior literature has consistently found associations between salinity stress and increased production of the monoterpenoids relevant for pest deterrence, which suggested a relationship with a faster time of death. Future replications of this experiment will be needed, in order to verify whether or not salinity truly is correlated with a slower time of death.

Discussion

Hippodamia convergens was chosen as the main species for this study in order to investigate the impacts of botanicals towards a likely non-target species. Besides being a common candidate for "natural predator" of common pests (Bjørnson, 2008), some research indicates that *H. convergens* is attracted to the volatile compounds of the Lamiaceae family (Al-Doghairi & Cranshaw, 1999). Prior studies investigating field exposure of the Coccinellidae family to commercial pesticides typically assess death several days post-exposure (Fernandez, n.d.; Rodrigues et al., 2020); future research investigating lethal and sublethal effects of botanicals on *H. convergens* will most likely use field studies, investigating the impacts after such lengths of time.



Pearson's Partial Correlations

Variable		Time of Death (seconds)	Salinity (mM)
1. Time of Death (seconds)	n	—	
	Pearson's r	—	
	p-value	—	
2. Salinity (mM)	n	107	—
	Pearson's r	0.223*	—
	p-value	0.022	—

Note. Conditioned on variables: Volume of the sample (ml).
* p < .05, ** p < .01, *** p < .001

Figure 17. A correlation test between salinity and time of death, with volume as a partial out. Although high outliers are present under the 100mM treatments, removing them does not make the correlation negative.

Of note is how the controls for water and isopropyl - the two solvents involved with the tested extracts - resulted in significantly longer and shorter times of death, respectively, compared to any of the treatment groups, while the pesticide used had highly similar measurements (Figure 18). The concentration of λ-cyhalothrin in the pesticide was 0.16% of its total mass; the extracts, for comparison, had concentrations varying from 0.42% (BB-C_0) to 4.01% (LB-B_{50}), depending on the plant species used and the samples' reduced volumes. Future research is needed with even closer approximations to pesticides' concentrations, in order to verify that Lamiaceae botanicals result in a similar lethality of *H. convergens* compared to λ-cyhalothrin.

It is necessary to clarify that while some studies have found cases of *H. convergens* populations developing resistance to λ-cyhalothrin (Rodrigues et al., 2020; Barbosa et al., 2016), the species overall

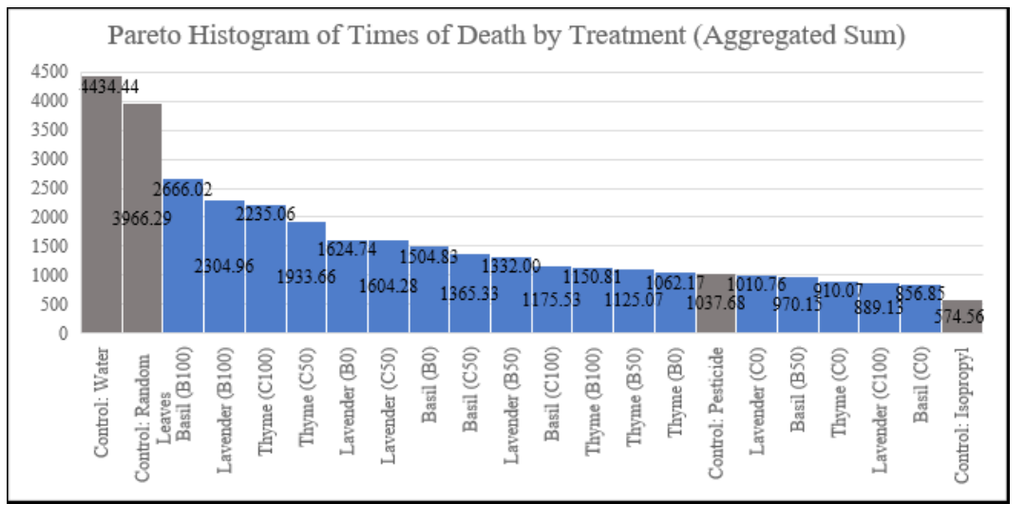


Figure 18. This Pareto Histogram displays the sum of the times of death (in seconds) under each treatment type. Please note that half of the "Control: Water" experimental units survived the test, as did one from the LA-C_{100} group.

remains highly vulnerable to the chemical, with findings indicating that "local extinction" (removal of all members from a specific area) was possible for adults under λ-cyhalothrin exposure (Fernandez, n.d.; Torres et al., 2015). Additionally, as stressed by Barbosa et al. (2016), the emergence of λ-cyhalothrin resistance underscores the widespread usage of commercial pesticides, which botanicals are largely being designed to replace. The findings in this study, showing similar death times when *H. convergens* is exposed to Lamiaceae botanicals and λ-cyhalothrin at a similar concentration, raises concerns that the issue of non-target species being impacted may not be addressed upon application of botanicals in place of pesticides. This, again, raises the need for future research to replicate the IAT tests performed, to determine how replicable this study's results may be.

Conclusion and Future Directions

This research found highly unprecedented relationships between salinity stress, *B. subtilis* application, and the growth of the plants. The impact that the extracts had on *H. convergens* were highly varied, but overall, a decrease in vertical surface adhesion was observed across the harborage and IAT tests. For the harborage tests, the growing conditions had different relationships depending on the species used in the botanical, with only *B. subtilis* holding significance ($p < 0.001$). For the IAT tests, the resulting biomass of the plants and volumes of the botanicals did not have a significant relationship with the time of death of *H. convergens*. Salinity, in contrast to the hypothesis, had a significant ($p < 0.05$), albeit trivial ($\eta < 0.1$), relationship with a slower time of death; *B. subtilis* mitigation had a similar positive relationship ($p < 0.01$, $\eta < 0.1$), except for *T. vulgaris*, which had a negative relationship. It is plausible that a larger % of isopropyl remaining post-boiling in this study, compared to the 0.16% concentration of the λ-cyhalothrin pesticide used, may have influenced these findings; despite this difference, λ-cyhalothrin did not lead to a significantly faster time of death compared to all tested botanicals, when a sample of Isopropyl solvent alone did lead to such. Future research should continue to investigate proposed botanicals with the environmental conditions highlighted in this study in mind, as well as how the reduced volume reached may impact its lethality, to further clarify how the application of Lamiaceae-derived botanicals in lieu of pesticides will influence the health and abundance of non-target organisms.

Further Reading

For original appendices and additional data, please go to this page:

https://www.dropbox.com/scl/fi/oigqws7wg6od1f-pe19hn8/Tritschler-K-2025-Paper-Abstracts.pdf?rlkey=njhpl6a5ldupi08ylmzaycbzb&e=1&st=bt0gmg74&dl=0

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Attitudes Towards AI in Healthcare Among High School Students and Healthcare Professionals in Toronto

Sushrut Lamsal

Abstract: Discussions about artificial intelligence (AI) in healthcare are becoming increasingly prevalent, given the growing influence and developments of AI. Furthermore, the scale of AI's influence renders it important to understand the views of both younger and older generations, as they represent the future and current attitudes of recipients and leaders of healthcare. This study explores the attitudes towards AI in healthcare among high school students – an underrepresented demographic in the literature – and healthcare professionals in Toronto, Ontario. Using an explanatory sequential mixed-methods approach, a survey was conducted using a 5-point Likert scale to assess participants' (n = 68) overall views and establish broad trends across four domains: confidence, concerns, ethical and regulatory concerns, and practical applications. Optional, structured interviews were then conducted with willing participants (n = 15) to provide context to survey responses. This study benefits policymakers and governments in ensuring that AI development in healthcare aligns with public expectations.

Keywords: artificial intelligence, healthcare, public attitudes, high school students, healthcare professionals, AI implementation

Introduction

In recent years, artificial intelligence (AI) has become a major driver of progress in the healthcare sector, having improved the efficiency and effectiveness of various repetitive tasks and has started to be implemented in clinical settings (MacDonald et al., 2022). For instance, AI systems have been used in telemedicine to automate routine work such as appointment booking and in surgical settings to improve diagnosis capabilities (Lee & Yoon, 2021). However, the general public's concerns about AI have slowed down its full integration into healthcare (Roppelt et al., 2024). For instance, Witkowski et al. (2024), after surveying 600 adults in Florida, found feelings of distrust towards AI and nervousness about potential lower-quality physician-patient relationships. Yet, public opinion is not one-sided, as evidenced by Wu et al. (2023), who found AI's perceived ability to reduce inefficiencies was a point of optimism for many. This range of opinions is further compounded by Gao et al. (2020), who analyzed 2315 social media posts about AI in healthcare and found a mix of both optimism and apprehension. Together, these results highlight the conflicted nature of opinions held on AI in healthcare and indicate a need for a clearer understanding of the public's attitudes.

Implementing an initiative as large as AI in healthcare hinges on public trust and confidence; therefore, understanding these perspectives is imperative. This notion is further supported by the fact that public sentiment is already a well-established predictor of technology adoption (Marikyan et al., 2023). However, current research predominantly focuses on the abilities of the technology and the potential benefits and inconveniences, causing a gap in focus. Additionally, the existing literature focusing on attitudes mainly samples healthcare professionals and older adults, leaving the perspectives of younger populations, particularly high school students, largely unexplored. This gap is significant as this population represents the people who will live in a society where AI is more readily used in healthcare. There are also very few studies looking at Canada, despite it being a global leader in both AI and healthcare. Furthermore, the mere nature of AI's constant development necessitates up-to-date research.

To address this gap, my study employed a Likert scale survey to quantitatively measure the attitudes of high school students and healthcare professionals to establish initial trends. An optional interview component with open-ended questions followed the survey and allowed participants to provide more context to their survey answers. Thematic analyses of these interviews further elucidate the recurring points of confidence and concern. Effectively, this study aimed to compare the views of the under-researched demographic of high school students with those of healthcare workers in Toronto, Canada. This leads to the research question: do the attitudes towards AI in healthcare differ between high school students and healthcare professionals, and if so, how? The findings of this study could inform healthcare policymakers and governments, providing insights to ensure future AI technologies are introduced in ways that align with the expectations and needs of current and future generations.

Literature Review

Assessing public attitudes entails subjectivity. Individual views towards wide-reaching topics are heavily influenced by experiences that are unique from person to person. Consequently, exploring the attitudes held by the public towards healthcare and AI, two large and actively growing aspects of society, necessitates a thorough understanding of existing data.

The Existing Research

The existing literature on this topic reveals both hopeful and cautious perspectives from the general public. Collectively, the conclusions and findings of the literature hint toward a broad range of public opinions, which sometimes vary when looking at specific demographics or aspects of AI in healthcare.

Concerns

Despite the limited presence of AI in healthcare systems so far, people have still expressed concerns about the possibility of AI being introduced more prominently in healthcare. Witkowski et al. (2024) surveyed 600 adults in Florida and found that many expressed distrust towards AI in healthcare, worrying that the quality of physician-patient interactions would deteriorate. This sentiment of distrust is also seen in online settings, as evidenced by the findings of Gao et al. (2020), which show that the majority of social media posts expressing concern towards AI in healthcare did so due to worries about unreliability and privacy issues.

However, these findings are challenged by Beets et al. (2023), who reviewed 11 publicly available surveys on AI in healthcare in the United States, finding that while most participants acknowledged AI's potential for healthcare, many struggled to explain its specific applications. This suggests that some opinions held by the public may be based on preconceived notions due to a lack of first-hand exposure to AI tools in healthcare (Abdullah & Fakieh, 2019). Esmaeilzadeh et al. (2021) substantiate this, finding in their experimental study that physical exposure to real-life AI healthcare systems had a considerable influence on participants' concerns about AI. Together, these results indicate that education to familiarize the public with AI tools could better inform their opinions, especially their concerns, on AI in healthcare.

Optimism

Optimism is not missing from the public opinion, however. Fast and Horvitz (2017), for instance, observed an increase in positive discussions about AI in healthcare since 2009, alongside a growing demand

for AI education, suggesting that there is a willingness and curiosity from the public to learn more about these technologies.

Additionally, Wu et al. (2023) note that both healthcare providers and patients view AI as a useful tool to reduce operational and logistical inefficiencies or improve waiting times. They also find that improved algorithms allow AI to be viewed in a positive light (Wu et al., 2023). This is supported by Fritsch et al. (2022), who analyzed patient perceptions in Germany and found that optimism toward AI in healthcare is usually grounded in the hope that it can improve access to healthcare resources for underserved populations and reduce overall costs for society as a whole. Lastly, Antes et al. (2021) point out that the public's openness to AI in healthcare is likely largely influenced by its ability to improve convenience and access, having discovered that trust in healthcare systems is strongly correlated with optimism.

Demographics

Certain studies have also looked to understand whether specific demographic markers shape attitudes towards AI in healthcare. Antes et al. (2021) and Fritsch et al. (2022), for example, did so with gender and age. Regarding gender, they found that females were more skeptical and less open than their male counterparts (Antes et al., 2021; Fritsch et al., 2022). Regarding age, they, alongside Rony et al. (2025), found that younger participants generally showed more openness and optimism towards AI usage, whereas older groups were more cautious (Antes et al., 2021; Fritsch et al., 2022).

Additionally, Laï et al. (2020) found, in their analysis of French healthcare professionals, that, while the benefit is acknowledged, healthcare professionals tend to voice their concerns on the ethical handling of health data and the effect on patient relationships. Furthermore, Teng et al. (2022), who looked at healthcare students across Canada, found that the "majority reported a positive outlook" (p. 14). The only available study on the high school population, however, deviates from these results, finding that high school students generally distrusted AI and were not that familiar with the technology (Parmar, 2024). That said, the results of this study ultimately contradict this conclusion.

The Gap

Current research reveals a spectrum of public opinion on AI in healthcare filled with conflicting opinions, particularly from older demographics, such as healthcare professionals, who exhibit both hope and skepticism. While many gaps exist in the literature on this topic, the most prominent one is the lack of focus on the younger population, who represent the next generation of healthcare decision-makers and recipients. Preliminary indications suggest that high school students may have a more optimistic view of AI in healthcare than healthcare professionals who often handle the ethical and practical implications of such technologies. However, further research is essential to clarify the range of opinions seen in the literature, which raises the question: do the attitudes towards AI in healthcare differ between high school students and healthcare professionals, and if so, how?

Methodology

This study employed an explanatory sequential mixed-method approach. This entailed collecting and analyzing initial quantitative data to identify trends, followed by qualitative data collection informed by the quantitative results to explain these trends in depth. The study was also approved by an ethics committee beforehand to ensure that the well-being of participants would not be compromised.

Quantitative Data Collection

The quantitative portion of this method involved administering a survey to all the participants. The survey was distributed using Google Forms and shared through in-school communication for the high school students and email for the healthcare professionals, with the help of an expert advisor and a school alumni network.

At the beginning of the survey, participants were given a form of consent and reminded of their right to withdraw and the anonymity of their data to protect their privacy. The survey then started with six demographic questions to gather background information

on age, gender, education level, and familiarity with AI. Following this, 13 Likert-scale questions were administered to assess the attitudes of AI, ranging from optimism, confidence, and concerns.

Qualitative Data Collection

Building on the quantitative results, the qualitative portion involved conducting semi-structured interviews with a subset of survey respondents who expressed interest in participating further.

The majority of these interviews were held through Google Meet, with the exception of a few in-person meetings that were recorded using Voice Memos. The purpose of these interviews was to explore the reasons behind participants' views by gaining context behind specific survey findings. The structure of the interviews and some of the questions used were based on previous studies conducted by Parmar (2024), Nair et al. (2024), and Laï et al. (2020).

Analysis

Once the interviews were conducted and recorded, the recordings were transcribed using Riverside AI's automatic transcribing software. The transcriptions were then separated into high school student and healthcare professional categories. Then, a thematic analysis was manually performed on each transcript. Each transcript was read twice over to identify recurring themes and ideas. Then, several quotes from the transcriptions were selected to accompany each theme. The collected themes and quotes were then distilled to only keep the prevalent ones (those with the most occurrences across the interviews). These themes were then compared with the quantitative data and collectively analyzed to find similarities, discrepancies, or unexpected results, which are elaborated upon in the discussion section of this paper.

Justification

Given the extensive nature of AI and healthcare in general, an explanatory sequential design was chosen to ensure a broad understanding of the ideas while still allowing for nuance in individual responses. The quantitative survey was intended to provide an overview of the trends in attitude by analyzing average Likert Scale values and the shape of the graphs, while the qualitative phase served to provide the context behind these trends. This combined approach enabled the study to accommodate a large number of responses, all while considering the subtle differences within participants' attitudes.

Findings

This study had 68 participants: 59 high school students and nine healthcare professionals. The survey quantitatively assessed the general attitude of the participants through four categories: confidence, concerns, ethical and regulatory considerations, and practical applications. Participants could select one out of five options ranging from strongly disagree to strongly agree, as per the 5-point Likert scale.

Following the survey, 15 interviews (eight high school students and seven healthcare professionals) were conducted, which gave qualitative insights into participants' attitudes. A thematic analysis was then conducted to find common themes.

Quantitative Findings (Survey Results)

This portion contains the Likert scale questions. For the analysis purposes, numerical values will be assigned to each of the Likert options: Strongly Disagree (1), Disagree (2), Neutral (3), Agree (4), Strongly Agree (5).

Demographic Questions

The survey began with demographic questions to identify high school students and healthcare professionals. Questions on gender and self-identification as either a high school student or a healthcare professional are excluded from the following figures, due to demographic imbalance and redundancy, respectively.

Initially, 61 participants reported that they were familiar with artificial intelligence, with seven responding neutrally or not familiar with artificial intelligence. None of the healthcare professionals reported neutral or below for familiarity. See Figure 1 for more details.

Fourteen participants reported that they were familiar (selected strongly agree/agree) with how artificial intelligence was used in healthcare, with eight being healthcare professionals and six being high school students. See Figure 2 for more details.

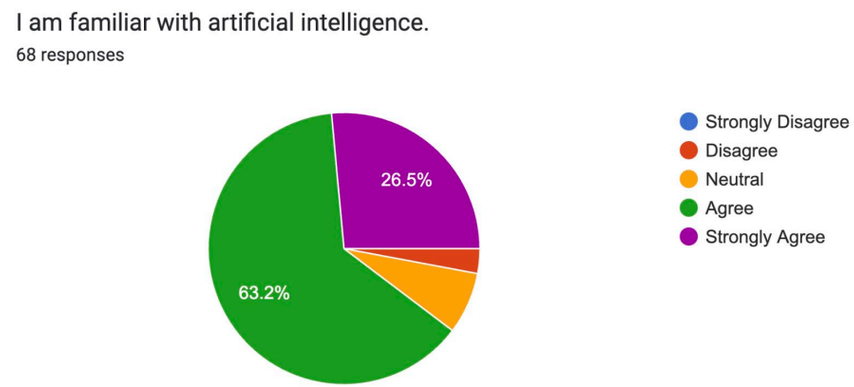

Figure 1: Question 4

Ten respondents reported that they had interacted with or used AI-based tools in a healthcare setting, with six being healthcare professionals and four being high school students. See Figure 3 for more details.

