JURA

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This edition features 4 extended abstracts and 2 research papers featuring hot topics such as osteoarthritis, cancer, obesity, and LGBTQ⁺ in sport.

Photo taken by Raied Aburashed

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Volume 8: Contents

Photo taken by: Paul Park

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Editors' Remarks

We are pleased to present the 2020 full edition of the Journal of Undergraduate Research in Alberta. This year's edition has been a greater challenge to publish as COVID-19 has decreased undergraduate research in Alberta. Nonetheless, the resilience of the undergraduate students has proven vital to the advancement of our journal and we thank them for their contributions.

This edition features four extended abstracts, one review article and one research paper on a multitude of topics from undergraduate students in diverse backgrounds. Hot topics such as osteoarthritis, cancer, and obesity are explored which provide further insight into their respective fields. The highly relevant conclusions drawn by our authors in their work emphasize the importance of undergraduate research and we at JURA are proud to be at University of Calgary's forefront in spurring more research of this quality.

We at JURA are currently encouraging further submissions of research from undergraduate students within the science realm. We understand that current circumstances have made lab visits troublesome for many students. However, students are encouraged to submit written portions from sources beyond extracurricular laboratory research such as their thesis dissertations and class research projects. Furthermore, students are encouraged to continue submitting review papers, as these are equally vital contributions.

JURA has been proud to be involved within the Albertan scientific community and create initiatives to stimulate more research from students. We have continued our outreach through the awards program at the 2019 Alberta Biomedical Engineering Conference, which offered the winners, Maria Baclig and Alexis Pawluk, $250 for their poster presentations. Congratulations!

We hope that readers enjoy the 2020 JURA edition, and we encourage you to share this journal with your peers. We would like to thank Raied Aburashed and Paul Park's contribution in photography featuring Albertan landscapes which are featured within this edition. We would also like to thank Walter Herzog and the Biomedical Engineering Graduate Program for their continued support towards JURA and we hope to see your submissions published in the following editions.

Sincerely,

Miriam Nightingale, *Editor-in-Chief*
Jak Loree-Spacek, *Managing Editor*
Catherine Swytink-Binnema, *Outreach Editor*
Rakesh Narang, *Publishing and Layout Editor*

A Socio-Historical Exploration of Gay Sport and Physical Activity Communities in Calgary

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AUTHOR BIO

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Abstract

Sport holds a prominent position in Canadian society. Historically, in the LGBTQ community, sport has had a paradoxical role: it can be a place of homophobia, and an opportunity for community building. Recent historic research has been done on the LGBTQ community in Calgary, Alberta. What is missing from this research is specific stories covering LGBTQ sporting spaces in Calgary. The goal of this research was to investigate how LGBTQ Calgarians constructed the role that sport and physical activity played in their lives in the second half of the last century. Three gay men, who resided within Calgary during the 1980s and 90s, participated in oral history semi-structured interviews. Transcripts were subjected to a thematic analysis where generated themes demonstrated the overlap and tension found in participants' ideas and stories. Participants constructed LGBTQ sporting spaces as a safe space to come out and be out, as well as a social outlet for HIV positive individuals. However, the spaces also seemed to reaffirm dominant sport and health discourse, through reference to the creation of "hard" bodies, "taking sport seriously", and sport being a healthier alternative to the bar scene. What can be concluded from this research is that sporting spaces created by and for LGBTQ participants are complex and paradoxical. They are spaces that resist some hegemonic narratives, while reifying others. Future research must focus on collecting the stories of lesbian and trans citizens of Calgary. This research adds to academic literature and augments the Calgary Gay History Project.

Keywords: gay, categorical sport, oral history, LGBTQ, health narratives, community building

Introduction

In Our Past Matters: Stories of Gay Calgary, Kevin Allen [1] explored LGBTQ (lesbian, gay, bisexual, trans, queer) history in the context of Calgary, Alberta, Canada. Allen [1] found that although Calgary was a toxic and had, at times, a dangerous culture for LGBTQ Calgarians, it was also a space for resistance and reclamation. Calgary's LGBTQ history is similar to LGBTQ history in Canada more generally, with plentiful examples of how Canadian culture has been hostile to LGBTQ identities, and how this hostility has been met with individual and collective acts of bravery and courage. Queer histories of Canada elucidate everyday lived experiences of homophobia within a context where homosexuality was only decriminalized in 1969, where sexual orientation was only added to the Canadian Charter of Rights and Freedoms as a protected identity in 1982 and gender identity and gender expression in 2017, and where same-sex marriage was not legalized until 2005.

As a microcosm of society, sport has also been largely unwelcoming to LGBTQ persons historically [2–10]. Because sport holds a prominent position within society, it influences conceptualizations of sex, gender, sexuality, and health. Consequently, sport both produces and reproduces limited and problematic ideas about women and men and femininity and masculinity, often resulting in heteronormative spaces that have been unwelcoming and even unsafe for those who "don't fit". While perhaps having improved from the past, some scholars suggest that LGBTQphobia remains an issue in sport in present times [11–13].

Despite prejudicial and discriminatory treatment in mainstream sport, LGBTQ individuals and groups have carved out their own spaces within sport and physical activity [6, 14], including specific teams, leagues, and organizations. Some examples include large international multisport events such as the Gay Games and the (now defunct) Outgames, national and regional events, and more locally-focused leagues and events. These sporting spaces organized by and for LGBTQ persons have been referred to as categorical sport [15]. The term categorical sport also aids in differentiating LGBTQ sport from mainstream sport. Although a large body of research has demonstrated that so-called categorical sporting spaces can allow opportunity for people to engage in counter discourse—subverting traditional and dominant sport and gender discourses—research has shown how categorical sport can also reify traditional ideas about sport, gender, and health [16–20].