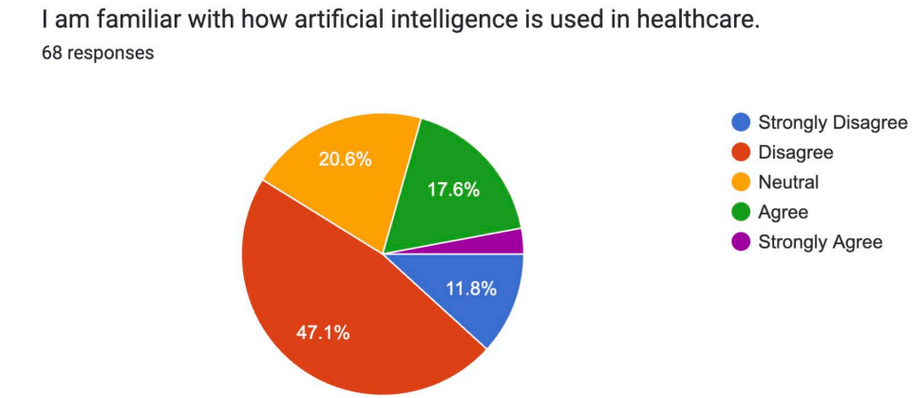

Figure 2: Question 5

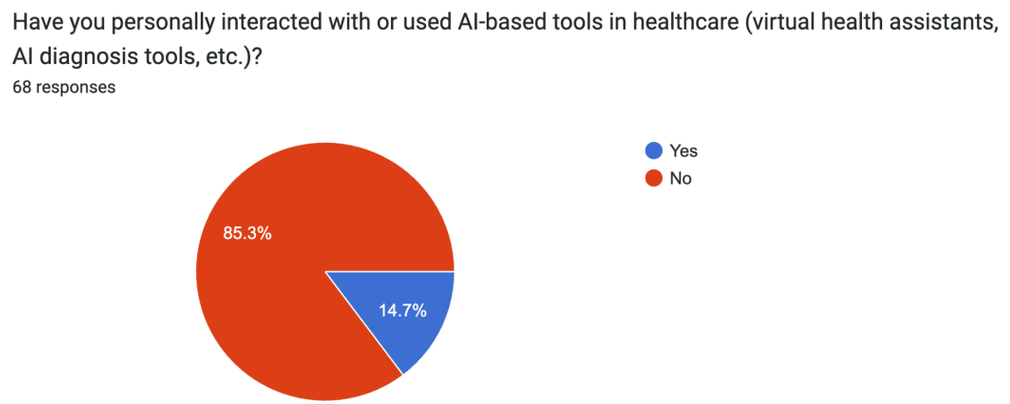

Figure 3: Question 6

Analysis Questions

Part 1: Confidence in AI in healthcare

The majority agreed that AI would significantly improve healthcare quality. However, when asked whether AI could make medical decisions as accurately as a human doctor or whether they trust AI for medical recommendations, responses for high school students followed a general bell curve distribution, while healthcare professional responses were slightly skewed to "Agree".

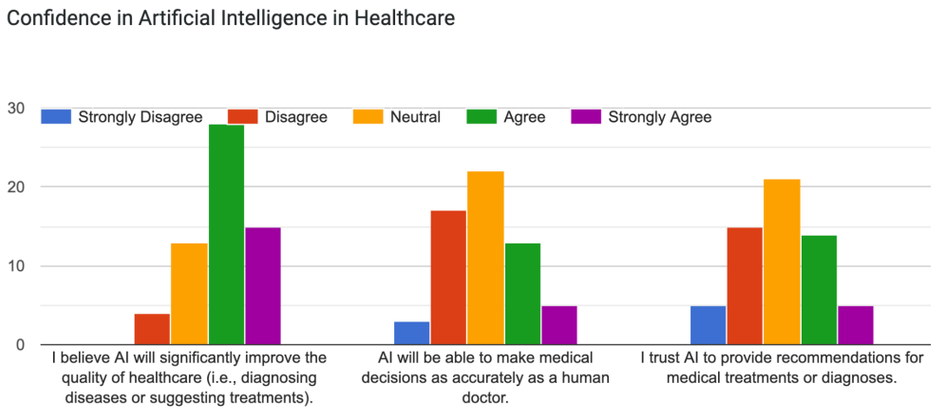

Figure 4: Questions 7-9 Results (High School Students)

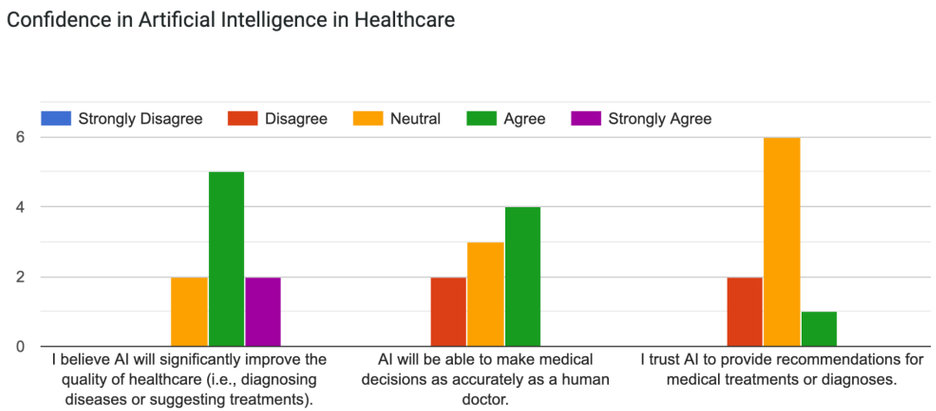

Figure 5: Questions 7-9 Results (Healthcare Professionals)

	Average Value High School Students	Average Value Healthcare Professionals
Question 7	3.900	4.000
Question 8	3.000	3.222
Question 9	2.983	2.889

Table 1: Questions 7 - 9 Likert Analysis

Part 2: Concerns about AI in healthcare

For high school students, the questions regarding AI replacing human doctors, its potential biases, and whether it might prioritize efficiency over patient well-being, all followed a bimodal distribution, with "Disagree" and "Agree" being the most selected options. Neutrality was demonstrated with privacy concerns, however. With healthcare professionals, thoughts on concerns were more skewed, with the questions about replacement and bias being skewed to "Disagree" and "Agree", respectively. A bimodal distribution was observed with the questions about data privacy and AI priorities.

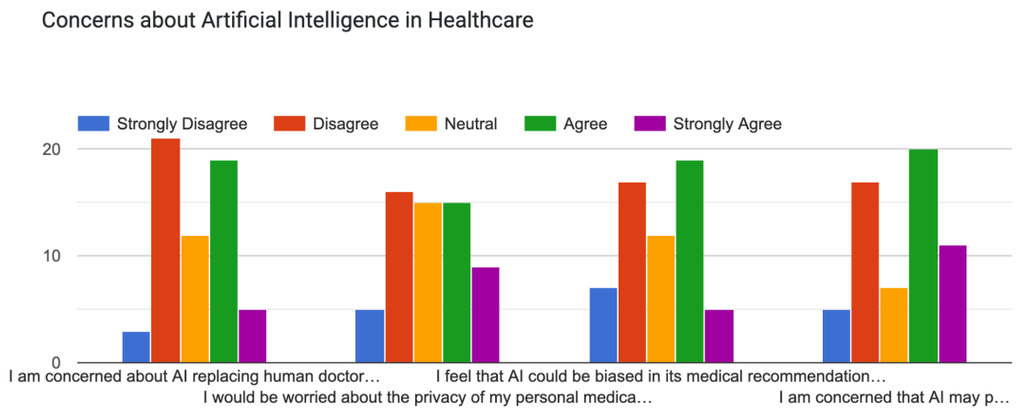

Figure 6: Questions 10-13 (High School Students)

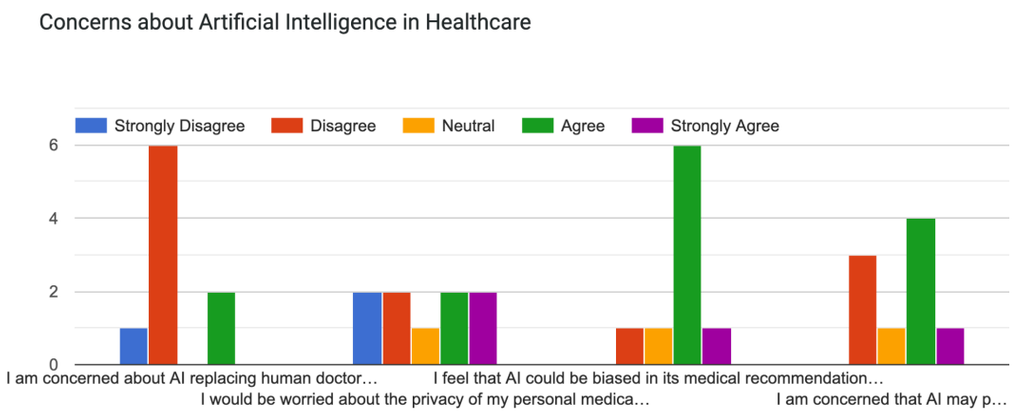

Figure 7: Questions 10-13 (Healthcare Professionals)

	Average Value High School Students	Average Value Healthcare Professionals
Question 10	3.033	2.333
Question 11	3.117	3.000
Question 12	2.967	3.778
Question 13	3.250	3.334

Table 2: Questions 10 - 13 Likert Analysis

Part 3: Ethical and Regulatory Considerations of AI in Healthcare

All participants were generally in favour of the implementation and ethical considerations of AI in healthcare. With high school students, all three questions (about AI's external regulation, AI's role in medical decision-making, and patient's choice when using AI tools) were skewed to "Agree" or "Strongly Agree".

A similar trend was observed with healthcare professionals, with the exception of the last question, where professionals were more ambivalent on whether patients should have the choice to opt out of AI-assisted tools.

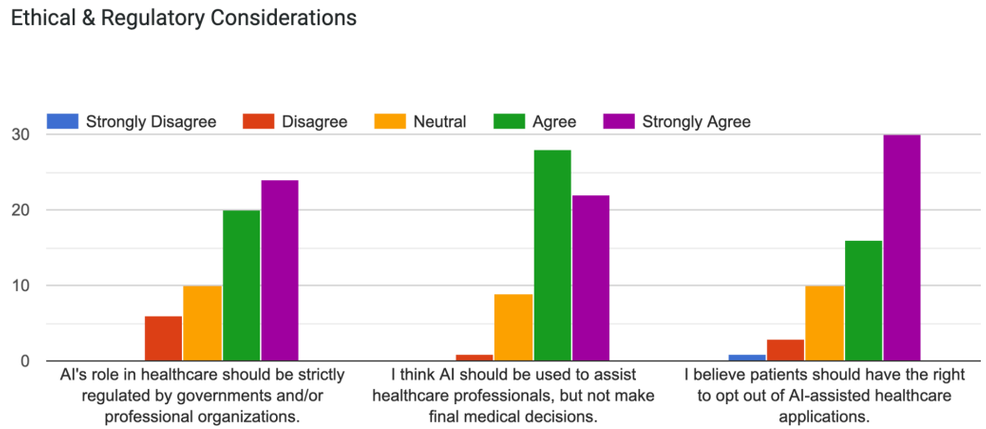

Figure 8: Questions 14-16 (High School Students)

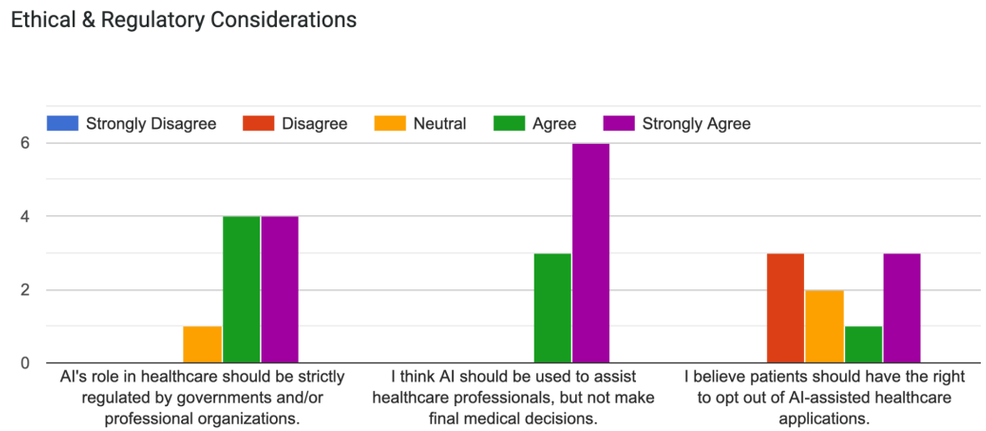

Figure 9: Questions 14-16 (Healthcare Professionals)

	Average Value High School Students	Average Value Healthcare Professionals
Question 14	4.033	4.333
Question 15	4.183	4.667
Question 16	4.183	3.444

Table 3: Questions 14 - 16 Likert Analysis

Part 4: Practical Applications of AI in Healthcare

Both high school students and healthcare professionals believed that AI could contribute to more personalized healthcare, and that they would be open to using AI tools themselves, with their responses both being skewed to "Agree" or "Strongly Agree". However, when asked whether AI should only be used in administrate settings, high school students generally expressed neutrality while healthcare professionals expressed slight disagreement.

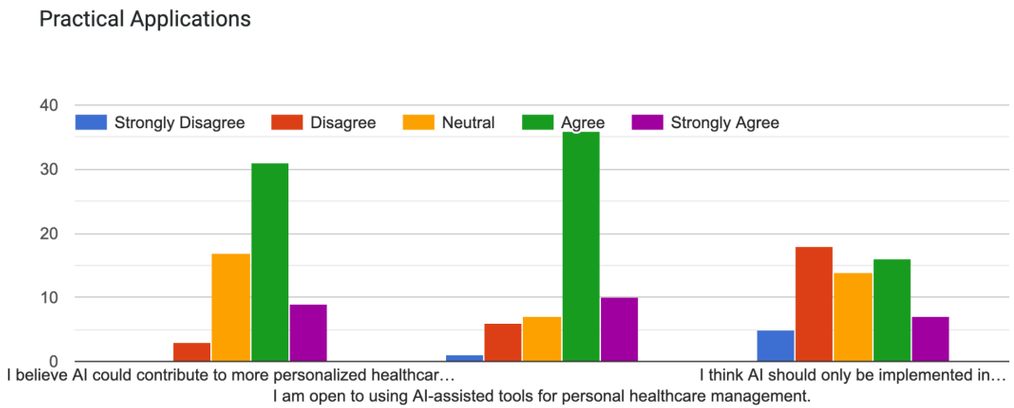

Figure 10: Questions 17-19 (High School Students)

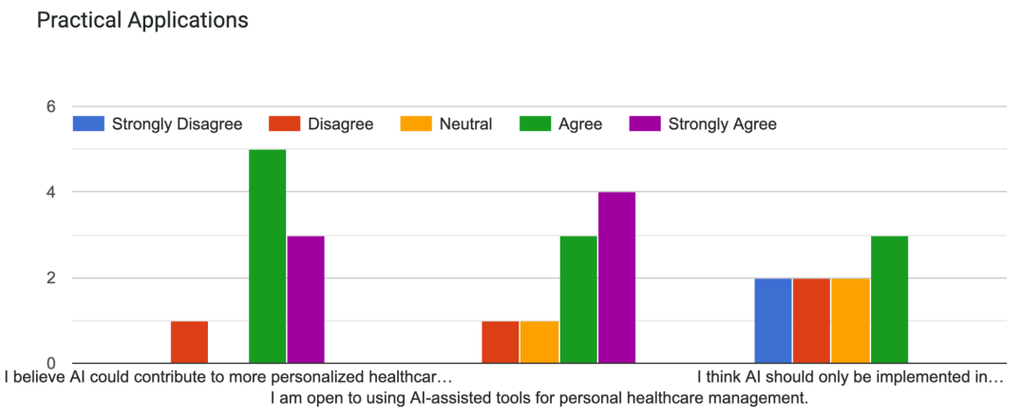

Figure 11: Questions 17-19 (Healthcare Professionals)

	Average Value High School Students	Average Value Healthcare Professionals
Question 14	3.767	4.111
Question 15	3.800	4.111
Question 16	3.033	2.667

Table 4: Questions 17 - 19 Likert Analysis

Qualitative Findings (interview results)

The interviews were used to conduct a thematic analysis to identify and analyze recurring trends in the high school student and healthcare professional subgroups.

Thematic Analysis Findings

Thematic analyses of the interviews found several themes within the participants' attitudes towards AI in healthcare. Some of the major themes from this portion were:

1. Trust in AI and Its Capabilities

Students expressed skepticism about AI's current reliability, given that it is a relatively recent implementation. They were, however, hopeful for the future, with most expecting AI to eventually be able to make medical decisions with the same, if not greater, accuracy as human doctors. Healthcare professionals acknowledged AI's potential but emphasized its current and potential future limitations. Due to this, many believed that patients are more likely to trust human doctors over AI systems, and suggested that there is a need for some sort of human input in all decisions.

2. Ethical Issues and Bias

Few students touched on the ethical concerns of using AI to handle data, but the majority referred to the intrinsic human bias in the training data used for AI, which would consequently result in biases in the AI as well. Professionals emphasized that AI being trained on data that reflects existing disparities in healthcare (i.e., racial or socioeconomic biases) would only exacerbate these problems further.

3. Influence of AI in Healthcare

Many students recognized and supported AI for administrative tasks, but not for making final critical medical decisions. Healthcare professionals regarded AI as unusable for final critical medical decisions and saw more specific uses in radiology and predictive analytics.

4. Limitations of AI

Students believed that AI's lack of emotional intelligence renders it incapable of making decisions that involve human compassion. Similarly, healthcare professionals acknowledged the limitations of AI, particularly its inability to pick up on subtle, often subjective, cues that professionals can.

5. Regulation of AI

Nearly all students agreed that AI should be used as a tool to support healthcare professionals, not replace them. The regulation and oversight of AI, potentially through government bodies, were also deemed to be an important factor. Healthcare professionals stressed the importance of having a clear system of accountability, especially for situations where AI makes an error. All professionals believed that liability should fall on the overseeing physician if they were to work with AI, with many referencing the current "most responsible physician" system used in many healthcare systems.