In Calgary specifically, there is some documented evidence of two lesbian softball teams that were formed in the 1960s [1] but the first formal LGBTQ sport organization was Apollo—Friends in Sports. Apollo was established in 1981 to organize a multi-sport event for gay and lesbian athletes. Also, the Alberta Rockies Gay Rodeo Association (ARGRA, now CRGRA—Canadian Rockies Gay Rodeo Association) was created in Calgary in 1991. Beyond some brief historical references on organization websites, and in Allen's [1] landmark gay history of Calgary, there remains limited research on the lived experiences of LGBTQ individuals in sport and physical activity in Calgary in the latter part of the last century, at a time when stigma towards LGBTQ persons and identities in Calgary specifically, and Canada more generally, was high [1, 21]. Thus, the overall objective of my research—inspired by the stories gathered and shared in Allen's work—was to learn about how LGBTQ Calgarians constructed the role that sport and physical activity played in their lives in the latter half of the last century (~1950 to 1990s).

Methodology

One approach to gathering rich knowledge about the past is through oral histories. Oral history interviews have been used quite frequently in socio-historical inquiries into queer lives in academia [22–24], and public projects like the Calgary Gay History Project [1]. The oral histories collected for this research allowed for what Cahn [23] calls "bottom up history": the inclusion, investigation, and promotion of traditionally disempowered groups' voices, like, for instance, LGBTQ persons. This inclusion of stories from disadvantaged or disenfranchised groups is important because of their omission from popular history and academic knowledge [25].

Three self-identified gay men who resided within Calgary during the 1980s and 90s responded to recruitment notifications for my project. Oral history interviews were conducted between December 2018 and January 2019. All participants were involved in one interview, each lasting between 2.5 to 3 hours; with their consent, interviews were audio recorded. The three men were asked about their lives in Calgary and Canada more generally, their experiences as gay men, and the role sport and physical activity played in their lives and the lives of queer Calgarians. The participants were provided the opportunity to choose a pseudonym or to have one assigned for them; their choices are reflected in the names assigned to quotes in this paper.

The interviews were transcribed and subjected to

thematic analysis [26–28]. Thematic analysis was used to organize the content of the interview transcripts into common ideas across the interviews as well as divergent opinions. Analysis was also informed by themes identified in existing socio-cultural literature on gender, sexuality, and sport/physical activity, and the guiding objectives of this research. NVivo 12.0 (QSR International), qualitative research software, was used to help organize themes and subthemes. All aspects of the research project followed ethical guidelines for research with humans and received approval from my institution's research ethics board.

Results

I created many different and often overlapping themes through my thematic analysis of the interview transcripts. Some of these themes included the construction of LGBTQ sport and physical activity spaces as community builders, as safe spaces, and as places that integrated with and connected to other spaces within the developing LGBTQ community. In this manuscript, I present findings related to health narratives (broadly defined) as they related to categorical sport and physical activity spaces in the geographical and temporal context that was of interest. The findings are also made sense of in relation to dominant ideas about sex, gender, and sexuality circulating in the Canadian context at the time.

A. Queer Sporting Spaces as Safe but also Exclusionary Spaces

In their interviews, all three participants discussed how LGBTQ sport and physical activity spaces were used as places for socialization and friendship building. The participants also constructed these spaces as a safe space where one could "come out" and feel safe doing so.

> *"…[T]hey're definitely safe spaces for people to come out early, and later in life. Like, I witnessed it. I can remember volleyball back in the early 90s, um... I saw a guy that was in his late 20s showing up and uh... playing volleyball with us, and not identifying as gay, and then, you know, a couple weeks later, 'I'm questioning.' Then, you know, 2 or 3 months later... 'I'm comfortable—a little bit more comfortable, but still struggling.'"* (Matt)

> *"[T]he Frontrunners [an international running organization for LGBTQ persons, with local groups around the world, including Calgary], you know, like, my gay best friend, you know, one of his first parts of coming out, was coming out to the Frontrunners… It's so safe to just go out to a—you know, try a drop in running group. You know, yeah so, that was a great way to meet people and bring them into the community."* (Gene)

Safety was set against the cultural backdrop of Calgary during the 1980s and 90s, which was constructed as having an air of conservatism that did not bode well for those considering coming out and being out. As such, these categorical sport and physical activity spaces, such as Apollo and ARGRA and other fledgling teams and groups, became places of openness and refuge. And for some, became the only reason they stayed in Calgary:

> *"I think that sports played, for me personally, sports played a big part of my fitting into Calgary. If I hadn't had... that connection... I probably would have left Calgary. I probably would have moved somewhere else, back to Montreal. And I actually contemplated it in the first 6 months, and it took me a good 2 or 3 years to feel comfortable here."* (Matt)

Although these categorical sport spaces provided an opportunity for belonging and acceptance, they were also frequently compared to mainstream sport; the emphasis on taking sport seriously and prioritizing it in one's life was also a theme that emerged. Interestingly, this comparison took place, even with the recognition that mainstream sport could be exclusionary and toxic.

> *"I've known tons of athletes, and met them, where being outed, or going to Apollo weekend was the last thing that athlete could do because that was their 'real' career, and they thought if [other people] found out, the world found out, they were gay or lesbian, they would never get taken seriously again. But that was the 80s and the 90s; that's what happened."* (Fred)

Another participant, Matt, described discussions he had with lesbian participants and the complaints they had of not feeling welcomed and/or being excluded from competition in recreational volleyball to international tournaments like the Gay Games in 1994 in New York. Both Fred and Matt pointed out how transgender people were not visible in these spaces and were in fact overlooked completely by the sport and physical activity groups in Calgary at the time. Because exclusionary aspects of sport were reproduced in

these categorical sporting spaces, not all LGBTQ persons could participate and gain in the same ways from the health benefits (i.e., physical, emotional, and/or social) ostensibly available [4, 29].

Another of the exclusionary elements reproduced from mainstream sport that the participants discussed was competitiveness. Competitiveness in sport is a discourse that demands sport be "taken seriously". In other words, competitiveness in sport, as it is understood in this article, is being framed alongside neoliberal principles of meritocracy, selfishness, and winning at all costs. The implications of this discourse is the exclusion of individuals in the pursuit of victory, the creation of harsh, demanding, and skill focused environments in sport clubs and leagues, and disregarding the health and wellbeing of individuals. Competitiveness, or "taking sport seriously", led Gene to put himself in a position of compromised health. Gene discussed how he trained for a particular distance running event until the point of injury; to his dismay, he eventually had to sit out the event for which he had been ardently preparing. More generally, Gene also talked about how the running group to which he belonged, the Frontrunners, would ignore stormy or unforgiving weather to run, thus, putting themselves—and their health—in jeopardy.

B. Sport and Physical Activity and Connections to Healthiness

Ideas of healthiness were connected to body shape and size (i.e., hard bodies). "Hard bodies" are the cultural constructions and understandings that bodies are meant to be muscular and strong. These corporeal conceptualizations of hardness lend themselves well to, and are reaffirmed by, discourses around masculinity and men's beauty standards. So, the production of a hard body was conceptualized as important in relation to attractiveness and, by extension, to health:

> *"I don't know, it's like... like-minded people so that's what their comfortable with, that's who they want to be around. I don't say this in a bad way, but I know a lot of guys who would go to Apollo weekend, cause they [were hoping] to date a healthy guy. And not somebody, you know, going through all those specifics like… no fats, no femmes, no drag queens, and stuff. If guys who wanted to date healthy men, they thought, in their opinion, it was an easier way to get a date out of it. 'Let's go to Apollo weekend, those guys are healthy.' .... You always joked about. Like the swimmers weren't shaped like me who went to Apollo swimming, let's be honest. Those people… they're serious."* (Fred)

> *"…then you'd go to the [YMCA]… and you know, the sad thing is like, gay people, we're really body focused. Not so much anymore, now the bears are a lot more popular, but back then, the gay community was all about like, looking good. Like really shallow and body focused and being slim and having big muscles. A lot of us went to the gym…. Then you can also go to the gym and you'll see those people…. We call them 'Gym Bunnies' so the people that really go to the gym. They're working out just so they can look as sexy as possible to get the hottest guys. Gym bunnies. They're not necessarily there for fitness. They're really to be as pretty as possible." (Gene)*

Dominant ideas about health, and what a healthy man is able to do and look like, were central to conversations with the participants when discussing the place of queer sport and physical activity spaces historically. Health maintenance and activities, not to mention the construction of hard bodies, were undoubtedly reinforced by dominant ideas about masculinity, something that was, and has been, a source of tension for several decades in the gay community, at the intersection of gender and sexuality [30, 31].

Also related to notions of healthiness, categorical sport teams and organizations, along with physical activity spaces like the YMCA, were constructed as healthy alternatives to the bar or club scene in Calgary at the time.

> *"Hopefully, most people are like, even if they stop going to the bars, which a lot of people do, hopefully, they still go to the choir and hopefully they still go to the gym. They might stop going to the gym once they found their man because now they've settled down and they feel like their guy isn't going anywhere… Hopefully, they'll keep at least their sporting event. Hopefully they're not there only to meet people. There are people who only do these only to meet people. I do know people that only join the sporting clubs because they're single and lonely and they want to meet a guy so they join a sports club just because they're not dating."* (Gene)

> *"Calgary, for a city of this size, there is not a lot of gay bars. Um... and it's not every body's thing: more people are healthier,*

they don't want to drink…. I found Calgary to be a much [healthier] city…. When I lived in Vancouver, it was…there was a lot of alcohol and drug abuse in the community, and in general." (Matt)

Alcohol is widely viewed as a negative health commodity and so constructing sport and physical activity as an alternative to the bar scene may have been because sport and physical activity were seen as a so-called healthy social alternative. At the same time, this contrast was somewhat paradoxical given the often close relationship between sport organizations and gay bars vis-à-vis sponsorship of teams and events [32] as well as drinking while participating in, or following, games or tournaments:

"It doesn't matter how many contracts I've seen people do; I've sat at the table myself and tell people, 'we drink'. You run out of booze. We drink!.... And I watched even the bowling alley sell out of booze. How do you sell out of draft booze, like get more draft beer? You know, and they're like, 'We have to order more.'" (Fred)

C. Sport, Physical Activity, and HIV/AIDS

Also related to health in the participants' narratives was the topic of HIV/AIDS. As one example, Matt referenced the impact that AIDS had specifically on sport and physical activity groups:

"[W]hen I first joined Apollo... they were sort of struggling, or... not struggling, but a couple of their earlier founders had been diagnosed HIV positive, and some had passed away, some were too ill to participate anymore. And um... so... that was also my first sort of contact with HIV in the sense of knowing people who were positive. I would say that would probably be the biggest secret in the leagues at that time. And people didn't talk about status. One because it was still, even within the gay community, unfortunately, taboo, and people would be a little bit... you know... uncomfortable, especially in contact sports, I think some people were fearful of, 'Oh, what if he falls on me and is bleeding?' That type of thing. So there was a little bit of that. That kind of... fear…. But, it never... it never kept people from participating. There was no, 'Oh, you can't participate.' You know we encouraged people, and for some people, it was their only outlet. It was their only way out of the house. Their only social activity. And so... I know some of the members made sure that, you know, they would pick people up to take them to bowling, even if they weren't bowling, they were just sitting there and watching, and having a beer. It was a way out of their house…. And to forget their illness, and things like that." (Matt)

Without doubt, AIDS had a tremendous impact on LGBTQ communities in Canadian cities in the 1980s and 90s [1]. It is evident from comments in the interviews, such as Matt's above, that sport and physical activity remained sources of community for some HIV+ individuals and those with AIDS. This further demonstrates the stated connection between sport and physical activity spaces and community building, at a time when gay men in particular were ostracized by other social institutions because of their health status. However, as Matt eluded, it was still a taboo subject and made some people in sport and physical activity spaces uncomfortable. It is evident that categorical sport and physical activity spaces were places of great paradox, as revealed through the participants' sometimes conflicting constructions of these spaces and their lived experiences in relation to them.