Discussion

Differences were found in the attitudes of high school students and healthcare professionals. While both groups recognized the potential of AI to improve healthcare efficiency as a whole, their confidence, concerns, and ethical considerations for both the present and future differ. These results attest to the conflicting public opinions found in existing research, yet simultaneously stress the importance of understanding these perspectives to ultimately inform future decisions and initiatives involving AI in healthcare to, at the very least, consider public views.

Confidence in AI's Capabilities

Both the survey results and thematic analysis revealed that all participants believed AI would improve the quality of healthcare in some way. However, the confidence in AI's ability to make medical decisions, especially at the level of human healthcare professionals, was more ambivalent between the two groups. High school students, for example, were more neutral in the survey, but more optimistic during interviews, with many citing AI's near-unlimited learning abilities that could be funneled back into optimizing it. On the other hand, healthcare professionals were slightly more reserved, emphasizing the limitations of AI in situations involving rare diagnoses or uncommon patient interactions. This disparity was reinforced through the thematic analysis, where many healthcare professionals stressed that AI's lack of subjective judgment and empathetic reasoning makes

it unsuitable for the type of patient care healthcare is built for. While high school students emphasized this, they were also considerably more concerned about AI replacing human healthcare professionals than the professionals themselves. This disparity is likely a result of differing levels of exposure to healthcare AI technologies (as shown in Figure 2), media and education influences, or personal beliefs about technological advancement as a whole. This aligns with previous research, showing that younger populations exhibit greater openness to AI, whereas professionals maintain a more cautious position due to pragmatic concerns (Antes et al., 2021; Laï et al., 2020; Teng et al., 2022). It also aligns with other research, where job replacement was seen as a major fear in the implementation of AI (Abdullah & Fakieh, 2019; Derakhshanian et al., 2024).

Concerns and Ethical Issues

Although general concerns surrounding AI in healthcare were prevalent in both groups, they varied slightly in nature. High school students focused less on ethical and privacy concerns, rather voicing their concerns about the current abilities of AI, believing it to be insufficient for current use in non-administrative settings. Healthcare professionals, on the other hand, mentioned the ethical challenges associated with AI but still emphasized a different problem: the lack of clarity on how an AI makes decisions. Despite this, however, healthcare professionals were still open to limited clinical uses of AI in healthcare, such as in radiology or predictive analytics. These findings are consistent with Stewart et al. (2023) and Hamedani et al. (2023), who found that most medical students, physicians, and nurses selected specialties and aspects such as radiology, pathology, medical tests and imaging where AI would be implemented or most likely to be impactful. Additionally, both groups agreed that AI had a level of emotional intelligence that rendered it unsuitable for personal, one-on-one patient interactions. Similar skepticism is noted in other studies, where participants often consider AI insufficiently flexible for intricate patient interactions (Rony et al., 2025; Sabra et al., 2023; Smoła et al., 2025).

Another major trend and concern that arose in the surveys and interviews was bias. High school students showed a broad awareness of bias, with many speculating the presence of human bias in the data used to train AI, which would affect the AI itself. Healthcare professionals also recognized this but were more worried about the implications, particularly the potential for existing disparities in healthcare due to human bias to be entrenched further, a concern that was also observed by Wu et al. (2023). Racial-based and gender-based biases were mentioned frequently. That said, both groups suggested the idea of using larger data sets when training AI to minimize bias and subsequently make AI as generalizable as possible.

There was minimal mention of AI handling data and the associated privacy concerns in the interviews. This contradicts the findings of Gao et al. (2020), who identified AI data handling as a large public concern, as well as the broader discussions on AI implementation, where data handling is a central focus of future healthcare policy (WHO, 2021). Collectively, these findings suggest that transparency on AI's functionality, data handling, and its decision-making processes, and education on the privacy challenges, could help alleviate the trust gap.

The Future Role of AI in Healthcare

A major aspect of the interview data was the suggested role of AI in healthcare. All interviewed participants fully supported the continued usage of AI for routine administrative tasks in healthcare settings. In non-administrative settings, where AI would likely play a more active role, both groups agreed that AI should play a supporting role rather than a lead one. In fact, participants consistently emphasized that healthcare professionals should retain greater influence than AI systems in these non-administrative settings, ensuring that the AI serves as an aid rather than the final decision-maker. For many participants, this was due to the healthcare professionals' years of training and perceived ability to handle anomalous situations more effectively. This is consistent with Fritsch et al. (2022), who found that AI is most widely accepted when used as a tool to enhance, not replace, human expertise. During the qualitative interviews with the healthcare professionals, many compared AI to self-driving cars, and how, like the cars, AI systems in healthcare must be tested strictly and in many different conditions before even being considered for release to the public.

Additionally, three healthcare professionals suggested some specific use cases of AI in non-administrative settings, such as in radiology, in analytics, and as replacements for expensive tools. Together, both groups exhibit a cautious yet hopeful look toward AI in the future through the shared lens of valuing the expertise and capabilities of human healthcare professionals.

Implications for Policymakers and AI Implementation

The findings of this study have several implications for healthcare policy and AI development in the future. First, transparency in AI decision-making processes must be prioritized to build trust among healthcare professionals and high school students. A concern raised during the interviews was the lack of clarity regarding how AI systems arrive at decisions, which participants noted contributed to uncertainty and reduced trust. This underlines the value of open discussions regarding AI's functionality to allow for confidence as it is being implemented. Second, educational initiatives aimed toward both younger and older populations should expand to teach beyond AI's capabilities and include proper usage and ethical considerations as well. This is also consistent with the findings of Rony et al. (2024) and Stewart et al. (2023), who found that people see value in incorporating AI topics or handling in formal education or training.

Additionally, given the variance in the two groups' views depending on the question, educational or discussion-promoting initiatives should be tailored to the population. For instance, education for younger people could focus on AI's limitations, while professional development for healthcare professionals could focus on ethical frameworks and bias prevention. Lastly, almost all participants emphasized the importance of regulatory guardrails for AI, at least in its early implementation, to prevent misuse. Therefore, regulatory laws in the early stages of AI's implementation should emphasize AI's role as an assistive tool to maintain human oversight in decision-making. As AI is used more in healthcare, promoting discussions on bias, data privacy, and the long-term societal impacts through education will ensure that current and future generations approach it responsibly.

Limitations and Future Research

Given the 8-month time frame for this study, one of the major limitations was gathering participants as a whole. The sample size, particularly for healthcare professionals, was small compared to the number of high school students, limiting the generalizability of findings. A lack of engagement from nearby schools limited the female participants in this study, thereby reducing the overall demographic balance in the participants. Additionally, interview responses from high school students interestingly revealed a bit more optimism than the survey results suggested, highlighting a potential gap due to the self-reported and structured nature of the survey and interview, respectively. Future research should explore different demographic groups, both narrow and broad, through longitudinal research designs to assess how perceptions and attitudes evolve as technology inevitably advances. Incorporating larger sample sizes would also be beneficial to allow the findings to be generalized and applicable to larger populations.

Conclusion

This study highlights the nuanced perspectives on AI in healthcare, which reveal generational differences in confidence, concerns, and suggestions. While high school students generally view AI as a promising innovation, healthcare professionals remain cautious, recognizing both its existing and future limitations and ethical challenges. The implementation of technologies as powerful as AI demands public input, and these findings collectively demonstrate the importance of public education and transparency required to achieve this. Accordingly, perspectives across all age groups must be considered. While current healthcare professionals represent the modern view of AI as it is slowly being implemented, high school students represent the values and attitudes of the recipients and leaders of these very AI technologies as they are more widely implemented in the future. Consequently, addressing these information gaps through education, policy, and responsible AI development will be imperative to ensuring the widespread acceptance and ethical integration of AI in healthcare systems worldwide.

Further Reading

For original appendices and additional data, please go to **this page**.

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Racial Identity and Student Satisfaction: Analyzing Black College-Student Experiences Through Social Media Expression

Kameron Drumright

Abstract: This paper explores how Black college students express their positive or negative experiences, opinions, and perspectives relating to racial/ethnic identity at their racialized learning institutions—either Historically Black College/University or Predominantly White Institution. Codes were identified from posts (n = 57) on X (formerly known as Twitter) by Black college students through keyword search based on issues prevalent in past scholarly literature as well as historical context—issues such as racial alienation, hostile interracial-interaction, and institutional discrimination. I analyzed these tweets primarily using a thematic analysis in order to triangulate the most commonly held views and opinions of these Black college students in conjunction with historical continuity and scholarly research. This paper identifies great psychological impacts of educational segregation and the concept of "triple consciousness"—an awareness of perception by institutions, by fellow Black peers, and self-reflection on how educational choices affect identity—expanded from W.E.B. DuBois' theory of racial authenticity, among other findings. These findings suggest consistent patterns and differences in racial identity navigation across institutions. High rates of expressed external identity consciousness, persistent alienation, and institutional criticism show that, while legal segregation has long since ended, its psychological legacy continues to shape Black educational experiences today.

Introduction

The treatment of Black students within the American education system has come a long way. Since the days of the Reconstruction Era following the Civil War, integration of Black people into schools from elementary to higher education became greatly common. Nevertheless, the lasting effects of educational segregation are still affecting Black students today. Two categories of institutions of higher education are discussed throughout this research: Historically Black Colleges/Universities (HBCUs) and Predominately White Institutions (PWIs). Both sets of institutions have a population of Black students that face different challenges in both social and education experience. Using X (Twitter) as the primary social media site for data collection, this research sought to present the thoughts, viewpoints, and insights of these students.

The guiding question for this research was: "How do tweets by Black College-Students represent the opinions and experiences—in relation to racial identity/ethnic background—of attending either an HBCU or a PWI?" The methodology of this research consists of a multi-step, thematic analysis followed by a coding process to categorize tweets from Black students across institutions. This approach was taken to find significant themes from the major grievances and experiences of these students. The steps are laid out in chronological order of how I formulated the research. These steps are:

- Initial Literature Review
- Methodology/Coding Process
- Findings/Analysis
- Conclusions/Implications

With this study, I attempted to put the experiences of Black students at HBCUs and PWIs into conversation to bridge the research gap not previously explored within this field. While past literature discusses these institutions in isolation, this research synthesizes the experiences of these students to help fellow Black students grasp a greater understanding of how their lives are similar to or different from those around them.

Literature Review

College Difficulties

The stress faced by students in higher education, meaning college/university, is well-documented and -discussed; there are many social impacts in adjusting to college education, dorm-life, and, in some cases, the new geographical environment of their institution (Goldrick-Rab et al. 2016; Pedrelli et al. 2015). These stressors have been well documented because college, to many, is the bridge to their professional future. Social isolation, academic inadequacy, and apathy are some of the many reasons why an undergraduate student or beyond might struggle in their college/university career; these social and psychological difficulties in adjusting and/or fitting into college environments are even more prevalent among Black students due, in part, to the lasting impacts of educational segregation as well as lesser educational funding and resources. Black students are disproportionately impacted by systemic neglect, racial inequality, and educational inequalities. Some social challenges faced the most by this subset of students, which will be explored in the results of this research, include racial microaggressions, stereotype threat, campus climate issues, and the necessity for "code-switching"—which means changing or censoring the way one speaks to present a certain personality.

Historical Context

From the cultivation of this country's thirteen colonies in the early 1600s, Black people—then Enslaved Africans—faced numerous structural barriers preventing their educational and economic prosperity. This was due, in large part, to American society and its economy benefiting through the Slave trade and the dehumanization of Africans that prevailed in American legislation for centuries. The process of extracting African people from different countries that spoke different languages disrupted both any possible connection that they could have with each other to unionize against their captors and the establishment of culture within these Africans' country of origin. The countries that were victim to this human extraction were often fighting wars against neighboring African countries and traded with European colonialists for weapons. When the resources that these colonialists sought had dissipated, they received humans as a resource to do labor with. This commodification of African people, along with the pre-established European ideal that darker-skinned people have inferior cultures to their own, helped cultivate American race-politics that would end up being embedded into American society for the foreseeable future. Education within what is now known as the United States of America was a privilege often only given to white people. The education that Black people had in the Antebellum Era consisted of either teachers being sent to teach young enslaved children reading and writing or clandestine learning, which was a tactic of enslaved people teaching others—this defied the era's laws that prohibited the formal education of enslaved people. Even then, knowledge and learning were a privilege that a large majority of Black people did not have (Cornelius 1991; Anderson 2010).

It would take more than two centuries for the enslavement of Black people in the pursuit of free labor to become illegal. Immediately following the Civil War and the emancipation of Black people across America, education and political power were a priority for Black people. After generations of labor and illiteracy being all that many enslaved Black people knew, establishing their own societal contributions became possible on a macro-scale. In 1865, the Reconstruction Era sought to reintegrate Southern states into the Union in order to establish new governments in the South to give basic rights to formerly enslaved people; constitutional amendments such as the 13th, 14th, and 15th were outlets for this integration. This was done to redefine the relationship between African Americans and white citizens. The interracial relationship of the time was hostile, which led to these efforts being undermined by later Jim Crow laws and white supremacist violence.

After the perceived success of the Reconstruction Era that saw the establishment of civil rights for Black people (DuBois 1935), the white resistance to this nationwide progressive movement in the formerly-Confederate states in the South manifested through violence groups, like the Ku Klux Klan, and the fight to erode the civil liberties Black people had fought for. During the late 1870s, state legislatures in the Southern U.S. passed laws requiring the segregation of Black and white people in such places as public transportation and schooling. This separation through race within learning institutions subsequently damaged the educational success of Black students by systematically underfunding and overcrowding schools that were poorly equipped with resources compared to white schools (Walker 1996). This economic inequality and inequity affected educational opportunities. The fight for the betterment of schooling for Black youth eventually led to the landmark case of *Brown v. Board of Education* of 1954, ruling racial segregation in public schools unconstitutional (Kluger 2004). Despite this ruling, it was not until the 1960s, after the Civil Rights Act of 1964 made racial segregation and discrimination illegal on an institutional level, that these segregation laws were fully abolished. While the abolition of racial discrimination and separation helped Black students' educational attainment through increased integration into white schools, de facto segregation within learning environments still persisted, perpetuating racial separation and inequality.

After institutional segregation had been ruled illegal, white communities responded by contributing to social segregation, psychological and emotional strain, and subtle or overt hostility to their Black student-counterparts. White communities also engaged in "white flight" with their access to higher-quality education (Kruse 2005). This contributed to further social segregation because it both perpetuated the idea of a racial hierarchy in American society and, once again, left Black students in learning institutions that were underfunded due to a lack of care for these communities. Within the following decades, as racial integration of educational environments has become normalized, Black communities have improved educational access, increased representation, and social and economic mobility (Harper 2009). Simultaneously, white communities encounter increased diversity, collaborative learning, and improved social and political awareness. Similar to many progressive movements, there are barriers to preventing the ideal learning environment for both Black and white communities. Some of these contemporary barriers include implicit biases and hostility towards non-white peers, unequal educational opportunities, racial tension, and the dissolution of safe racial spaces.

College Life for Black Students and the Development of HBCUs

During the early to mid-1800s, the first HBCUs were created with the mission of educating the nation's poorest and least academically prepared students, particularly from communities of color; these institutions sought to prepare first-generation students of color for stable careers to ensure their contribution to the American economy (Allen & Jewell 2002; Gasman 2007; Johnson et al. 2017). These institutions were greatly funded and developed during the Reconstruction Era within the South, which was a short period of over a decade where African Americans gained citizenship, access to education and political office, and career opportunities among other rights.

The legacy of HBCUs is exemplified by pivotal Civil Rights leaders that have graduated from these institutions, including Reverend Martin Luther King Jr.; Thurgood Marshall, Former Associate Justice of the Supreme Court of the United States; Ms. Rosa Parks, Civil Rights activist; and Spike Lee, acclaimed Black director, actor, and activist. HBCUs have a tradition of nurturing students' talents and skills and contributing to the development of the African-American middle class.

In the present-day, HBCUs have seen a decrease in attendance of Black students—with a decrease of 254,286 to 208,785 Black students from 2010 to 2022, respectively (AIBM; IPEDS 2022). This could be indicative of the perception that HBCUs might not be as sought out or necessary in America's current educational environment. Still, for the students that are present at HBCUs, research suggests that the Black-student body tends to have more positive experiences than their Black counterparts attending PWIs. For instance, Pascarella & Terenzini (1997) state that HBCUs appear to have a small positive effect on occupational status and both academic and social self-image among Black women. This sentiment is mirrored in James Earl Davis' journal (1994) discussing the suggestion that Black students have greater support and reported higher levels of satisfaction when attending HBCUs. Considering the more positive learning outcomes of Black students in HBCUs, it posits whether the contemporary Black college student sees their experiences as positive or not from their own personal perspective.

The interracial relationships vary greatly within institutions of higher learning between Black and white students, whether it be at HBCUs or PWIs. Within the Black-student body at both HBCUs and PWIs, the sense of community is supportive, but there is a specified comfortability between Black students at HBCUs. Leath et al.'s (2022) study on Black students' community at PWIs identified strong Black intraracial-support themes—meaning within the Black community—such asbuilding professional support networks, acknowledging racism in academic settings, feeling like a family, and engaging in activism. But, at PWIs, Black students are exposed to more opportunities for racial discrimination than Black HBCU students. The work of Campbell et al. (2016) reports that students themselves identify that these occurrences of racial discrimination, whether large or minuscule, occur at higher rates than they do at HBCUs. Chen et al. (2014) supports this in their journal that discussed the increased drop-out rates of Black students being due in part to their common feelings of isolation and/or alienation. These racial stressors, among others, affect the psychological well-being of many Black students (Dancy 2018).

Research Connection

The historical context connects to my research process at three points: 1) in what topics were chosen as the focus in the tweet-collection process; 2) what categorization/coding was done after data collection; and 3) what conclusions and implications were drawn from the research. All steps of the research process and conclusions drawn from findings were influenced by previous works within this field of research, and they were performed to best represent the lives of Black college students; past literature and historical continuity substantiates the impact of systematic racism and educational segregation on these students. Exploring how these students feel these impacts and how they express themselves catalyzed this research. These Black college students are tasked with navigating complex social environments while learning to communicate and represent their own racial identity. Simultaneously, this all takes place in a new environment that is their college/university, where they must maintain an adequate educational career. There is much to be done to improve the lives of these students. With their voices tending to go unheard, taking the time to understand their experiences and opinions can be the necessary step towards change. My research recognizes the necessity of listening to these young college perspectives and the institutional/societal improvements that can come about. By comparing the realities expressed online regarding two different racial learning environments, those being HBCUs and PWIs, the middle ground of what impacts Black college-students most in contemporary higher education could be observed.

Method

Positionality Statement

Before exploring the method of this research, it is crucial to recognize any implicit biases that could have impacted the research process and the work's conclusion. The majority of my own previous research has related to negative historical policies and societal ineptitudes that have impacted Black people in America, including inadequate mental health initiatives for Black high school students and economic mistreatment of Black veterans leading to high rates of homelessness. As a Black student approaching college decisions myself, with educational experience researching and reporting within the fields of humanities and social sciences, I recognize that I bring a personal perspective to this research. Considering that I have personal interest in possibly attending Howard University or another prestigious HBCU, Black college-students' discussions and opinions are useful in my own college searching process as well as for other Black students deciding on their learning institution of choice. Given these factors, there may be preconceptions about which environment might be more accommodating or nurturing for Black students. To

address any potential biases, I implemented the strategy of using a multi-step categorization process in addition to coding and also included both positive and Beattie perceptions of HBCU and PWI institutions for diverse sources. This sudy also had multiple external reviewers consisting of educational advisors, professional researchers within this field such as Dr. Marlena Debreaux, and peers who are familiar with the research process. While this position requires careful attention to remaining objective, it also provides valuable insight into the nuanced conversations surrounding the college decision process and the racial environment that HBCUs and PWIs , respectively, cultivate for Black students.

Ethical Considerations

For this research project, no direct human subjects were used. IRBs are only necessary when a research project is conducted with direct involvement of human subjects. Because this research only involved the tweets from Black students and not direct contact with the students, no IRBs or other ethical reviews were necessary.

Data Gathering

The data that was gathered were tweets on the social-media platform "X" (formerly known as Twitter) by a number of HBCU/PWI students and graduates. I chose the tweets based on their relevance to keyword searches on the platform's search engine. Most keyword searches included topics from scholarly literature within this field alongside the inclusion of the acronyms "HBCU" or "PWI" to specify the topic to either learning institution. Once keyword searches were made, tweets relating to the following criteria were chosen:

- User self-identifies as Black/African-American/Afro-Caribbean/Continental African
- User self-identifies their attendance at either an HBCU or non-predominantly Black college/university through their Twitter Bio, username, or prior posts
- Tweet relates to their own experiences, opinions, and perspectives with their chosen learning institution regarding their racial/ethnic identity in relation to student satisfaction
- Tweet comes from an individual, rather than an organization/representation of a larger group

Approach

Initially, the data analysis and triangulation would have used codes from Williams et al. (2018) source focusing on the microaggression categories that any Black students could face at either institution, but this would have led to different, unintended implications for the experiences Black students have at HBCUs vs. PWIs. To remain focused on comparing the opinions and perspectives of Black students across learning institutions, which helps specify the gap this research attempts to target, I developed more specific codes after identifying multiple categories for each tweet; the first category was given using a sentiment analysis—"Is this tweet positive, negative, or neutral?"— and the following two steps identified specific attitudes within any given college students' tweet using adjectives descriptive of the Black-college student experience—"Is this student angry, supportive, regretful, etc.?"—and the subject of discussion in each tweet—"What is the specific issue or topic that is being addressed in this tweet?"

Participating Media

The final sample size of tweets consisted of 56 posts from 56 individual, Black students. 30 tweets were from HBCU attendees and 26 tweets from PWI attendees, meaning 30 tweets were about the Black HBCU-student experience and 26 were about the Black PWI-student experience. The institution with the most tweets relating to it was Howard University, which is an HBCU. No more than one tweet from the same individual was included in the data to increase representation.

Data-Analysis Process

I completed initial coding using the aforementioned three-step identification process for comprehensive categorization: This process allows tweets of different focuses and varying positivity levels to be recognized before thematic analysis. This thematic categorization further narrows down tweets before final thematic analysis.

I. Sentiment Analysis of Tweets

Sentiment Analysis was conducted first for each and every tweet using language queues to denote whether the tweet is positive, negative, or neutral. This is useful when observing the commonality of positivity students had towards certain institutions, racial groups—interracial or intraracial, or any other discussion topic.

II. Attitude-Language Distinction

Expanding on the Sentiment Analysis step above, each tweet was given one or multiple words to describe the tone they used within the tweet. Words were effective in encompassing the attitudes of these students.

III. Subject Observation

After the previous two steps, the last step before actual coding was to identify the subject of discussion within each tweet. This was simple and useful for observing where the greater swath of HBCU or PWI students were targeting their energy through their posts.

After the three-step identification process, each tweet was given codes for further categorization, with some tweets being given more than one code. All 12 codes were decided on using the most recurring themes of past literature and the three-step identification. No codes of past research were used to differentiate this research beyond past works. The following is a table of all codes used:

All codes fall under one of two categories—positive or negative codes—with two exceptions [*Self-Identity Conscious* and *Identity Conscious of Others*]. *Interracial Appreciation* and *Interracial Judgement* can be defined by Black students' expressed positive or negative interactions with or insights on White/non-Black peers in regards to their social or educational experience. Similarly, *Intraracial Appreciation* and *Intraracial Judgement* can be defined by the interactions expressed by Black students towards other Black students; these discussions, issues, and gripes remain within the Black community while interracial codes denote interaction across racial groups. *Student Connection* and *Student Alienation* are defined by either the expressed acceptance or isolation a student has felt in their institution. All *Institutional* codes [*Institutional Appraisal (PWI), Institutional Criticism (PWI), Institutional Appraisal (HBCU), Institutional Criticism (HBCU)*] relate to a student's experience, either positive or negative, at either racialized learning environment—HBCU or PWI. Finally, *Self-Identity Conscious* and *Identity Conscious of Others*, are the aforementioned exception codes. These codes are not inherently positive or negative. Each tweet given one of these two codes contained a student either discussing their own Blackness or how another student chooses to express their own Blackness. "Blackness," within this study, is defined by one's expression of African-American identity, culture, and/or ethnic background in social settings—how they talk, dress, portray themselves, communicate ideas, etc.

Fig.1: All Codes Used for Tweets from Black College-Students/Alumni

Positive Codes	Negative Codes
Interracial Appreciation	Interracial Judgement
Intraracial Appreciation	Intraracial Judgement
Student Connection	Student Alienation
Institutional Appraisal (PWI)	Institutional Criticism (PWI)
Institutional Appraisal (HBCU)	Institutional Criticism (HBCU)
Self-Identity Conscious	Identity Conscious of Others

Findings/Analysis

Below is a table showing each code from most frequent to least frequent, an example of a tweet found for each given code, historical context, and a connection to past literature in order to triangulate what themes are most commonly expressed and why.

Fig. 2: Black Student Experiences at HBCUs and PWIs: Triangulation Analysis

Code	Frequency	Frequency in Percentage (%)	Twitter Evidence	Historical Context	Research Connection
Identity Conscious of Others	32	21.3%	"It aches my heart when I see privileged, suburban, well versed & well-spoken black girls attending HBCUs"	Rooted in educational segregation history and integration challenges	Williams et al. (2018): "identity monitoring" as coping mechanism
Student Alienation	24	16.0%	"Being a black kid at a PWI is experiencing constant alienation when the professor asks for everyone to get in groups"	Classroom segregation and the act of bussing Black elementary students to colored schools during the Jim Crow Era has had lasting social impacts on racial integration in schooling.	Chen et al. (2014): Black students, particularly at PWIs, identify feelings of isolation and alienation that lead to higher dropout rates
Institutional Criticism (HBCU)	17	11.3%	"Howard University should be ashamed for the housing crisis at hand"	Higher expectations for institutions created specifically for Black students	Amanishakete Ani (2013): Productive and Progressive attitudes lead Black students to remain hopeful while pushing for better educational resources and opportunities.
Institutional Criticism (PWI)	15	10.0%	"Attending an elite PWI this past semester has severely depleted my mental health because of the intense anti-blackness" "I had never been to a predominately white school until college...it was still psychological warfare"	Legacy of explicit exclusion followed by reluctant integration	Chen et al. (2014): Black students at PWIs encounter both overt and covert racism from various sources
Intraracial Judgment	14	9.33%	"Black PWI students appropriate HBCU Culture everyday" "Are we not supposed to have fun because we're black and attending a PWI that's not even classified as a PWI anymore? Going to a university with Caucasians does not make us any less black than students who go to an hbcu."	Reflects tensions about educational choice within Black community; Identity policing has historically been performed to create more positive perceptions of Blackness and combat stereotypes	Larry J Walker (2018): Mental health stigmas within the Black community can prevent mental health support and perpetuate a hurtfully-quiet mindset
Self-Identity Conscious	13	8.66%	"One of my fears coming to a PWI as a Black woman was trying to fit in professionally"	Fear of racial-uniqueness in any environment is not new to American society; any "out-group" in American society has been discriminated against until assimilation.	James Earl Davis (1994): Necessary attention to identity development in educational contexts

Student Connection	11	7.33%	"HBCUs are such an experience... everybody was a smart black kid...I'll never experience something like that again"	HBCUs historically created spaces for Black community building; racial congruence contributes to comfort	Williams et al. (2018): Importance of same-race peer networks for retention
Institutional Appraisal (HBCU)	9	6.0%	"Sometimes I be forgetting racism even exists when I'm at my hbcu"	Recognition of HBCUs' historical mission and cultural significance	James Earl Davis (1994): Black students tend to receive greater support, and they seek out this support more to great rates of satisfaction
Intraracial Appreciation	6	4.0%	"Black students at PWIs please do not let anyone make you feel like you deserve to deal with racism because you don't attend a HBCU"	Reflects solidarity within Black educational communities	Amanishakete Ani (2013): In-group support systems
Interracial Judgment	6	4.0%	"If you aren't BLACK AMERICAN, you will NEVER understand the depths of the SOUL people"	Reflects ongoing racial tensions in integrated spaces	Sue et al. (2007): Normalization of racial discrimination can manifest in less acceptance, equality, and diversity.
Institutional Appraisal (PWI)	1	0.66%	"If a black university refused to care for their own, yet you expect PWI school to treat them better. My son is at PWI and he does not have all these issues. It is disgusting what [the administration is doing]."	Historical context of PWIs as sites of exclusion	Leath et al. (2022): Strong intraracial-support networks and engaging activism are necessary in acknowledging and combating systemic racism at PWIs.

Interracial Appreciation	1	0.66%	"So say a white person that grew up in the area knows most of the people of color in the area and is most comfortable around them… you think that white person is weird for wanting to attend [an HBCU]?"	Racial integration has become more and more common as diversity has been normalized through policies, like the Civil Rights Acts of 1964.	Amanishakete Ani (2013): Hope Theory—the stated importance within Black Youth of remaining hopeful towards a greater political and social future.

Quantitative Observations

The *Identity Conscious of Others* code was the most frequently observed code at 21.3% of observations. *Student Alienation* followed with 16.0% frequency, while *Institutional Criticism (HBCU)* and *Institutional Criticism (PWI)* appeared at similar rates of 11.3% and 10.0%, respectively. *Intraracial Judgement* was coded at 10.0% of observations. *Self-Identity Conscious* followed with 8.66% frequency, with *Student Connection* at 7.33%. *Institutional Appraisal (HBCU)* appeared at 6.0% of observations. Both *Intraracial Appreciation* and *Interracial Judgement* were coded at 4.0%. The least frequent codes were *Institutional Appraisal (PWI)* and *Interracial Appreciation*, each at only 0.66% of observations.

Putting these quantitative values into the context of the qualitative research, the significant gap between *Identity Conscious of Others* (21.3%) and *Self-Identity Conscious* (8.66%) suggests individuals were more attuned to navigating their racial identity in relation to others than to internal identity processing. This external focus coincides with the disparity between *Student Alienation* (16.0%) and *Student Connection* (7.33%), revealing environments where belonging remains challenging. Despite different institutional contexts, criticism rates for HBCUs (11.3%) and PWIs (10.0%) were similar, though the subjects of the tweets with those codes differed—resource and administration at HBCUs vs. social climate and discrimination at PWIs. This contrast is further emphasized by the substantial difference between *Institutional Appraisal* rates, with HBCUs (6.0%) receiving nearly ten times more positive appraisal than PWIs (0.66%). Within racial dynamics, judgment outweighed appreciation in both intraracial contexts (10.0% versus 4.0%) and interracial contexts (4.0% versus 0.66%), highlighting persistent tensions around educational choices, authenticity, and cross-racial relationship development in higher education settings.

Thematic Triangulation

The pre-coding categorization process of evaluating tones and subjects of tweets was purposefully inductive in order to gather the direct opinions of the students with the experience in these institutions and develop the appropriate codes. Through combining the historical context of Black education and past studies, both past and present experiences, opinions, and perspectives on the racial environments that higher learning can create for Black students were triangulated.

Identity Navigation (Identity Conscious of Others 21.3%, Self-Identity Conscious 8.66%)

The most frequent codes reflect Black students' heightened awareness of racial perception in educa-

tional spaces, with external consciousness appearing nearly three times more frequently than internal identity discussion. This consciousness stems, partially, from the legacy of educational segregation, where Black students' transition to predominantly white spaces created what Williams et al. (2018) terms "Identity Monitoring" as a coping mechanism. Chen's research connects this to "stereotype threat," creating cognitive burdens that impact academic outcomes and social comfort (2014).

Belonging Dynamics (Student Alienation 16%, Student Connection 7.33%)

Experiences of isolation significantly outpaced those of connection, with alienation appearing more than twice as frequently, particularly at PWIs. Despite racial integration, Black students remain psychological outsiders at many institutions, reflecting the reporting that Black students feel heavily isolated within PWIs due to non-racial congruence—the lack of seeing peers of the same race—and at HBCUs due to the higher expectations some subsections of the Black community hold each other to (Everett et al. 2016, Chen et al. 2014). Still, positive reinforcement amongst Black adolescents in higher education supports high morale and student connection (Ani 2013).

Institutional Evaluation (Criticism HBCU 11.3%, Criticism PWI 10%, Appraisal HBCU 6.0%, Appraisal PWI 0.66%)

Criticisms appeared at similar rates despite different focal points of discussion—HBCUs for resources/administration and PWIs for social climate/discrimination. Notably, positive appraisals of HBCUs appeared at much greater rates than at PWIs. The dual criticism reflects Black students' nuanced position; they are critical of PWIs for lacking inclusion while holding HBCUs to higher standards despite their historical underfunding. Sue and Williams' work on racial microaggressions explores how everyday interactions at PWIs create hostile and unwelcoming environments, while public opinion demonstrates the many benefits HBCUs provide despite resource limitations (Sue 2007; Williams 2018). Sylvia Hurtado's (1992; 2007) campus climate research reveals the gap between institutional diversity statements and actual Black student experiences at PWIs, explaining the dramatic difference in positive appraisal rates.

Racial Relationship Dynamics (Intraracial Judgement 10%, Intraracial Appreciation 4%, Interracial Judgement 4%, Interracial Appreciation 0.66%)

Judgement consistently outpaced appreciation in both intra- and interracial contexts, with positive cross-racial interactions appearing particularly rare. Dating back to the debates between Black scholars Booker T. Washington and W.E.B. DuBois on the purpose of Black education, the choices surrounding adequate education within the Black community remain politically charged. This tension reflects DuBois' "double consciousness" concept, expanded by contemporary sociology research by Cherise Harris and Nikki Khanna on racial authenticity where educational choices become "proxies" of identity politics (Khanna and Harris 2014). The minimal positive interracial experiences echo America's segregated educational history, while Sue's microaggression framework helps explain the subtle forms of interracial judgement, and historical racial identity development clarifies why positive interracial connections remain challenging.

Post-Triangulation Findings

Historical Continuity and Institutional Impact: Despite decades since legal desegregation, the psychological impacts of educational segregation persist through high rates of identity consciousness and alienation. The nine-fold difference in positive institutional appraisals (6% HBCU vs. 0.66% PWI) demonstrates how institutional histories directly shape student experiences of belonging. Despite resource advantages at many PWIs, the cultural affirmation provided by HBCUs offers psychological benefits that Davis (1994), Ani (1997), and Pascarella and Terenzini (2013) connect to overall Black student satisfaction and wellbeing.

Complex Identity Navigation and Discourse Evolution: The high frequencies of External Identity Consciousness, Intraracial Judgement, and Self-Identity Consciousness reveal what extends beyond DuBois' "double consciousness" to a what could be coined a "triple consciousness"—an awareness of perception by institutions, by fellow Black peers, and self-reflection on how educational choices affect identity. Adding this third dimension of self-reflection is important to acknowledge because it addresses the individual's choice to identify themselves based on their own merit and achievement rather than just how their institution or their peers perceive them. This "Triple Consciousness" framework can assist Black students in triangulating where their own idea of Blackness fits with the broader spectrum of Black people. There is no "correct way" to be Black, even if a considerable amount of *Identity Conscious of Others* codes say otherwise. The significant gap between judgement and appreciation codes across all relationship types suggests that contemporary racial discourse in higher education remains problem-oriented rather than solution-oriented. This complex identity navigation appears most pronounced in discussions about institutional choice (HBCUs vs. PWIs), where students negotiate between cultural affirmation and perceived professional advantages that reflect the evolving expectations of a new generation balancing racial consciousness with career/education/professional aspirations.

Conclusions and Implications

Connecting past literature with contemporary sources and the codes developed for this research revealed how Black college students in this sample appear to navigate racial identity at different institution types. This research bridges the gap between isolated studies of either HBCUs or PWIs by comparing Black student experiences across both racialized learning environments.

The most significant patterns in the findings reveal a complex landscape of Black student experiences. First, the frequency of *Identity Conscious of Others* (21.3%) over *Self-Identity Conscious* (8.66%) indicates Black students invest considerable time in managing external perceptions rather than internal identity development. This extends beyond what previous literature concluded, suggesting a "burden of representation" that persists, regardless of institutional/racial makeup. Second, while criticism rates were similar for HBCUs (11.3%) and PWIs (10.0%), their foci differed as mentioned—resource allocation and administrative inadequacy at HBCUs vs. social climate and racial discrimination at PWIs. This suggests Black students hold different expectations of these institutions that reflect distinct historical goals. Third, the disparity between institutional appraisal rates (HBCU: 6.0%, PWI: 0.66%) quantifies the significant psychological benefit HBCUs provide despite resource challenges, which supports James Earl Davis' (1994) conclusion that HBCUs offer superior social support, similar to the conclusion of Ani and what is supported by the racial-congruence framework leading to greater resilience found in the results of Leath et al. (2013; 2022). This research introduces the concept of "triple consciousness"—extending DuBois' "double consciousness" to include the awareness of institutional perceptions, fellow Black peers, and self-reflection on how educational choices affect one's racial identity. This helps in explaining the frequency of negative/judgement codes and highlights complex identity navigation Black students persist in higher education.