Discussion

The gay men interviewed, all who lived in Calgary in the 1980s and 90s, spoke not only about the role of LGBTQ sport and physical activity in their lives, but they also offered insights into life as gay men in relation to broader social realities in Calgary and in Canada during the same time period. These latter insights helped to contextualize the findings of my research and so it is worth elucidating those comments to lead into further discussion about the place of sport and physical activity in the lives of LGBTQ Calgarians in the latter half of the last century. For example, with its discriminatory policies and laws, and the toxic culture espoused by politicians and the media, Canada was an unsafe place for LGBTQ persons in the latter part of the last century [1, 21]. In Calgary, gay bashing occurred in known cruising spaces like downtown parks and, as noted previously, Calgary experienced tremendous loss during the AIDS epidemic in the 1980s and 90s [1]. It is important to recognize that in the Global North in the 1980s and 90s, an HIV/AIDS diagnosis was still considered a death sentence and the illness was homophobically labeled a "gay plague" [1]. The time period discussed in this research also comes before the creation of the first antiretroviral treatment for HIV in 1996 and the understanding

that HIV/AIDS was treatable [33]. The number of deaths of gay men in the Calgary community, especially related to AIDS, brought the entire LGBTQ community closer. Consequently, activism was prevalent in the city at the same time. Fueled also by the origins of World AIDS Day in 1988, Calgary's LGBTQ groups began to mobilize by writing editorials for mainstream newspapers that openly challenged the toxic rhetoric about AIDS and people living with the illness, while also calling for municipal, provincial, and federal governments to take action [1].

Sports and physical activity groups and spaces seemed to serve an important purpose in this same time period, offering a place for individuals to integrate and to get, and perhaps stay, involved in Calgary's growing LGBTQ community. These were places where sexuality could be openly discussed and where socialization could take place. They were safe spaces in an otherwise conservative and closeted city. They were spaces where one could come out and be out—and feel safe doing so. This could have contributed to social and emotional health and wellness by providing opportunity to interact with others who shared similar experiences. They were also spaces where there was some evidence of the contestation of heteronormative sport culture and the dominant health narratives reproduced therein [15, 16].

Paradoxically and problematically, there were also many exclusionary aspects to these spaces, one example being the promotion of competition over participation. Additionally, the often unproblematized reproduction of traditional gender norms suggests that in Calgary, categorical sport reinforced traditional notions of sporting masculinity, similar to categorical sport in other cities in Canada and elsewhere [16, 20, 30]. Ideas about health often reproduced dominant ideas that equate healthiness with a particular body type, which in this case was connected to masculine ideals of the hard body, as well as the participation in "appropriate" activities. In this regard, the LGBTQ bar scene was simultaneously constructed as being integral to categorical sport and physical activity groups and also the less healthy choice. So, while the intentions may have been to provide safe and inclusive alternatives to mainstream sport and physical activity at the time, this did not always play out in practice. Rather, these spaces seemed to be largely safe for and inclusive of white, cisgender, gay men subscribing to dominant ideas about gender and about sport [20].

The juxtaposed narratives about inclusiveness and about health demonstrate how history, and its interpretation, is a messy process. Ambiguity, in this case, is accuracy; no one history is the "truth". But here also appears one of the limitations of this paper: I was only able to interview three white, cisgender, gay men. This may speak to the dominant make up of these sport and physical activity groups, but it also means only certain perspectives are captured. For example, women—and specifically Indigenous women—were mentioned as participating in queer sporting spaces, but they did not always feel welcome or included. While not monolithic, many aspects of the three participants' stories were similar. The gathering of histories and the interpretation of those histories is also affected by the identity and context of the researcher; thus, my identity as a white, cisgender, asexual man, who did not grow up during the time period studied, must be recognized and taken into account. That said, this limitation was also mitigated by putting my interview materials into conversation with Allen's rich history of Calgary's LGBTQ community [1].

Conclusion

This research adds to the body of knowledge on the complexities of categorical sporting spaces; they can be spaces that simultaneously reaffirm and challenge dominant sport and health discourse, that are exclusionary yet inclusive in limited ways. Future research should explore more diverse stories and experiences in categorical sporting spaces in Calgary and other parts of Alberta, with a particular focus on including lesbians and transgender persons present in Calgary during the same time period, and/or older participants from the 1950s, 60s, and 70s. Giving space and platform to the voices of the unheard highlights that history is not a linear, singular, true story; history is cyclical and often a clash between progress and backlash. Listening to the stories of marginalized groups can help to contextualize the present as well as point to directions we are headed. Even with the progress that has been made in the Canadian context, history collection and interpretation with a social justice and activist arc challenges the overly-simplified idea that "we're better than we used to be" by demonstrating that the tireless work of oppressed groups is what creates progress. It is not that we have gotten better but rather that we have learned from individuals and from groups who had to fight ignorance and violence to be heard in order to create a more just society. There remains work to be done. The stories graciously offered in the three oral histories collected for this study offer important insights into Calgary's LGBTQ history in relation to sport and physical activity, but there

are, without question, other stories to gather and more insights to gain to capture the complexity of a history that should not be forgotten.

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The Role of Host-Microbiota Interactions in the Development and Functionality of the Gut-Brain Axis

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AUTHOR BIO

Anthony Marullo has taken a roundabout path to get to where he currently is. After graduating high school, he studied computer engineering before, by chance, falling into a career as a chef. After a little more than a decade of the restaurant experience, he decided to pursue an education in science. He is currently going into his fourth year of the Health Science major at Mount Royal University and has taken a keen interest in microbiology and physiology. After spending a few summers volunteering for a developmental neurobiologist and a respiratory scientist from Mount Royal who utilized his computer engineering background for data analysis, Anthony has realized that the pursuit of research is where he wants to be.