Implications

For PWIs, the near absence of positive institutional appraisal codes coinciding with high student alienation signifies an urgent need for change/expansion in diversity initiatives to address systemic issues in campus climate. These institutions should provide more resources in creating genuine and welcoming spaces for community-building while addressing microaggressions and social isolation discussed by Sue et al. (2007), Chen et al. (2014), and Williams et al. (2018). For HBCUs, the mixed perceptions of institutional criticism and appraisal suggests that these institutions provide crucial cultural and racial affirmation, but that administrative and resource challenges need to be addressed. Policymakers should address this historical funding inequity that has impacted HBCUs. For Black high school students considering college options, this research provides evidence-based insight into institutional choices. PWIs offer resource advantages, as evident through history, and HBCUs provide significant psychological benefits through racial and ethnic congruence. The high frequency of intraracial judgement codes (9.33%) still suggests that navigating identity politics within the Black community remains a challenge, regardless of institutional choice.

This topic of inquiry and its data collection required maintaining a level of distance from the opinions and experiences of these Black college students, as stated within the positionality statement. There are parallels between the lives of the students/alumni and the lives of students who identify as Black within my own educational environment.

Limitations

Personal bias can be a limitation within this work; maintaining the proper tone in order to collect and assess tweets from these students was a crucial strategy for objectivity. Similar to possible researcher bias impacting research conclusions, the biases of X's algorithm could have possibly impacted the tweets collected during the data collection process. This was combated through the creation of a new account on the platform in order to reduce algorithmic bias. It's also important to note that the smaller, more concise number of tweets chosen does not represent all Black college students. So, a greater sample size of tweets could be targeted, possibly including more positive instances, if this research project was expanded upon.

Other notable limitations that persisted were the site's common recommendation of accounts, people, and news organizations that had a right/far-right political ideology—some include Donald Trump Jr., Tucker Carlson, Elon Musk, and Fox News—and the keyword-searching method of my data collection phase having varying degrees of consistency; the longer it took to find quotes for data collection, the more the algorithm could impact the recommended tweets to what it believed the account used for research was most interested in. The X (Twitter) algorithm is constantly changing through adaptive technology and evolving free-speech regulations, which creates challenges in deducing reliability.

Conclusion

This research bridges previously separate conversations about the Black student experience, which reveals both consistent patterns and crucial differences in racial identity navigation across institutions. The high rates of external identity consciousness, persistent alienation, and patterns of institutional criticism show that, while legal segregation has long since ended, its psychological legacy continues to shape Black educational experiences today. The contrasting experiences of students across the two racialized learning environments suggests Black students face a largely implicated choice between institutions offering greater cultural affirmation versus greater resources despite a more complex racial climate. By focusing on Black student voices through this social media analysis, this research adds to the understanding of contemporary higher education as experienced by those simultaneously navigating racial identity, institutional contexts, and educational excellence.

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When AI Joins the Scope: Canadian Endoscopists' Perceptions of NodeAI Versus Conventional Methods for Identifying Lymph Node Malignancies in EBUS Imaging

Ria Datta

Abstract: This study investigates the potential impact of integrating NodeAI, an AI-assisted tool, into endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) procedures for cancer staging and lymph node (LN) biopsy. Using a convergent parallel design mixed-methods approach, nine experienced participants, including thoracic surgeons and pulmonologists in North America, were surveyed and participated in a focus group to assess their perspectives on AI integration into EBUS-TBNA cases. The baseline and endline surveys measured shifts in opinions regarding NodeAI's usability in diagnostic accuracy, procedure time, and ease of use. Qualitative insights were gathered through open-ended questions during the focus group to explore clinicians' views on AI's potential role, while quantitative data was captured using scales/ratings. The study found that, while most participants expressed satisfaction with current EBUS-TBNA practices, concerns around over-reliance on AI, data privacy, and the technology's accuracy surfaced during discussion. However, following exposure to NodeAI, participants' views became more favorable, with an increased likelihood of incorporating AI into clinical practice. Key benefits identified included improved diagnostic speed, reduced false positives/negatives, and potential cost savings. The findings suggest that AI tools like NodeAI could enhance decision-making, reduce procedure time and resources, while also presenting challenges related to workflow integration and over-reliance, especially for less experienced individuals.

Keywords: artificial intelligence, NodeAI, EBUS-TBNA, lymph node diagnostic imaging, clinician perceptions, convergent parallel mixed methods

Introduction

As artificial intelligence (AI) increasingly transforms healthcare, its potential to revolutionize diagnostic procedures elicits both excitement and skepticism among providers. Since its introduction in the early 2000s, endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) has transformed the diagnosis and staging of lung cancer, providing a minimally invasive method to assess lymph nodes (LN) in the thoracic region. EBUS is a minimally invasive technique for assessing LNs in the thoracic region, aiding in the diagnosis of lung cancer (Navani et al., 2015). By combining a bronchoscope with ultrasound technology, EBUS enables real-time imaging of LNs, allowing clinicians to evaluate their size, shape, and location, which is crucial for accurate cancer staging (Czarnecka-Kujawa & Yasufuku, 2017). TBNA involves a needle that passes through the bronchial wall to sample tissue from LNs or other structures within the chest. This combination is effective in diagnosing a variety of conditions, including lung cancer, infectious diseases, and airway lesions, while also facilitating the staging of diseases through mediastinal LN analysis (Aziz, 2012). In Canada, the Canada Lymph Node Score (CLNS) is used to assess EBUS-TBNA biopsies (Hylton et al., 2020; He et al., 2023). This introduces potential variability in diagnoses due to the subjective nature of human interpretation, highlighting the need for more standardized and reliable methods to assess LN involvement in lung cancer.

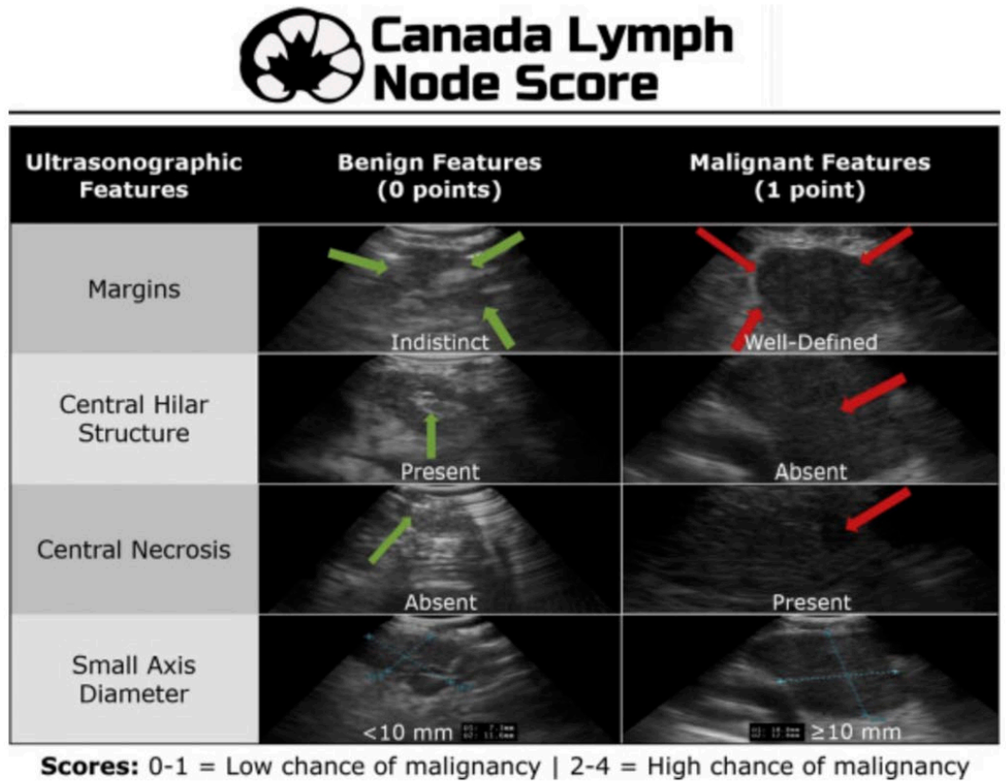

Figure 1. Comparison of benign and malignant versions of ultrasonographic feature that comprise the Canada Lymph Node Score.

To address this issue, a team at McMaster Hospital developed NodeAI, formally known as NeuralSeg, an AI program that uses GPU processing to automatically segment LN images during EBUS procedures (Churchill, 2022; NeuralSeg, n.d.). By automating the scoring process, NodeAI aims to reduce human error and provide a more consistent interpretation of the images. This has the potential to standardize the staging while using GPU processing for rapid image analysis, ultimately enhancing diagnostic consistency, accuracy, and timeliness.

Through this research, I investigate endoscopists' perceptions of NodeAI efficacy in improving diagnostic accuracy for cancer staging compared to conventional methods. As AI continues to shape healthcare, successful integration into clinical practice depends on healthcare professionals' acceptance and trust. Understanding these perspectives is crucial for broader adoption of AI in lung cancer diagnostics. In order to explore expert perceptions of AI integration into EBUS procedures, the next section reviews existing literature on EBUS and addresses the gap in research. This is followed by a discussion of the design and methodology of this study. The results of the study and discussion of findings are provided thereafter, followed by the conclusion.

Literature Review

This literature review explores current research on the application of AI in EBUS imaging, the accuracy and efficiency benefits AI provides, the perceptions of healthcare professionals towards its adoption, and identifies the gap in research that this study aims to address.

2.1 Current Research on Identifying LN Malignancies through EBUS

In a 2018 narrative review published in *Lung Cancer*, Danielle A. Hylton, from Thoracic Surgery, McMaster University, and colleagues examined ultrasonographic features of LNs to predict malignancy during EBUS procedures. McMaster University is considered Canada's top health sciences research university and #36 in the world in Clinical and Health (Hylton et al., 2018; U15 Group of Canadian Research Universities, 2023) by the Times Higher Education World University Ranking (McMaster University, 2024). The review found that certain features, such as the absence of a central hilar structure and presence of central necrosis (dead tissue), were often linked to malignancy. Hylton and her colleagues believed that by understanding which features are linked to malignancy, this assessment has the potential to reduce the number of EBUS procedures. Building on this, Hylton et al. (2020) developed the CLNS as a predictive clinical tool to determine the likelihood of malignant LNs and to guide biopsy decisions during EBUS procedures. It is based on four characteristics: distinct margins, absent central hilar structure, present central necrosis, and a large axis diameter (size). A score of 0–2 suggests a lower chance of cancer, while a score of 3–4 suggests a higher chance of cancerous LNs. The CLNS was validated through the analysis of 300 LNs from 140 patients by 12 endoscopists, demonstrating its potential as a reliable predictive tool for identifying malignant LNs during EBUS procedures (Hylton et al., 2020). Richard He, Thoracic Surgery, University of Alberta, further demonstrated the validity of the CLNS. Over one year, CLNS scores for 367 LNs biopsied during endobronchial ultrasound were linked to malignancy outcomes. Higher scores (≥3) showed 84.4% specificity, while 10.1% of nodes with scores < 2 and negative CT/PET scans were still malignant (He et al., 2023).

Triple-normal LNs are those that appear normal on CT scanning, PET scanning, and the EBUS procedure, with a CLNS of less than two (Hylton et al., 2021b). An observational study was conducted by Hylton et al. (2021b) to determine whether LNs classified as triple-normal require routine biopsy. The study assessed 143 triple-normal LNs from 57 patients and found that they had a specificity of 60% and a negative predictive value of 93.1%, with only 5.6% of the nodes being malignant upon pathologic examination. This study suggests that routine biopsy is not required for patients, allowing for a more targeted approach to biopsy in EBUS staging. Sanz-Santos et al. (2022) further affirm this by hypothesizing that "targeted sampling (TS), which omits biopsy of triple-normal LNs during endobronchial ultrasound, is not an inferior staging strategy to systematic sampling (SS) of all lymph nodes" (Hylton et al., 2021a; Hylton et al., 2021b; Sanz-Santos et al., 2022). EBUS patients were randomized to TS or SS. Results showed that TS had a faster procedure time (3 minutes vs. 19 minutes for SS) and missed only 5.45% of cancer cases, below the 6% threshold. This suggests that TS may offer a more efficient and focused approach to staging, minimizing unnecessary biopsies and associated risks, while still ensuring accurate cancer detection.

2.2 Advancements of AI in Diagnostic Imaging Regarding Procedures

The integration of AI into diagnostic imaging has become increasingly significant, with studies demonstrating its ability to enhance both image quality and diagnostic accuracy. For example, Dr. Hosny and colleagues from Harvard Medical School (2018) found that AI improves medical imaging by enhancing image quality, increasing diagnostic accuracy, and reducing interpretation time. To validate this, Isabella Churchill and her team at McMaster Hospital (Churchill et al., 2022) conducted a two-phase study using the NodeAI algorithm, achieving 73% accuracy in Phase A by training and comparing on past and known LN images. In Phase B, accuracy increased to 76% when assessed on unseen new images, improving NodeAI's potential for real-time diagnostic scoring.

NodeAI is an advanced machine learning algorithm that evaluates EBUS images and provides real-time diagnostic scores based on the CLNS. It is also used in other imaging procedures, like MRI, to automatically segment images, thereby reducing time and costs. Leveraging deep learning and GPU processing, NodeAI speeds up analysis from days of manual work to just minutes (Gatti, 2024; NeuralSeg, n.d.).

Dr. Anthony Gatti, a graduate student from Stanford Medical and McMaster University, first developed NodeAI (Gatti, 2018) to automate femoral cartilage segmentation from high-resolution MRI data. The study included 172 MRI scans from 86 individuals, split into training, testing, and validation sets. Segmentation accuracy was assessed using the Dice Similarity Coefficient (DSC), comparing automated results to manual segmentations. NodeAI achieved a mean accuracy of 88.3% for 28 images, indicating high segmentation accuracy. The average segmentation time was 56 seconds, demonstrating the method's efficiency (Gatti, 2018; Gatti, 2024). Furthermore, NodeAI was used in a study by Yogita Patel and colleagues from McMaster University, including Gatti, aimed to validate a stiffness area ratio from endobronchial ultrasound elastography images for diagnosing mediastinal LN malignancy in non-small cell lung cancer. NodeAI assessed bronchial tissue stiffness and created a map to differentiate tissue layers through shades of blue based on elasticity. They analyzed 210 LN images from 124 patients and found 70.59% accuracy, 43.04% sensitivity, 90.74% specificity (Patel et al., 2024). This reveals that NodeAI is adaptable, versatile and beneficial to various medical procedures, building credibility in its incorporation in EBUS procedures.

2.3 Perceptions of AI Among Healthcare Professionals

Despite AI's potential, its integration into healthcare has been staggered due to the controversy and barriers that come with it. The implementation of AI in healthcare faces several ethical barriers, primarily concerns about, "privacy, trust, consent, and conflicts of interest," (Ahmed et al., 2023). Molla Ahmed, Pediatric Respiratory Medicine, University Hospitals of Leicester and colleagues noted that among 59 articles reviewed, 20 highlight confidentiality as a substantial concern. The General Data Protection Regulation (GDPR) emphasizes patient control over their data and the need for informed consent when sharing data with AI developers (Marcu et al., 2019).

Trust in AI is another significant barrier, noted in 25 studies (Ahmed et al., 2023). Healthcare professionals often lack training to evaluate AI tools, and the lack of rigorous, randomized controlled trials creates skepticism (Ahmed et al., 2023; Chen & See, 2020). Many AI algorithms operate as "black boxes," where users can see the input and output but lack insight into the process in between, and how AI arrived at those results (Sakamoto et al., 2020). This reduces confidence in validation and puts strain on doctor-patient relationships, raising concern and hesitance about AI's integration into healthcare treatment (Brady & Neri, 2022).

2.4 Gap Analysis

Despite research on AI efficacy in EBUS procedures, there is a gap in understanding clinicians' perspectives on adopting these technologies. Current literature often overlooks the factors influencing acceptance among medical providers, impacting the successful implementation of AI in diagnostics (Churchill et al., 2022). My research aimed to explore how Canadian endoscopists perceive the effectiveness, usability, and potential barriers to incorporating NodeAI into EBUS procedures. The findings of this study hope to provide valuable insights into the barriers and facilitators of AI adoption in clinical settings, ultimately guiding the successful integration of AI technologies like NodeAI into routine diagnostic practices (Koseoglu et al., 2023). By understanding clinicians' perspectives, this research could inform strategies to improve AI adoption, enhancing diagnostic accuracy and efficiency in EBUS procedures.

3. Methodology

This study used a convergent parallel mixed methods (CPMM) design, incorporating a qualitative nominal group technique (NGT) alongside baseline and endline surveys to capture shifts in opinions and facilitate discussion among expert participants. The following methodology section will detail the study design, participant selection, data collection process, and ethical considerations.

3.1 Design and Approach

This study employs a CPMM design developed by Creswell (2009), which "consists of taking quantitative and qualitative data collection and analysis and comparing the two and then interpreting them" (Harvard Catalyst, 2014). Mixed methods combine both quantitative and qualitative approaches, collecting both numerical and non-numerical data, integrating numerical data with in-depth insights, offering a fuller understanding. The CPMM design collects and analyzes two types of data simultaneously but separately (Damyanov, 2023). Also, it allows for cross-validation of results, as findings from qualitative and quantitative sources can support or contrast with each other, increasing the reliability of the conclusions (Ahmed et al., 2024). In CPMM, qualitative data often comes from interviews, focus groups, or observations, while quantitative data is typically derived from surveys, tests, or statistical measures (Damyanov, 2023).

This design is particularly useful when researching a new phenomenon with limited existing knowledge and literature. The CPMM is primarily used in healthcare and medical research to evaluate new interventions, technologies, and treatment strategies, especially when there is minimal prior research (Tomasi et al., 2018). It is beneficial in contexts where both broad patterns and individual experiences are valuable, as it enables researchers to compare different data types (Alele & Malau-Aduli, 2023). An example of a CPMM design is a study by Rosenkranz, Wang, and Hu from the University of Western Sydney School of Medicine (2015), which aimed to explore what motivates and demotivates medical students to pursue research. The study collected quantitative data through surveys and qualitative data via semi-structured interviews. The data were analyzed separately, and the results were then compared and integrated. Since this study aims to analyze perceptions, which, like motivations, are driven by values and are subjective, this design is particularly well-suited to capture these complex factors.

Alternatives, such as the Delphi method, which relies on iterative rounds of surveys to build consensus among experts, are often time-consuming and can be challenging for busy healthcare professionals to fully engage with. Additionally, the lack of real-time interaction in the Delphi method limits the opportunity for in-depth discussions, making it harder to explore the complex implications of AI in clinical practice and how it could impact decision-making (Nasa et al., 2021). Similarly, the NGT, which generates consensus through structured face-to-face discussions, is effective in gathering diverse perspectives but does not capture shifts in opinions over time. Without a baseline to measure changes, the NGT falls short in understanding how expert views evolve, particularly when new information or perspectives are introduced (Burke et al., 2019). These limitations make the CPMM a complete and nuanced understanding by combining qualitative insights from focus groups with quantitative survey data. This approach informs AI implementation and future research, addressing the research question of the impact of NodeAI on diagnostic accuracy and efficiency.