Abstract

Numerous novel studies suggest that due to a long evolutionary history and constant host-microbiota symbiotic interactions, bacteria facilitate the development of a functional nervous and immune system creating the ideal environment for bidirectional communication between both host and symbiont through the gut-brain axis. This paper reviews several of these studies and analyzes studies on how perturbation of the gut microbial ecosystem can contribute to abnormal physiological and cognitive conditions such as Autism Spectrum Disorders.

Keywords: Microbiotas, Horizontal Gene Transfer, Immune Imprinting, Neuronal Development, Gut-Brain Axis, Autism Spectrum Disorders

Introduction

Billions of years before modern day humans made an appearance, unicellular microbes were the only organisms present on earth [1]. Over time these unicellular organisms diverged and evolved, eventually leading to the emergence of multicellular organisms, including humans. With modern-day technology, we are able to study microbial evolution and the complex interactions we have with the microbes that inhabit our own bodies. It is becoming clear that these particular microbes, our microbiota, play important roles in human physiology. Furthermore, there is evidence to suggest that the establishment of a "healthy" microbiota during early life may be crucial for normal human development.

Although while it may seem farfetched to think that something as small and seemingly insignificant as a unicellular organism could affect the development of something as large and complex as a human, it is important to take in the entire scope of what we know of the evolutionary history of our planet. The entire time our species has been evolving intricate organ systems and intercellular communication pathways, microbes have been present. It seems reasonable to speculate that each of our evolutionary ancestors underwent physiological development in the presence of its own microbiota and that natural selection and evolution were also influenced by the presence of these microbiotas.

Within the last decade, numerous novel studies have been performed to understand the role that microbiotas play in various multicellular species, including ourselves. It is becoming evident that our microbiotas are tightly entwined with human physiology, development, and evolution. In fact, this paper will present current evidence that human gut microbiota influences immune and nervous system development and function. However, because the human system is extremely complex and exceedingly difficult to study, it is worthwhile to first discuss the vital roles that microbiotas play in the lives of animals much simpler than ourselves. It is also important to consider how prokaryotes, which are major components of our microbiota, use common genetic and biochemical components to communicate with eukaryotes. Before discussing the human system, several methods for studying the effects of microbiotas on mammals are discussed. With this background, the reader is introduced to evidence that bacteria facilitate the development of a functional nervous and immune system in humans, creating the ideal environment for bidirectional communication between both host and symbiont through the gut-brain axis. Finally, this paper will summarize studies on how perturbation of the gut microbial ecosystem can contribute to abnormal physiological and cognitive conditions.

Host-Microbiota Interactions in Simple Eukaryotes

Host-microbiota symbiosis is believed to be responsible for guiding the evolution of eukaryotic organisms. The endosymbiosis theory states that the interaction of bacteria led to the development of the first organelles leading to the first eukaryotic cells [2]. As ancestral multicellular organisms branched into the metazoans, the coelom evolved [1]. This body cavity allowed for gut elongation and regional specialization allowing for a larger diversity of microbes to take root [1]. Within gut locations specialized in both ingestion and storage, more intricate crosstalk and metabolic collaboration could take place.

Host-symbiont microbe interactions allow for metabolic collaboration within the host ecosystem that, in some cases, create a beneficial mutual symbiosis giving all the organisms involved a survival advantage [3]. These relationships can allow a host organism to adapt to an environment that may not otherwise be the most suitable for the host [3].

A well-studied host-microbiota model is that of the Pea-aphid. The Pea-aphid has coevolved alongside the genus of the bacterium, Buchnera. Pea-aphids are missing the necessary pathways to obtain vital amino acids from their native diet. Similarly, Buchnera lacks the necessary pathways to produce the metabolites they require for survival [3]. Through coevolution, Pea-aphids have developed specialized cells referred to as bacteriocytes that house Buchnera allowing for beneficial metabolic collaboration to aid in the survival of both species [3].

The host-microbiota interactions in simple eukaryotic organisms can help us to understand the more intricate and complex exchanges that may aid in the development of human physiology.

Common Inter-Kingdom Languages Between Microbes and Animals

To allow for efficient symbiosis there must be bidirectional communication between the host cells and the microbial population residing within. Recent studies into microbial endocrinology have observed the ubiquitous nature of signaling molecules between microbial populations and their human hosts. Of more importance is that the biochemical pathways used to synthesize these

signaling molecules are the same as within host cells [4]. The authors of one study postulate that this is due to horizontal gene transfer (HGT) leading to traceable phylogenetic lineages.

It is generally accepted that proto-mitochondrial endosymbiont HGT is responsible for the development of eukaryotic organisms within the unicellular phase of eukaryotic evolution [5], but new evidence is indicating that later HGT may be responsible for the prevalence of many first-messenger metabolic genes found within different animal species. Cell to cell communication within animal systems involves the use of first-messenger molecules. These molecules can be divided into amino acids, amino acid derivatives, nucleosides, histamine, and lipid and fatty-acid derivatives [4]. These molecules are synthesized through biosynthetic pathways. One study compared information from databases to establish any phylogenetic trends of vertebrate enzymes responsible for the biosynthesis of first-messenger molecules. The databases compared were the non-redundant protein sequence database and organism-specific databases containing information on orthologs of the enzymes [4]. It was observed that out of seventeen of the major enzymes involved in messenger metabolism, fourteen of them were shared between one or two eukaryotic lineages and bacteria [4]. To further understand the relationships, phylogenetic analysis and statistical tests were performed. Two clear groupings were observed upon completion: genes found within eukaryotes and bacteria, and genes found specifically within animals and bacteria [4].

Within the grouping of genes found in eukaryotes and bacteria the most common pattern observed involved decarboxylases. Decarboxylases are required for the initial biosynthetic steps for creating amino-acid-derived messengers. The decarboxylases were further divided into two clades. The first clade consists of aromatic amino-acid decarboxylases involved in the synthesis of catecholamines, 5-hydroxytryptamine, and melatonin, and histidine decarboxylases involved in the synthesis of histamine [4]. The topology of this clade is indicative of a mitochondrial origin as the branching includes the eukaryotic cluster and α-proteobacteria, the progenitor of mitochondria [6]. The second clade consists of glutamate decarboxylases involved in GABA synthesis and cysteine sulfinate decarboxylases. This clade lacked the plant and α-proteobacteria representation observed in the previous clade, indicating that the HGT may have occurred after the common ancestor of animals and fungi radiated from their ancestral precursor [4]. Further evidence of more refined gene dispersion within the eukaryotic taxa is reported in the study.

The clades found within animals and bacteria also include only two groupings. The grouping amino-acid hydroxylases are found in all animals and bacteria. Hydroxyindole-O-methyltransferase, involved in melatonin synthesis, is found only within vertebrates and bacteria. [4]. This indicates that HGT may have occurred after the animal and fungal evolutionary precursors parted ways.

The evidence of microbiota having the same biosynthetic pathways as humans can be further exemplified by the range of hormones that are found within microorganisms. These hormones include, and are not limited to, somatostatin and acetylcholine [7]. Both hormones play vital roles in the everyday regulation and functionality of fundamental human physiological systems. It is also evident that normal microbiotas also have cognate receptors for these hormones. The ubiquitous nature of the biosynthetic pathways within both bacteria and their hosts paired with the expression of the corresponding receptors on both symbionts paves the way for bidirectional communication, thus facilitating an environment for symbiotic harmony.

The bidirectional communication occurring between host and symbiont, particularly between normal gut microbiota and humans, is being heavily investigated by many disciplines. Many of the signaling molecules proposed to be introduced through HGT have been observed to be key factors within the gut-brain axis.

Methods for Studying the Effects of Microbiota in Mammals

One way to begin to understand exactly how our native microbes affect our physiology is by looking at what not having them around can mean for our overall health. As society becomes more urbanized, we find ourselves interacting with microbes less and less. The hygiene hypothesis proposes that the lack of diversity of microbial interaction during postnatal maturation contributes to an underdeveloped immune system, leading to an increase in allergic and autoimmune diseases [8]. As technology advances, there are multiple techniques that can be used to observe and speculate what this lack of diversity can mean within diverse living systems.

The primary source of our understanding of the complex interactions that microbial metabolites have with their hosts is studied using mice with specific microbial states. Gnotobiotic mice are mice that have known microbial makeup [9].

Gnotobiotic mice can also include axenic (germ-free) mice that are delivered via caesarean section or from an axenic mother and raised within a completely sterile environment, making sure they are devoid of all microbial interaction [9]. Gnotobiotic mice can also be genetically altered with human genes, cells, or organs to mimic human physiology for experimental study. Using these humanized gnotobiotic mice, we can study the effects that the microbial interactions might have on our own cells and organ systems [10]. Specific pathogen-free mice are mice that are free of known pathogens and contain a normal microbial population [17]. Researchers can also introduce specific types of bacteria whose metabolic activities are known and observe the effect on the organ systems and health of the mice. On top of introducing specific types of bacteria, one can also perturb existing colonies to observe the effects of dysbiosis within a system. Dysbiosis refers to a microbial imbalance on or within a living system. Studies involving mice will be further discussed in the following sections.

To observe and confirm microbial populations within living systems, metagenomic techniques have been utilized. Metagenomic techniques have allowed scientists to explore host ecosystem relationships further than ever before. The most accessible and abundant location from which to obtain and sequence microbial DNA is the gut. Fecal samples can be analyzed allowing one to assess the full scope of the ratios and diversity of microbiotas found within an organism's digestive tract. Trends can be established by comparing this data with that of other organisms allowing for correlations to be inferred creating a more accurate depiction of the inter-organism interactions.

While the use of humanized and non-humanized gnotobiotic mice can help to understand what modification of the gut microbiome can mean to a physiological system, it may not directly translate to human models. However, the ethical boundaries in mouse models are less restricting than those of humans and allow for more in-depth studies to form inferences regarding host-microbial interactions within the human model.

The Roles of Microbiotas in Human Immune System Development

In order for microbiotas to thrive within a living ecosystem they must be able to interact with their environment in a manner that will not result in an adverse response from the host's immune system. One controversial hypothesis is that microbiota first interacts with an individual within the womb. It has been suggested that microbes could exist within the placenta [11], but it may be more likely that the maternal microbiota produces metabolites that interact with the fetus [12]. This early life interaction is important for developing a fully functional immune system and a healthy symbiotic relationship between microbiota and their hosts [12]. When the immune system is functioning optimally it creates a protective environment for both mutualistic and commensal microbiotas to exist [13]. Examples of how these early life interactions benefit the development of the immune system will be discussed further in this section.

Some studies on microbiota facilitated development have indicated that the neonatal period is the most essential time for the microbial host interaction, referring to it as the window of opportunity [13]. Increased susceptibility to allergy and inflammatory bowel disease (IBD) have been observed in adults who have experienced perturbations of the mucosal microbiome within this window [14], [15]. Stemming from these observations, the concepts of early life imprinting and pathological imprinting have been coined to explain further observed trends [12]. 'Early life imprinting' is used to explain the host-microbiota crosstalk that occurs during the perinatal period and is required for establishing immune function, while 'pathological imprinting' is used to explain the increased susceptibility to inflammatory pathology in individuals who have experienced microbial perturbations during the window of opportunity [12].

In one study, germ-free mice and specific pathogen-free mice were utilized to begin to understand the effects of early life bacterial colonization on allergic airway inflammation [16]. The germ-free mice lacking the native bacterial populations demonstrated a lack of maturation and recruitment of dendritic cells, macrophage populations, an increase in basophil response, and an increase in allergic airway inflammation compared to the specific pathogen-free mice[16]. This suggests that early bacterial colonization is important for the development of certain immune cells and functions.