3.2 Sampling and Recruitment

For this study, purposeful sampling was employed to select nine thoracic surgeons and pulmonologists with expertise in EBUS and NodeAI technology (Patton, 2015). This sampling strategy ensures diversity while maintaining relevance to the research question, ensuring knowledgeable and insightful feedback. Recruitment was conducted via email, using an email list provided by an external advisor, a cardiothoracic surgeon. Once participants were confirmed, they were asked to commit to the duration of the study to ensure consistent and reliable participation throughout the research process.

3.3 Data Collection

3.3.1 Surveys

The first questionnaire was sent to participants to gather baseline data for the study. The survey included both open and closed-ended questions to explore demographic and professional information. The demographic section collected data on the expert's name, institutional affiliations, and credentials, helping to gauge the diversity of perspectives and the expertise represented. The professional section focused on the participants specialization, years of experience with EBUS and satisfaction with biopsy yields. Closed-ended questions, including Likert scale ratings, collected quantitative data on practices such as LN selection and the use of endosonographic scoring systems (like CLNS).

Following the focus group discussion, an endline survey was administered to capture shifts in expert opinions. The survey assessed changes in perspectives on the use of NodeAI in diagnostics, including perceived improvements in diagnostic accuracy, procedure time, and ease of use. Questions measured the likelihood of incorporating NodeAI into clinical practice. This allows for comparison with baseline data, providing insights into how group discussions have influenced individual views. This design is advantageous as it offers a clear evaluation of how specialist perceptions evolve, enhancing the study's understanding of AI's impact on EBUS procedures.

3.3.2 Focus Group

The guide aimed to explore participants' approaches to mediastinal staging, LN biopsy criteria, and their opinions on AI's potential role. The focus group included open-ended questions that provide deep qualitative data, such as how ultrasound features and the CLNS influence their decisions, and how they perceive AI's use. After an online demonstration of NodeAI, participants shared their initial thoughts, assessed its usefulness, and discussed how it might be incorporated into clinical practice. These qualitative findings were recorded in the quantitative survey that measured shifts in participants' views on AI immediately at the end of the focus group discussion. The integration of both qualitative and quantitative methods allows for a more comprehensive understanding of NodeAI's impact on clinical practices and its potential for implementation.

3.4 Data Analysis

Following my data collection, participants responses were synthesized into a comprehensive report. Qualitative data analysis was used to identify any changes in expert opinions and determine if a consensus had emerged regarding the use of NodeAI in EBUS procedures. Thematic analysis was applied to the open-ended responses: qualitative data was coded into categories, such as expected themes, unexpected findings, and significant insights, to uncover underlying patterns and trends, while quantitative data, including binary responses and Likert scales, was summarized and expressed in graphs and figures. Less emphasis was placed on statistical analysis compared to the qualitative insights, which form the core of the findings.

3.5 Ethics Memorandum

As this research did not involve the use of patient data or information, patient consent was not required. All participants were adults aged 25 and above. Before data collection, all participants signed an informed consent document that details their rights as participants. To ensure confidentiality, all results collected from the survey remained anonymous and were not used to identify any of the respondents. Participants were given pseudonyms, and their data is kept in password-protected files. This study was approved by the school's Internal Ethics Review Board and all procedures adhere to the ethical standards of the institution.

4. Results and Discussion

The study included nine participants with diverse specialties, professional experience levels, and practice settings (Table 1). This range of backgrounds provides a well-rounded perspective on the potential role of AI-assisted tools like NodeAI in EBUS procedures.

4.1 Concerns or Factors Influencing Confidence of AI and EBUS

Data collected from the baseline form, endline form, and focus group uncovered trends regarding the factors influencing people's perceived confidence in EBUS-TBNA and AI, specifically in NodeAI. In the baseline and endline surveys, participants were asked to distinguish their concern level regarding the accuracy of AI algorithms in medical diagnostics.

As shown in Figure 1, the double bar graph shows that after the focus group, participants' concerns surrounding the accuracy of AI algorithms increased. The number of participants who were somewhat unconcerned dropped to zero, while those who were somewhat concerned and concerned rose, reflecting increased awareness and skepticism about AI's reliability. Figure 2 reveals specific reasons.

Table 1. Subject Information

Participant	Practice	Affiliated organization(s)	Years of Practice
1	Thoracic Surgery	Academic or university hospital, research practice	>10
2	Thoracic Surgery	Academic or university hospital, research practice	6-10
3	Interventional Pulmonology	Academic or university hospital, community practice	0-5
4	Both	Academic or university hospital	6-10
5	Interventional Pulmonology	Academic or university hospital, research practice	0-5
6	Thoracic Surgery	Academic or university hospital	>10
7	Thoracic Surgery	Community practice, research practice	0-5
8	Thoracic Surgery	Academic or university hospital	>10
9	Interventional Pulmonology	Academic or university hospital	>10

Figure 2 highlights the primary concerns clinicians have regarding AI in medical diagnostics, specifically in EBUS-TBNA. The most significant concern, identified by 78% of participants, is the potential for over-reliance on technology, indicating a fear of losing human oversight in decision-making. Participant 4 expressed their concern for over-reliance on AI among less experienced bronchoscopists like trainees: "I am afraid that they become so dependent on it [NodeAI] they can't read lymph nodes anymore," referring to trainees potentially losing critical diagnostic skills. While initially viewed as a limitation, this concern was later reframed as an educational opportunity: "For those just starting, NodeAI could be a game-changer in helping them decide which to biopsy." With NodeAI real-time feedback and training, participant 4 highlighted that it could be used to teach trainees the differences between malignant and benign LNs, which could improve future clinicians and help reduce operator variability.

Ahmed and his colleagues' concern about "privacy, trust, consent, and conflicts of interest" appears to be common among the participant sample with 33% of participants believing that reliability of AI-generated results, data privacy and security issues, and cost of implementation were of concern (Ahmed et al., 2023). The concern about lack of training and support was the least prevalent, at only 11%, suggesting that clinicians may feel less concerned about educational gaps than about the tangible risks and challenges associated with implementing AI.

Additionally, challenges remain regarding accuracy, with one participant commenting, "Accuracy (79%) is not great, and if NodeAI is supposed to be the final source to decide on whether to biopsy a LN, we need higher accuracy." This concern underscores the need

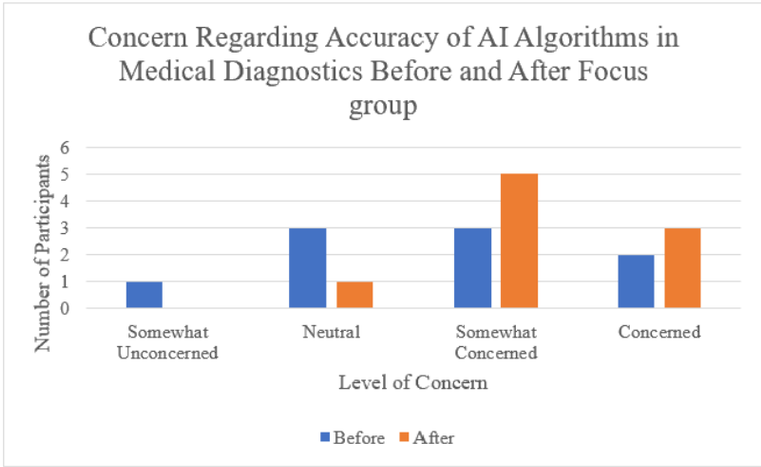

Figure 1. Double bar graph displaying concern regarding the accuracy of AI algorithms in medical diagnostics before and after focus group.

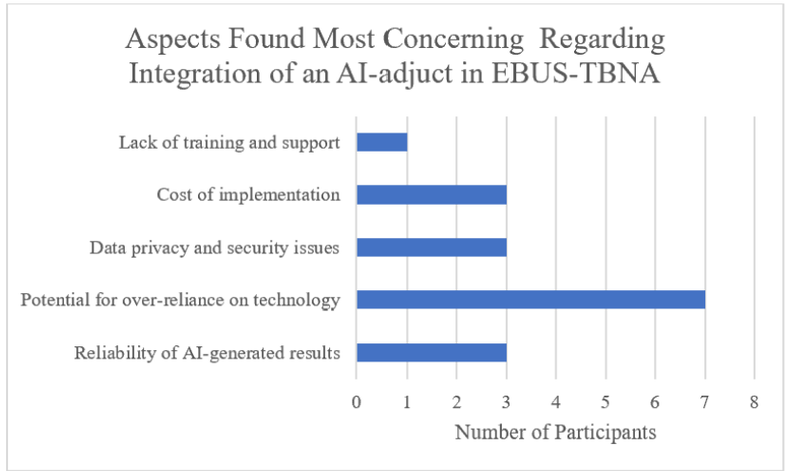

Figure 2. Bar graph of aspects found most concerning regarding accuracy of AI algorithms integrated EBUS-TBNA cases.

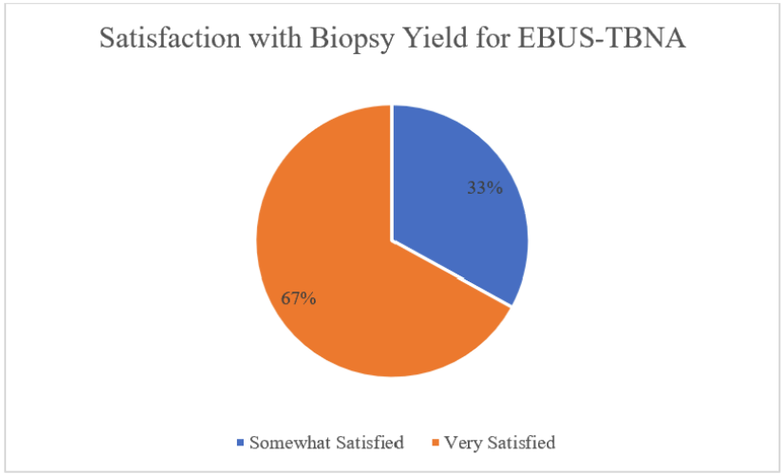

Figure 3. Participants satisfaction with biopsy yield for current EBUS-TBNA practices.

for continuous refinement of the AI model to ensure its practical applicability in clinical decision-making.

Despite the many concerns, overall satisfaction determined in the baseline survey seems positive.

In question 4 of the baseline survey, participants were asked to rate their satisfaction with the current EBUS-TBNA practice. As shown in Figure 3, 67% of participants reported that they were very satisfied with the biopsy yield from EBUS-TBNA, and 33% were somewhat satisfied. This indicates that most clinicians felt that the procedure was effective in yielding satisfactory results. Yet, most clinicians have concerns regarding current methods, as shown in Figures 1 and 2.

4.2 Current Methods

Figure 4 reveals that the majority of participants (78%) prefer using both CT/PET and endosonographic criteria for LN selection in EBUS-TBNA. In contrast, a smaller group (22%) prefers to biopsy all LNs regardless of imaging criteria or pre-test probability. This suggests a preference for a more targeted sampling as described by Hylton et al. (2021b). Guidelines recommend systematic staging, but in practice, many clinicians choose a more targeted approach, particularly for small or triple-normal LNs. According to three participants during the focus group, there is a desire to avoid unnecessary sampling of low-yield or likely benign nodes, supporting Sanz-Santos et al.'s (2022) theory that TS is not inferior to SS. However, in the focus group, Participant 2 was strongly against targeted sampling despite its 80% accuracy rate, stating, "I think more information is better… If you don't try, you don't know for sure, and you're still saying that 5% of triple-negative LNs can have cancer in them. It's still not 0%, right?" Participant 7 challenged this perspective, arguing, "I think these imaging devices will be extremely important because there are so many lymph nodes. We're not just talking about one lymph node within one station; you might see two or three lymph nodes, and we cannot sample all of them. You really want to look at the ultrasound image and sample the lymph nodes that are the most suspicious." Like Sanz-Santos and Hylton, supporters of targeted sampling emphasized that it saves time and resources, ultimately reducing waiting times, human availability, and costs.

Figure 5 shows a wide range of usage of endosonographic scoring systems, with 44% of participants reporting they always use the system, while 22% never use it. A small number of respondents rarely (11%) or occasionally (11%) use the system, and 11% use it fre-

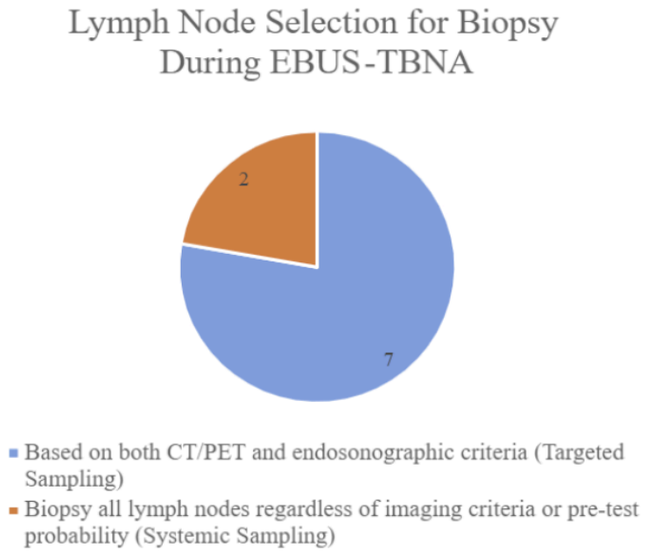

Figure 4. Pie graph of participants selection process for biopsy during EBUS-TBNA.

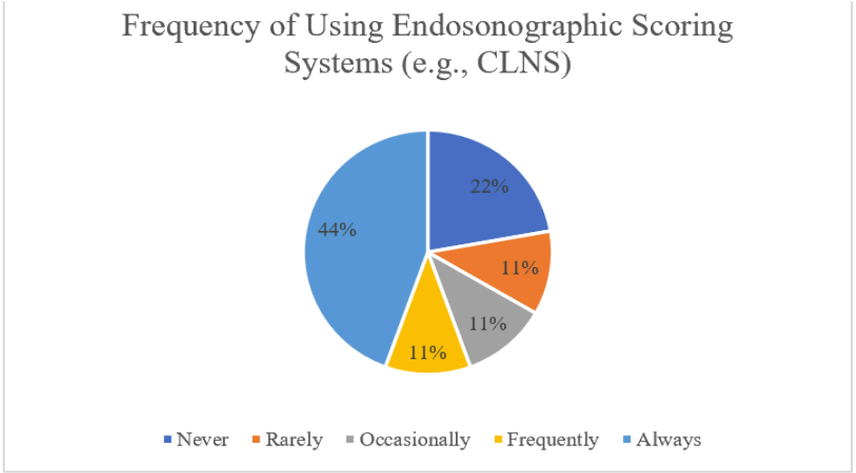

Figure 5. Pie graph showing participants frequency of using endosonographic scoring systems (e.g. CLNS) during EBUS-TBNA cases.

quently. This variability suggests that while some clinicians consistently use scoring systems, others may either lack familiarity or face barriers to incorporating them into routine practice. This is further supported by Participant 5 from Edmonton, who, after completing respirology training, noted that they did not use a scoring system like CLNS. Instead, their approach was to wait for substantial LN biopsies to return as non-diagnostic, and if that occurred, they would typically repeat the procedure. However, after coming to McMaster Hospital in Hamilton and learning about the CLNS, their approach changed significantly. Additionally, Participant 9 from Oshawa, Southern Ontario, reported not following the CLNS, highlighting regional variations in standardized protocols.

4.3 Perceptions and Potential Surrounding AI Integration and EBUS-TBNA

Figure 6 shows a slight increase in clinicians' perceptions of NodeAI's benefits for predicting malignancy in LNs during EBUS-TBNA. In the pre-survey, 33% of participants found it not beneficial, 33% viewed it as slightly beneficial, and 33% considered it beneficial. Post-survey results showed an increase in the number of participants who rated NodeAI as slightly beneficial (44%), a decrease in those who rated it not beneficial (22%), while beneficial remained unchanged (33%). This shift suggests that, through further understanding and discussion of AI adjuncts, clinicians may develop a more positive view of its application.

Like positive perceptions, the likelihood to use an AI-adjunct (NodeAI or other) for predicting Malignancy in EBUS-TBNA cases also increased. Figure 7 shows an increase in the likelihood of using an AI-adjunct (NodeAI or similar) for predicting malignancy in EBUS-TBNA cases after exposure to the technology. Initially, 78% of participants were likely to use AI, with only 22% neutral, and none were unlikely. After exposure, the number of participants who were likely to use AI rose to 89%, while only one participant indicated neutrality. This suggests an overall more positive perception of NodeAI specifically as supported by Figure 8.

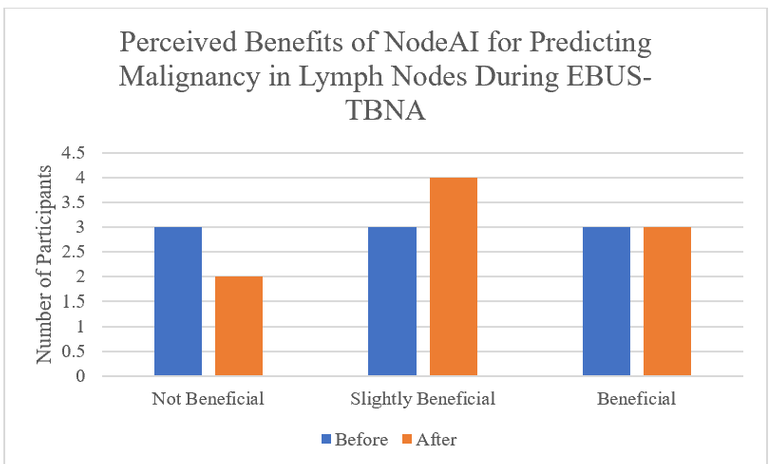

Figure 6. Graph of participant's perceived benefits of NodeAI for predicting malignancy in lymph nodes during EBUS-TBNA.

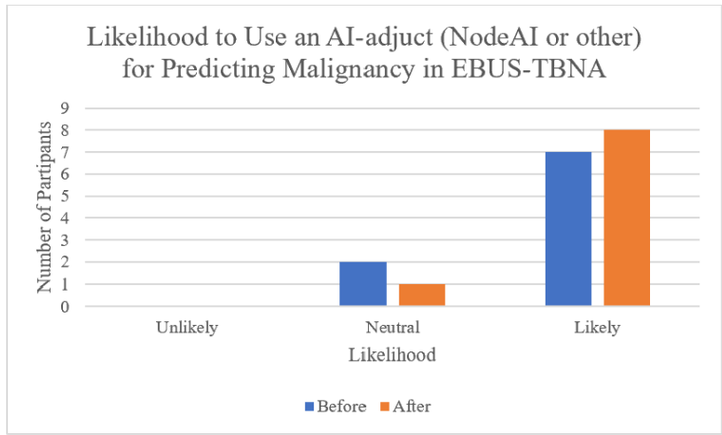

Figure 7. Graph showing participants likelihood to use an AI-adjunct (NodeAI or other) for predicting malignancy on EBUS-TBNA before and after the focus group.