Other studies, as reviewed in [12], have observed the direct effect colonizing microbes have on the development of the intestinal immune system. Germ-free mice do not develop lymphoid follicles, exhibit lower numbers of intraepithelial lymphocytes and specific types of regulatory T cells, and show a decrease in the substitution of yolk-sac-derived macrophages with bone marrow-derived macrophages. Some of these conditions may be reversible with supplementation of

microbial colonies [12], but other irreversible conditions can persist into adult life. Studies involving non-specific antibiotic treatment of specific pathogen-free mice within different developmental windows have yielded similar results indicating a strong correlation between microbial perturbation in key developmental windows and pathological imprinting. Antibiotic treatment limited to the neonatal period yielded mice with increased susceptibility to allergy in the gut and lungs, and skin and lung fibrosis within a model of systemic sclerosis [14]. Similarly, mice that were colonized with microbes only after the window of opportunity experienced similar pathological imprinting [12].

This evidence highlights the importance of the development of a properly functioning host immune system to maintain a healthy host-microbiota symbiotic relationship. As will be discussed in the next section, this relationship is necessary for the development and functioning of other physiological systems.

Interactions Between Microbiota and the Human Nervous System

It is becoming evident that a healthy host-microbiota relationship is vital in the development of key neuronal pathways and brain development. Neural development is one of the earliest systems to begin during fetal life and is not completed until after birth. This complex and lengthy process leaves an open window in which the development is susceptible to environmental influence [17]. Perturbation of host-microbial populations both pre- and post-natal has been observed to have significant implications in the development of the brain and nervous system. The term developmental programming has been used to describe these observations [18].

It is understood that a preterm infant's microbiome is greatly influenced by its surroundings within the hospital. Factors such as type of birth (vaginal or caesarean), antibiotic usage, interaction with caregivers, and the physical environment can all contribute to considerable variability within the infant [10]. One study looked to find connections between an individual's microbiome and nervous system development by creating mice with microbiota mimicking those of preterm infants with either fast or slow growth rates. These mice were created through the transfer of the preterm infants' fecal samples into pregnant germ-free mice so that their offspring would be born naturally colonized by the desired communities [10]. The preterm growth phenotype was successfully transferred to the offspring with the mice containing the microbiota associated with the high growth rate exhibiting significantly more weight gain [10]. The results further showed that the poor growth group demonstrated altered neuronal development and myelination that could likely have stemmed from the reported lower levels of circulating and brain localized insulin-like growth factor 1 (IGF-1) and higher levels of inflammation [10].

Similar trends are being observed in greater frequency as more studies explore the different developmental aspects of the brain and nervous system. A region that seems particularly influenced by microbial influence is the dorsal hippocampus. One study used bromodeoxyuridine to assess hippocampal cytogenesis and neurogenesis, as previous studies have indicated that some brain processes and substrates known to affect adult hippocampal neurogenesis having significantly different effects on germ-free mice versus regular mice [19]. The study investigated the direct effect that microbial influence had on neurogenesis within the region. It was observed that germ-free mice exhibited increased regional neurogenesis and that colonization outside of the sensitive period of development, or window of opportunity, had no effect on the adult hippocampal neurogenesis [19]. Unmodulated neuronal development has been linked to disorders such as autism spectrum disorder (ASD) [20].

Microbiotas have also been shown to affect neuronal development through the facilitation of the development of the blood brain barrier (BBB). The BBB works to maintain the microenvironment of the central nervous system (CNS) allowing for neuronal growth and specification [21]. This barrier is formed by a combination of astrocytes, pericytes, and tight junctions formed by endothelial cells. Much like the intestinal epithelium, tight junctions of the BBB are composed of transmembrane proteins like claudins and occludin [21]. One study compared the permeability of the BBB within germ-free mice and specific pathogen-free mice during different stages of development. Results involving prenatal development indicated that the fetal mice with germ-free mothers displayed significantly higher BBB permeability than that of the specific pathogen-free mice. This was associated with the disorganization of tight junctions and decreased expression of occludin and claudin-5 [21]. These effects were reversed through the transfer of fecal samples from the specific pathogen-free mice, the introduction of species of bacteria that synthesize short-chain fatty acids (SCFAs), or supplementation through oral

administrations of SCFAs [21]. SCFAs are produced via anaerobic bacteria through the fermentation of dietary fibers. SCFAs include butyrate, propionate, and acetate, and are believed to contribute to the upregulation of tight junction proteins [21].

Outside of direct developmental changes, microbiotas have also been observed to influence cells responsible for the maintenance of brain development. On top of their roles as macrophages within the BBB, microglia also play a critical role in brain remodeling in perinatal and adult life [22]. Through complement fixation activated phagocytosis, microglia undertake the important task of synaptic pruning. Synaptic pruning shapes neuronal connections and refines neuronal networks for the brain to function properly [17]. When comparing germ-free mice or antibiotic treated mice with controls, it was observed that microglial cells present within the perturbed models displayed significantly altered developmental states and morphology [22]. A lack of synaptic pruning paired with unmodulated neurogenesis during the development of the CNS could potentially be contributing to existing neurological disorders [23].

In several well studied neurological disorders such as anxiety and depression, it has been observed that there is a significant downregulation of GABA and 5-hydroxytryptophan (5-HTP). As mentioned in a previous section, there is evolutionary evidence that the biosynthetic pathways required for GABA and 5-HTP production were obtained from bacterial interactions via HGT. GABA is one of the main inhibitory neurotransmitters in physiological and psychological systems and is readily used within the gut-brain axis [24]. One study observed that supplementation of the *Lactobacillus rhamnosus* displayed regional dependent modulation of GABA mRNA resulting in both up and downregulation within different regions. These alterations resulted in reduced observed anxiety and depression within mouse models but were not observed in mouse models whose vagus nerve had been severed [24]. 5-HTP is a precursor for serotonin and is synthesized via an interaction between tryptophan and the enzyme tryptophan hydroxylase [15]. The importance of tryptophan/5-HTP is further investigated in a following section.