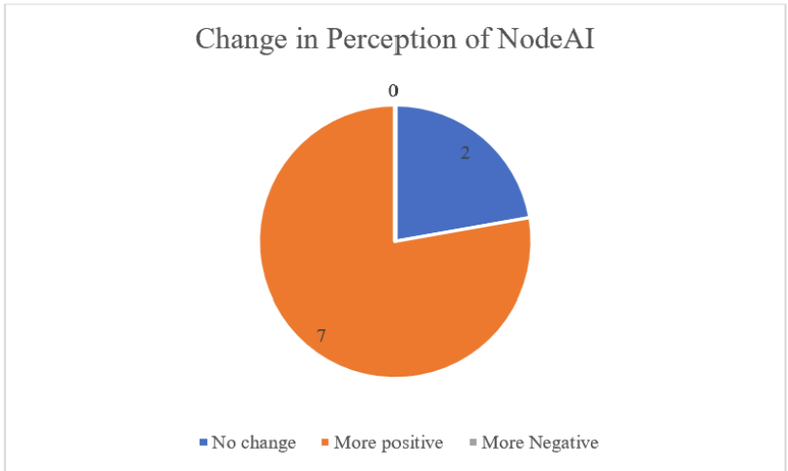

Figure 8. Graph displaying participants change in perceptions of NodeAI after the focus group.

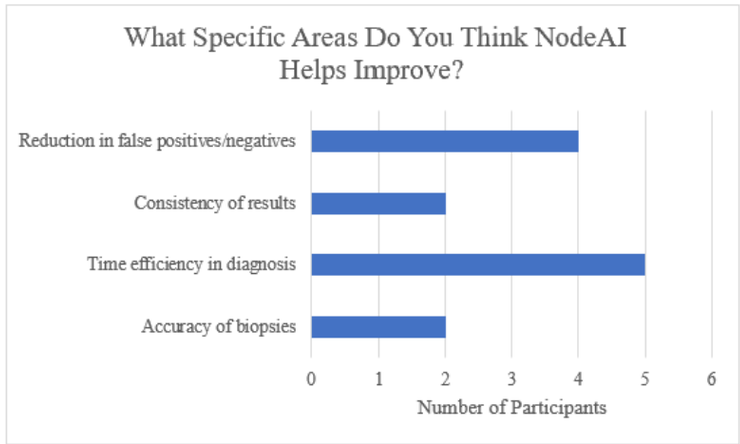

Figure 9. Graph showing specific areas NodeAI is perceived to improve after focus group by participants.

Determined by a question in the endline survey, Figure 8 reveals that after exposure to NodeAI, 78% of participants reported a somewhat more positive perception, while 22% experienced no change in their views. None of the participants reported a more negative perception, suggesting that exposure to NodeAI had a generally positive influence on clinicians' attitudes. Many factors and perceived benefits were both reported during the survey and discussed in the focus group.

Figure 9 highlights the areas where clinicians believe NodeAI could improve EBUS-TBNA. The most commonly identified benefit was time efficiency in diagnosis, with 56% of participants noting this as a key improvement. This was consistent in the focus group, as many participants believed that NodeAI could reduce procedure times as it speeds up decision making. However, Participant 1 thought that it could prolong procedure time due to its unfamiliarity, yet this concern was quickly diminished after the proposal of creating training modules and tutorials on NodeAI. Furthermore, anesthesia complications, such as sedation issues, have been reported. Participant 2, a clinician from Quebec, mentioned, "We sedate patients, but sometimes they cough and fight back, so we have to change plans. We can't biopsy all the nodes, and we have to skip some." Participant 9 said, "These difficulties worsen in settings where anesthesia availability is limited which reduces ability to access LNs and procedure time on patient due to their consciousness," further emphasizing the importance of speed and targeted sampling to ensure the procedure's success.

This led one participant, currently practicing in California but with prior training in Ontario, to explain, "In Canada and other public systems, reducing procedure time is a major priority. But in the United States, time and reimbursement concerns are different." Despite these differences, Participant 3 emphasized that overall cost savings, such as fewer pathology tests and repeated procedures, are still valuable. Thus, shortening procedure time should not be the main selling point if NodeAI wants to reach the US as well.

Reducing false positives/negatives was also a significantly desired area of improvement, cited by 44% of respondents. Accuracy of biopsies and consistency of results were selected by 22% of participants. This smaller percentage may be due to NodeAI's role in aiding decision-making, but its limitations, such as not assisting with scope movement, capturing clear ultrasound images of the LN, and ensuring conclusive biopsy yield, were also factors. This suggests that Canadian clinicians see the most immediate value of NodeAI in enhancing diagnostic speed and accuracy, with other valuable, yet less obvious benefits that may attract a broader range of users.

Also, achieving high-quality ultrasound images of LNs is objectively challenging without proper experience. This is true for those who are less experienced, learning and have trouble visualizing the LNs. Furthermore, obtaining a sufficient biopsy yield is challenging, but confirming takes even longer. Turnaround time for pathology (especially in the absence of on-site cytology, such as ROSE) can delay treatment decisions. As Participant 2 shared, "If we don't have ROSE [rapid on-site evaluation] the delay in getting pathology results can really impact treatment timelines." While NodeAI cannot directly help with the technicalities of EBUS-TBNA, it can help minimize variability when determining abnormal or normal LNs, a critical concern agreed by the majority of the sample during the focus group. This variability can result in inaccurate LN assessments, making it difficult to determine the best nodes for biopsy.

To improve EBUS-TBNA, the integration of AI tools like NodeAI offers many benefits. According to Figure 6, 44% of participants saw NodeAI as at least slightly beneficial and 33% of participants as beneficial for predicting malignancy. For beginners and less experienced clinicians, AI can assist in identifying the right LNs to biopsy, enhancing decision-making. An AI tool (like NodeAI) that predicts malignancy in real time could help clinicians decide whether or not to biopsy a particular LN. This might reduce passes, limit overall sedation time, and spare pathology resources.

5. Future Research

Looking forward, the future of AI in medical procedures like EBUS-TBNA depends heavily on its integration into work-flow and its ease of use. According to Figure 7, after exposure to NodeAI, 89% of participants were likely to use AI in predicting malignancy, indicating interest and a willingness to adopt the technology. However, participants agreed that testing is crucial to ensuring that clinicians are comfortable using the technology. As Participant 8 explained, "We need to test it out in our individual practices to ensure it is feasible to integrate." All participants in the focus group agreed that the tool must be adaptable to various clinical environments to ensure widespread adoption. Moreover, while ease of use is important, NodeAI must continue to evolve to meet the accuracy standards required for clinical decision-making to build confidence among users. Long-term testing and the integration of training modules will be key in overcoming initial hesitations. As the tool undergoes further development, NodeAI has the potential to improve diagnostic accuracy, reduce procedure times, and offer valuable educational opportunities for clinicians, ultimately enhancing EBUS-TBNA cases and patient outcomes.

6. Limitations

Limitations of this study include groupthink, in which participants align their opinions to the dominant narrative, limiting the diversity of feedback and authenticity of the data (Jhangiani, 2022). To address this, clear expectations were set at the beginning of the session, encouraging participants to express their own views and actively asking for differing opinions. Additionally, since AI is a relatively objective topic and less likely to evoke emotional responses compared to other subjects, it reduces the likelihood of personal biases or discomfort affecting the discussion. Lastly, to prevent any one participant from dominating the conversation and ensure all voices are heard, a round-robin approach was used for certain questions. This ensures that everyone had an equal opportunity to speak and that all perspectives were represented in the discussion.

Moreover, the results are limited by the perspectives of the nine recruited experts. With all participants being either interventional pulmonologists, thoracic surgeons, or both, their views are largely from a scientific perspective. While it is no doubt important to have scientific voices represented, gathering experts from outside the scientific and medical spheres could yield a greater range of perspectives. For instance, incorporating EBUS-TBNA patients could provide valuable insight into the patient viewpoint, including their concerns, expectations, and perspectives on AI-assisted diagnostics. Additionally, another limitation is the overrepresentation of participants from the

Greater Toronto Area, which may not capture practices in other regions. Expanding the study to include participants from diverse geographic areas, such as rural centers or regions with varying levels of technology, workflow and resources, could provide a more comprehensive understanding of how NodeAI might be implemented in different clinical settings.

7. Conclusion

This study aimed to examine the extent to which clinicians perceive NodeAI as a valuable tool in comparison to traditional diagnostic methods. Research revealed current use of sonographic methods such as the CLNS to determine abnormal lymph nodes, and shifting towards a more targeted approach as opposed to systemic as supported by many of the studies and participants. A CPMM approach was used, combining both qualitative and quantitative data from surveys and focus group discussions with nine experienced endoscopists across North America. Through data collection, I found that while all participants are currently satisfied with EBUS-TBNA, all had significant concerns including biopsy yield and nondiagnostic samples, decision making (identifying which nodes to biopsy), and variability in operator experience and diagnosing. After the discussion of NodeAI and AI-adjuncts, participants reported NodeAI's potential, including improved targeted sampling, support for decision making, and education, with most participants citing reduced time and resources. These findings align with existing literature that suggests AI has the potential to improve diagnostic outcomes, but also underscores the importance of workflow integration and proper training to prevent overreliance on technology.

Future research should investigate patients' perceptions of integrating NodeAI and other AI-adjuncts into medical procedures. Further test-trials and development to increase NodeAI's accuracy (currently at 79%) must be done, as all participants identified that as a critical barrier. In addition, determining its value proposition to reach a large audience, as other countries (such as the United States) may be less motivated solely by time savings. Also, participants unanimously expressed a desire to pilot the device in real-time clinical settings to demonstrate workflow feasibility and design larger trials to prove clinical impact, cost savings, and user adoption. Overall, the findings communicate the importance of balancing technological innovation with clinical expertise to deliver the most effective patient care.

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The Effect of Glass Fiber Reinforcement on the Thermal Properties of PETG

Zayden Chambers

Abstract: In the additive manufacturing industry, many polymer composites have properties - thermal and mechanical - that are unknown. The purpose of this study was to determine the key thermal properties of Polyethylene Terephthalate Glycol (PETG) with and without 15% glass fiber to understand the extent to which the glass fiber (GF) reinforcement affected the thermal properties of the thermoplastic. The PETG and PETG GF samples were produced via Fused Deposition Modeling (FDM). The samples were then weighed and tested using Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and a Dynamic Mechanical Analysis (DMA). The change in thermal properties given by these instruments suggests that the glass fiber has a minimal effect on the key thermal properties when reinforcing PETG. These findings imply that using a material like PETG GF is not recommended for applications that require high temperatures.

Keywords: Additive Manufacturing, Fused Deposition Modeling, Polymer Composites, Glass Fiber Reinforcement, Poly Ethylene Terephthalate Glycol, Thermal Properties.

1.1 Introduction

Over the years, many industries have utilized additive manufacturing (AM) to effectively produce desired products. Additive manufacturing is used in many ways to create these products, each having its own advantages and disadvantages. Common additive manufacturing processes include material jetting, where the machine produces droplets of a material layer by layer. Selecting laser sintering (SLS) is a process in which a laser combines power-based layers into three dimensional objects. Fused deposition modeling (FDM) uses a nozzle - fed with thermoplastics - to eject the plastic in a controlled manner with increasing layers. Among the plethora of AM methods, FDM is the most widely used due to its accessibility.

Numerous thermoplastics can be used as filament in FDM, all of which have their own properties. For instance, the most popular thermoplastic for FDM is Polylactic Acid (PLA), which is a completely biodegradable plastic made from plant starch. Acrylonitrile Butadiene Styrene (ABS), however, is a thermoplastic known for its stiffness and its production of an unpleasant odor when extruded. Polyethylene Terephthalate Glycol (PETG) is another popular thermoplastic that is known for its chemical resistance and mechanical properties. Hence, PETG is widely used in food packaging and medical applications. It is evident that each thermoplastic has a weakness, so manufactures use different types of reinforcements - an additive that is combined with a thermoplastic in hopes of strengthening performance of the plastic - to assist the material with performance.

Reinforcements are commonly used to create numerous types of polymer composites (a thermoplastic and a reinforcement). There are many different types of reinforcements; the most common ones include carbon fiber (CF), glass fiber (GF), and Kevlar fiber (KF). Similar to thermoplastics, each reinforcing fiber has its different benefits and characteristics. For instance, carbon fiber is known for its excellent thermal resistance, whereas glass fiber is popular for increasing strength in the composite. There are numerous thermoplastics, with numerous reinforcements; the combinations of composites are nearly endless. Researchers have been trying to optimize different properties by combining a thermoplastic and reinforcement, but many combinations have yet to be studied. For example, the use of PETG combined with GF has not been assessed thermally. In turn, the properties of this composite have not been tested, making it difficult to evaluate the composite's full potential.

Literature Review

2.1 Overview of FDM and Polymer Composites

As time progresses, the use of additive manufacturing - the act of creating an object one layer at a time - has become prominent in the production process [1]. There are numerous types of additive manufacturing, with fused deposition modeling (FDM) becoming one of the most utilized [2]. FDM works by extruding thermoplastic filament through a heated nozzle one layer at a time until the object is fully created. FDM is most commonly used because of its ease of use compared to other techniques of additive manufacturing, and material variability [2].

There are plenty of thermoplastics available to use for FDM, each with its own benefits. The three most common thermoplastics for FDM are Polylactic Acid (PLA), Acrylonitrile Butadiene Styrene (ABS), and Polyethylene Terephthalate Glycol (PETG) [3]. PLA is used for its biodegradability and its low risk of warping when produced [4]. However, PLA can be brittle and does not have optimal heat resistance [4]. ABS is a strong and heat-resistant thermoplastic, but has a higher chance of warping and requires ventilation when conducting FDM [5]. PETG is known for its ductility and chemical resistance, but needs specialized settings for FDM and has moderate heat resistance [6].

In an attempt to mitigate the disadvantages of thermoplastics, reinforcement fiber is added. The addition of reinforcement fibers with traditional thermoplastics results in a polymer composite. Reinforcement fibers are known to increase strength, thermal stability, and wear resistance, all while reducing weight. Common reinforcement fibers include Carbon Fiber (CF), Glass Fiber (GF), and natural fibers [7].

2.2 Thermal Role of Glass Fiber Reinforcement

The addition of glass fiber significantly changes the thermal properties of thermoplastic polymers including PLA, ABS, and Nylon [8]. When combined with PLA, the glass fiber commonly improves the thermal stability (to an extent) and tensile strength of the composite [8]. ABS acquires enhanced heat resistance and dimensional stability when glass fiber reinforcement is added. Nylon with added glass fiber reinforcement is known to increase thermal conductivity and the decomposition temperature of the composite [8].

Generally, the addition of appropriate fiber to a thermoplastic will most likely increase the glass transition temperature (Tg), decomposition temperature (Td), and thermal conductivity, whereas the specific heat capacity (Cp) will decrease [9]. The glass transition temperature increases due to the restraint on the polymer chain mobility. The decomposition temperature generally increases, as most fibers improve thermal stability, which has a direct effect on the temperature at which the composite starts to decompose. Generally, the fibers will store less heat than the thermoplastic that is being reinforced. This will cause a decrease in the specific heat capacity. The thermal conductivity of the composite [10] will increase because of the fibers allowing for improved heat transfer pathways [9].

It is important to note that the type of fiber reinforcement and morphology will have an effect on the properties of the composite. For instance, short fibers and continuous fibers have differing effects when reinforcing a composite [11]. Short fiber reinforcement is randomly orientated, which decreases the mechanical

strength of the composite, whereas continuous fibers drastically improve the mechanical properties. The fiber length, orientation, and volume fraction need to be considered when reinforcing a thermoplastic [12].

2.3 Thermal Testing in Polymer Composite Research

In the literature on thermal testing with polymer composites, many studies use thermal characterization instruments in order to gain credible thermal properties for the material [13]. One of the most commonly used instruments for thermal characterization is a differential scanning calorimetry (DSC). The DSC requires two crucibles - one where the sample is placed and a reference crucible. Both crucibles are heated in a controlled environment. While the heating occurs, the DSC records the difference in the heat flow between the sample and reference crucibles. The DSC records phase transitions of the sample, ultimately providing values such as melting point, heat capacity, and stability [14]. In the DSC, the specific heat capacity is calculated with the equation:

$$C_p = \frac{q}{m \cdot \Delta T}$$

where q is heat flow, m is mass, and ΔT is the temperature change.

Another common instrument used in thermal testing is a thermogravimetric analysis (TGA). The TGA requires a sample to be placed in a furnace. The furnace gradually heats up in a controlled environment, as the instrument measures the weight of the sample. The sample is exposed to varying gases, while the TGA records the temperature [13].

A dynamic mechanical analysis (DMA) is a testing instrument that can be used to obtain both mechanical and thermal data. The DMA gives data relating to the viscoelastic properties of the sample. A DMA requires a sample that will respond to a constant, oscillating force while simultaneously heating. The DMA records values such as strain, stress, storage modulus, and loss modulus. The damping factor (tan) is calculated by the equation:

$$tan\,\delta = \frac{E''}{E'}$$

where E'' is loss modulus and E' is storage modulus.

Most studies using these instruments widely accept the rules and guidelines set by the American Society for Testing Materials (ASTM). These rules give procedure protocols to guarantee safety while testing. Commonly used protocols include ASTM D3418 for DSC and ASTM E1131 for TGA [15].

2.4 Existing Gaps in PETG GF Literature

Although there has been a significant amount of research dedicated to polymer science, a multitude of gaps still need to be addressed. More specifically, there gaps remain regarding certain polymer composites, like PETG GF. Most of the research in this topic focuses on carbon fiber, or graphene reinforcement, which has been shown to enhance the mechanical and thermal properties when combined with most polymers [17,20]. However, many researchers do not consider the advantages of glass fiber, such as its lower cost, printability, and thermal insulation properties; glass fiber should be prioritized over its counterparts for specific applications where these factors are crucial. Despite these benefits, studies on glass fiber have not identified key thermal properties like the specific heat capacity and thermal conductivity, especially when combined with PETG for 3D printed applications.

Existing studies investigating glass fiber with other polymers like PLA or ABS suggest that the glass fibers help increase the thermal and mechanical properties of the material [18,25]. However, these findings cannot be assumed with PETG, as each polymer has its own molecular structure, as well as thermal behavior. Additionally, these studies specialize in observing the mechanical properties of the composites, leaving little to no focus on the thermal properties of the glass fiber composite. All of these factors leave a gap in the literature that will need to be addressed.

The proposed research question is, "To what extent does the addition of glass fiber reinforcement affect the thermal properties of Polyethylene Terephthalate Glycol (PETG) polymers in 3D-printed parts?" Based

on studies surrounding the topic and the mentioned trend of improved properties of polymer composites, the initial hypothesis is that the addition of glass fiber reinforcement will have a significantly positive effect on the thermal properties of PETG. This study proposes to discover the thermal properties of PETG when reinforced with glass fibers, in order to compare the results with PETG in its pure form, to determine how the glass fiber affected the polymer. There are many purposes for conducting this study. One is to determine if PETG, reinforced with glass fiber, can be a cost-effective counterpart to expensive thermoplastics such as polyetheretherketone (PEEK) or polyetherimide (PEI).

3.1 Materials and Methods

This study used an experimental method to investigate the thermal effect that glass fibers have when combined with PETG produced via FDM. This method was chosen in order to gain accurate numerical results of numerous thermal properties, which is important in understanding the materials performance, and determining fitting applications. A quantitative approach was chosen due to the need for numerical data and statistical analysis.

In order to assess the thermal properties of PETG, thermogravimetric analysis, differential scanning calorimetry, and a dynamic mechanical analysis were conducted using a discovery DSC, discovery TGA, Simultaneous TGA-DSC Q600 SDT, and a Q800 DMA. The TGA measured weight loss and thermal stability, while the DSC provided information regarding heat flow, crystallization temperature, glass transition temperature, and melting temperature. The DMA provided viscoelastic properties such as Storage Modulus (E'), Loss Modulus (E"), and tan delta (δ). These methods are standard in polymer research and have been utilized in similar studies for characterizing thermal behavior (Blanco, 2022).

The Glass Fiber Reinforced PETG Filament from TINMORRY (Guangdong, China) was chosen because of the popularity of PETG, and the potential performance enhancement of the glass fiber. The PETG-GF filament had polymer volume content, Vw, of 85%, and GF volume content, Vw, of 15%. The samples were produced via Fused Deposition Modeling (FDM). The FDM production method was chosen because it is the most popular method of producing thermoplastics, and is openly accessible for users.

Table 1
Printing Parameters of PETG and PETG

FDM Parameters	PETG	PETG GF
Extruder temp	250°C	260°C
Printing speed	40mm/s	40mm/s
Bed temp	60°C	85°C
Filament diameter	1.75mm	1.75mm
Fill density	100%	100%
First layer height	0.2mm	0.2mm
Layer height	0.2mm	0.2mm

The validity of the data collected was guaranteed during the data collection of PETG and PETG GF. Internal validity was maintained by standardizing the sample preparation process, including consistent drying conditions and uniform sample sizes for DSC and TGA tests. This technique minimized variation and guaranteed that the differences observed were due solely to the material properties rather than external factors. The content validity of measurement instruments was ensured by calibrating the Discovery DSC and Discovery TGA systems before testing. Furthermore, replicating measurements for each sample confirmed the construct validity and consistency of the data.

3.2 Testing Procedures

Both PETG and PETG GF were dried in a commercial filament dryer set at 70°C before being extruded via FDM. This was done to remove any moisture in the thermoplastic, which will assist with the printing quality. The two materials were printed with an Ender 3-S1 commercial 3D printer, with a hardened steel nozzle attached.

The 3D printed samples were carefully crushed to create small enough pieces weighing approximately 5 mg for the DSC and TGA tests. All samples were ac-

companied by a desiccator before testing to make sure that the samples remained dry.

The Discovery DSC was first calibrated in order to determine accurate results for the tests. The PETG and PETG GF were placed onto the aluminum crucibles, which were sealed and crimped to prevent leakage while testing. All tests in the DSC produced a heating and cooling cycle, with the temperature ranging from ambient to 300°C. The heating rate of 5°C/min was chosen to accurately capture all significant changes during the tests. The test was conducted under a nitrogen environment with a flow rate of 50 mL/min to prevent oxidation. The DSC recorded glass transition temperature (Tg), crystallization temperature (Tc), and melting temperature (Tm) during each thermal cycle.

The Discovery TGA was also calibrated in order to determine accurate results for the tests. The PETG and PETG GF were placed onto the aluminum crucibles, which were sealed and crimped to prevent leakage while testing. All tests in the TGA produced a heating and cooling cycle, with the temperature ranging from ambient to 700°C. The heating rate of 5°C/min was chosen to accurately capture all significant changes during the tests. The test was conducted under a nitrogen environment with a flow rate of 50 mL/min to prevent oxidation. The TGA recorded values such as the onset decomposition temperature (Td), the weight loss profile, and the residual mass percentage after thermal degradation.

The Q800 DMA was also calibrated in order to determine accurate results for the tests. PETG GF with dimensions of 30.0mm x 6.00mm x 2.00mm were attached to the machine with the clamps. All tests in the DMA produced a heating cycle, with the temperature ranging from ambient to 150°C. The heating rate of 2°C/min was chosen to accurately capture all significant changes during the tests. The test was conducted under a nitrogen environment with a flow rate of 50 mL/min to prevent oxidation. The DMA recorded values such as the Storage Modulus, Loss Modulus, Stress, and Tan Delta.

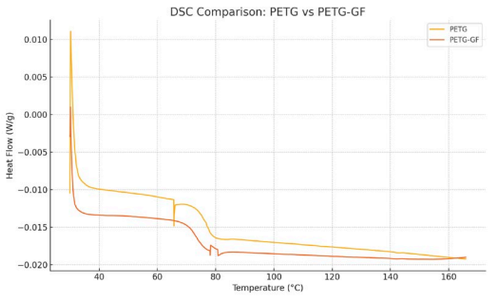
After these results were collected, the TA Universal Analysis software was used to analyze the data. The TA Universal Analysis software is an analytical tool used for TA instruments. The software visualizes data with interactive graphs, providing necessary data points for the research study.

4.1 Results

A quantitative method was used in order to determine the thermal properties of PETG and PETG GF. Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and a Dynamic Mechanical Analysis (DMA) were used in order to record desired properties.

4.2 Results - DSC Data

Figure 1 Heat Flow



Thermal Data from DSC tests:

Table 2

	PETG	PETG GF	% Change
Glass Transition (Tg) (°C)	65.66	78.16	19.04
Crystallization (Tc) (°C)	165.64	154.36	-6.81
Melting Temperatures (Tm) (°C)	323.80	227.42	-2.31
enthalpy of fusion (ΔHf)	-1.19	-1.25	5.04
Specific Heat Capacity (Cp) (J/g·K)	1.27	1.18	−7.09

The glass transition temperature increased from 65.66 to 78.16, which is a 19.04% increase. This implies that the glass fibers being added to the PETG had a substantial effect on the stiffness of the material. Granted, the crystallization temperature decreased from 165.64 for pure PETG to 154.36 for PETG with glass fiber. The glass fiber most likely had a negative effect of the crystal growth, hence the lower value than that of neat PETG. The melting temperature saw a very minimal decrease, going from 323.80 for neat PETG to 227.42 for PETG with glass fiber. The small -2.31% change in melting temperature implies that glass fiber has a minimal/no effect on how PETG melts when heat is applied to it. The enthalpy (ΔH) showed a 5.04 percent increase, going from -1.19 to -1.25. This implies that the glass fiber addition minimally increases the thermal energy exchange for the PETG.

4.3 Results - TGA Data

Figure 2 Mass %

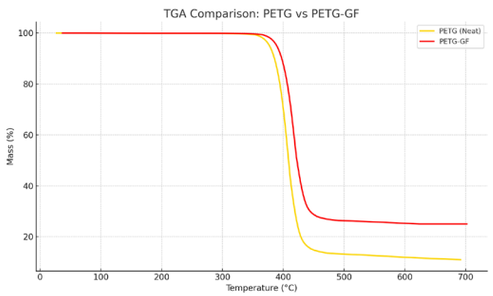


Table 3

	PETG	PETG GF	% Change
Onset Decomposition Temperature (Td)(°C)	377.32	389.2	3.15
Total Weight Loss (%)	89.01	75.0	-15.74
Residual Mass (%)	11.00	75.0	127.27%

The onset decomposition temperature saw only a 3.15 percent increase, having 377.32 °C for Neat PETG and 389.2°C for PETG with Glass Fiber. This implies that the thermal stability increased when glass fiber was added onto PETG. This suggests that PETG with glass fiber is harder to break down (decompose) than neat PETG. In addition, the total weight loss for PETG saw a -15.74 percent reduction. This is because the glass fibers assisted in minimizing the degradation of the PETG when combined with it; the neat PETG degraded normally. However, the residual mass percentage for PETG with glass fiber is nearly double when compared to PETG. This is simply because of the glass fiber residue that was left after the test was complete. It is expected that the glass fibers did not decompose with the TGA tests, explaining why there is glass fiber residue at the end of the test.

4.4 Results - DMA Data

Figure 3 Storage Modulus

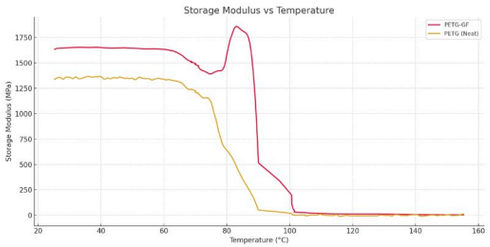


Figure 4 Loss Modulus

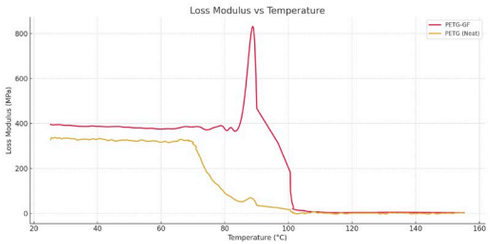


Figure 5 Tan Delta

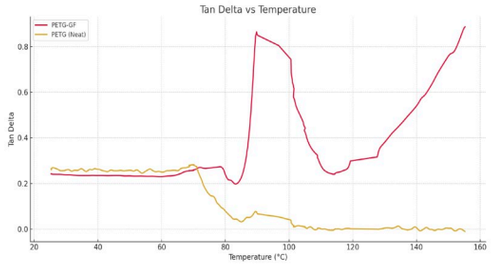
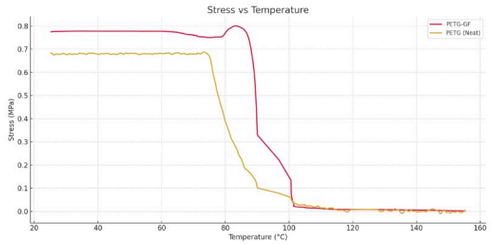


Figure 6 Stress



Overall, the graphs show a higher storage modulus, a higher peak for loss modulus, a higher tan delta, and a better stress graph when comparing PETG GF to PETG. The graphs show that PETG GF maintained a higher storage modulus than PETG. This means that PETG GF is stiffer than neat PETG because of the glass fiber added. PTEG GF had a higher loss modulus than the neat PETG. This means that PETG GF is better at absorbing mechanical energy than PETG. PETG shows a higher tan delta graph, meaning that PETG GF has a higher glass transition temperature, which is verified by the DSC data. The stress graphs show that PETG with glass fiber does better than neat PETG when stress is applied.

5.1 Discussion

The purpose of the study was to answer the question "To what extent does the addition of Glass Fiber affect the thermal properties of PETG produced via Fused Deposition Modeling?" Since the purpose of this study was to determine the thermal properties of PETG and PETG GF and compare the values, an experimental procedure with instruments such as a DSC, TGA, and DMA was taken because these instruments would yield the numerical values needed to analyze the effect of glass fiber. Overall, the data showed that the glass fiber had a minimal, but noticeable effect on the thermal properties of PETG. More specifically, the DSC showed a 19.04% increase in the glass transition temperature and a 7.09% decrease in specific heat capacity when glass fibers were added to PETG. The TGA showed a 3.15% increase in decomposition temperature, and a 15.74% decrease in thermal stability. The DMA showed a ≈ 25% increase in storage modulus. Overall, the glass fiber had a minimal effect on the PETG's thermal properties, but can be important for applications in the medical field.

5.2 Implications

There are many implications of the finding that the glass fiber had a minimal effect on the thermal performance when combined with PETG. This research can be applied to industries that need materials for high-temperature applications. To reiterate, the addition of glass fiber reinforcement does not substantially affect the thermal properties when combined with PETG. A study conducted by Saidova et al. [26] agrees with the results presented in this study, as they found that the addition of glass fiber affects the thermal properties, but other factors that the composite exhibits have a more direct impact on the thermal properties. This said, one cannot assume that since a reinforcement (like glass fiber) improves the mechanical properties of the composite, the thermal properties will also be positively impacted in turn. The results in this study found similar findings to those of Arrakhiz et al. [27], in which they found that doum (low-density polyethylene) drastically increased the mechanical properties of composites, but that the higher the doum concentration, the lower the thermal properties. A direct analysis of the glass fiber and doum should be conducted to observe similarities between the two reinforcements. In applications that require high temperatures in the automotive, medical, and aerospace industries, this study proposes to disregard glass fiber as a candidate for reinforcement.

For high temperature applications, other reinforcements should be explored, or plastics like polycarbonate or PEEK should be used.

5.3 Limitations and Future Research

This study helped develop the current body of knowledge surrounding polymer reinforcements, specifically glass fiber. This study shows that the effectiveness of glass fiber on thermoplastics like PETG is minimal when considering the thermal properties. It is currently acknowledged in the literature that the addition of glass fiber significantly increases the mechanical properties when combined with a thermoplastic, such as PETG. However, the thermal properties of the glass fibers when combined with PETG had a slight change. That said, this studu had many limitations that could have potentially influenced the data acquired.

Firstly, the inevitable porosity in PETG GF (as well as all thermoplastics produced via FDM) could have an effect on the results given by the instruments. In fact, porosity can be attributed to a lack of interfacial bonding strength between the GF and the PETG matrix, which could result in a reduction in the mechanical and thermal performance of FDM printed parts.

In addition, this study only tested PETG combined with 15% glass fiber. Although multiple trials were conducted to verify the composite's performance, having different concentrations of glass fiber content in PETG (ex. 10%, 20%, and 30%) could provide a better understanding of the results and the effect of the glass fibers.

In the future, research should be conducted to find out what reinforcement (ceramic, nylon, carbon fiber) optimizes the thermal properties when reinforcing PETG. This will reveal the extent to which PETG maximizes its thermal properties when in combination with a reinforcement. Additionally, using different manufacturing techniques to produce PETG GF (Material Jetting, SLS, etc.) should also be explored. This will reveal the extent to which different manufacturing methods have an effect on thermal and mechanical properties of polymer composites.

6.1 Conclusion

In conclusion, the results of this study suggest that glass fiber reinforcement does not have a positive effect on the thermal properties of PETG. These results, however, did affirm glass fibers' ability to increase the stiffness and strength of the composites. These conclusions were drawn after conducting three tests of the material: the DSC, which gave insight on the heat flow; the TGA, which gave insight on the weight loss; and the DMA, which gave information regarding stiffness and strength. The study helped fill the gap in the body of knowledge about the thermal effect glass fiber composites have on PETG. This study added that the thermal properties had only minimally increased for this composite.

The study conducted has many implications, focusing on industries that need certain materials for different applications. There is a widely debated issue on whether materials like glass fiber should be used for applications that require heat and high strength. This study supports the idea that glass fiber increases the mechanical strength when combined with a thermoplastic, but the thermal increase is minimal. This study can serve as a warning for industries like manufacturing and aerospace that this material is not suggested for high temperature applications, despite the conclusions drawn from other studies in the past. Instead, industries should invest in polymer materials with better molecular structures (like PEEK & polycarbonate) to better fit the need. This study had many limitations, so future research is needed to further investigate this phenomenon and affirm this notion for glass fiber reinforced composites.

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Kameron Drumright conducted this research through obtaining tweets that reflect the experiences and opinions of Black students attending either a Historically Black College/University (HBCU) or a Predominately White Institution (PWI). The tweets were coded into 12 codes that range from "Student Alienation" to "Intraracial Appreciation/Judgement" to "Institutional Appraisal/Criticism" and many others. These codes, in the context of the historical background of education for Black Americans and past scholarly literature from education scholars and historians, were used to deduce themes in the students' tweets on what could be done to recognize complex racial-identity navigation and target different issues expressed by these Black college students on a personal level as well as an institutional level.

Harshavardan Gunasegaran is a Grade 12 student at Royal St. George's College with a strong interest in AI technologies and the impact of AI on the humanities and engineering disciplines. He has participated in hackathons such as PeddieHacks and CirFin Create, where he received first place for his innovations and software. He hopes to pursue a degree in computer science at university.

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Kevin J. Tritschler is a graduating high school senior with an interest in the environmental sciences. Since he began his school research program in 10th grade, he has had ample time to learn the basics in research analysis, media literacy, and hands-on experimental work. In his most recent summer project, he has investigated whether botanicals - plant extracts made to deter pests - would remain effective if their source plants were grown under saline stressed soils, and what confounding impact they might have in the broader context of an IPM system, in terms of impacting non-target organisms. When he was not working on his project this summer, he had instead assisted as a tutor for schoolhouse.world, savored his last scout camping trip, and ended up with more down time than he expected. His long-term career goal is to get into public policy, but for his undergraduate, will be pursuing a degree in Environmental Studies. Regardless of what happens to him, all he hopes for is that he doesn't lose sight of the bigger picture, and sticks to his morals the best he can.

Consulting Editors of the Journal

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Guidelines for Contributors

The Young Researcher is a peer-reviewed journal dedicated to publishing the best original research from secondary school students.

The journal's mission is to provide a larger audience for the original academic research of ambitious secondary students, provide a forum for peer-review, and create a community of young researchers. In addition, the journal strives to advance the quality of academic writing in secondary schools.

The Young Researcher is edited by secondary school students working closely with scholars and active researchers at universities and in the community. The journal operates a blind peer-reviewed review process, following those found in academic research journals.

The journal encourages submissions of original research (including relevant replication studies) from a wide range of academic disciplines within the social sciences, humanities, and sciences.

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- Articles should have the following sections or equivalent:
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  - Literature Review
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  - Discussion, Analysis, and/or Evaluation
  - Conclusion and Future Directions
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- Papers should be formatted using discipline-appropriate methods (MLA, APA, and Chicago are acceptable).

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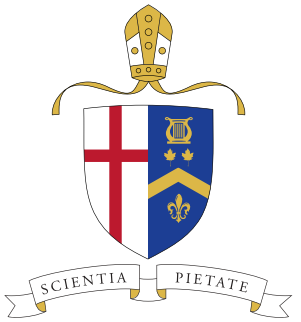

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