The establishment of the gut microbiota appears to have profound effects on the development of the CNS within the perinatal stage, facilitating the creation of an environment for healthy cellular growth and signal conduction. Optimal signal conduction allows for efficient feedback from the body's largest sensory organ, the gut, and the corresponding responses from the central nervous system, via the gut-brain axis, promoting systemic homeostasis.

Gut-Brain Axis

The gut-brain axis is a multifaceted and complex bidirectional communication system between the enteric nervous system (ENS) and the CNS. The axis consists of parts of the CNS, autonomic nervous system (ANS), ENS, and the hypothalamic pituitary adrenal axis (HPA). This system works to maintain gastrointestinal homeostasis and is also believed to affect higher cognitive functions [25]. Both clinical and experimental evidence are starting to indicate the importance of the interactions between enteric bacteria and intestinal cells, as well as their ability to manipulate neuroendocrine activity and metabolic pathways [25].

As discussed previously, the presence of microbiota helps to facilitate the development of the BBB within the CNS. This barrier works to maintain the microenvironment of the brain while allowing the passage of soluble molecules such as hormones and neurotransmitters [18]. Recent studies have also observed the presence of a lymphatic vessel network within the dura mater of the mouse brain that drains the cerebrospinal fluid in the adjacent arachnoid space and the brain interstitial fluid [28]. The BBB and lymphatic system can both function as portals of entry/exit for microbial metabolites and hormones allowing for crosstalk within the gut-brain axis.

The main pathway for both efferent and afferent communication between the ENS and CNS is the vagus nerve. The vagus nerve is the tenth cranial nerve, extending from the brainstem to the abdomen. Along its path to the gastrointestinal tract, the vagus nerve innervates the muscles of the pharynx and larynx in the neck, the heart, and other viscera providing both afferent sensory information to the CNS and parasympathetic activity to the innervated regions [26]. The parasympathetic innervation is responsible for regulating digestion, heart, and respiratory rates, as well as functions such as vasomotor activity. More importantly, the vagus nerve's afferent activity relays information from vital viscera such as the gut, liver, heart, and lungs to the brain [26]. The abdominal afferents consist of mucosal mechanoreceptors, chemoreceptors, and tension receptors, thus allowing for complex feedback from the external environment making it an important sensory organ [26]. The vagal nerve afferent pathways report environmental stress to

the CNS, which coordinates an adaptive response through the HPA to the stressor [26]. The HPA responds to environmental stressors by secreting corticotropin-releasing factor from the hypothalamus; this in turn stimulates the release of adrenocorticotropic hormone from the adenohypophysis. Adrenocorticotropic hormone stimulates the adrenal glands to produce cortisol. Cortisol has many downstream effects on organs [26].

Microbiotas influence the surrounding cells within the gastrointestinal environment and have been shown to have a role in modulation of stress response via the HPA. One study using germ-free mice reported that enteric microbiota modulated stress reactivity and anxiety-like behavior. The colonization of bacteria within the window of opportunity regulated the set point of HPA activity resulting in an anxiolytic behavioral phenotype with decreased levels of cortisol [27]. Another study observed that specific pathogen-free mice experience different levels of stress response when compared to germ-free mice [27]. The type of bacteria that colonized the mice caused variable responses ranging from adverse to beneficial [28]. Colonization with *Bifidobacterium infantis* was observed to decrease stress response while colonization with *E. coli* caused an increase [28]. Mice supplemented with *Lactobacillus rhamnosus* experienced modulation of GABA receptors within the brain causing a decrease in depression, anxiety, and stress like behaviors via the parasympathetic activity of the vagus nerve [28]. Similar tests were done using cecal transplants from exploratory and timid mice. The behavioural phenotype was observed to be transmissible through fecal transplantation to other mice [29].

Dysbiosis of gut microbiota has also been observed to contribute to gut dysfunctions in conditions such as irritable bowel syndrome (IBS). Studies have indicated that enteric microbial colonies are less diverse and unstable in individuals experiencing the disorder [15]. Although identifying dysbiosis of enteric microbial communities as a causative mechanism is still speculative, several studies have noted the amelioration of some IBS symptoms through supplementation with certain probiotic strains [30]. The study also reported that antibiotic usage and small intestinal bacterial overgrowth have been linked with an increased risk of IBS. Perturbations in the enteric microbiota cause changes in intestinal motility and secretion resulting in visceral hypersensitivity contributing to alterations in the entero-endocrine and immune system [15]. This visceral hypersensitivity phenotype has been transferred via fecal transfer from IBS patients to germ-free rats [31]. It is also indicated that psychiatric comorbidities such as anxiety and depression accompany the gastrointestinal symptoms of IBS [15]. A potential mechanism used to explain this is the decreased levels of plasma tryptophan, a precursor of serotonin that places a key role in the regulation of gastrointestinal motility, secretion, and perception as well as cognition and mood [15]. Humans have lost the ability to synthesize tryptophan endogenously and rely on diet and metabolic activities of certain microbial species to obtain it [15].

The bidirectional function of the gut-brain axis allows for an intricate host-microbiota communication system bridging the ENS and the CNS. Perturbations within the enteric microbial communities have been observed concomitantly with psychological disorders [32]. The importance of the gut-brain axis in facilitating both neurological and immune development is emphasized and demonstrated by current research on autism spectrum disorders.

Gut Microbiome Composition and Autism Spectrum Disorders

Autism spectrum disorders (ASDs) are broad and complex neurodevelopmental disorders that affect around 1% of children in the United States [32]. The symptoms of ASD are impaired social interaction, delayed and disordered language, repetitive or stereotypic behavior, and a restricted range of interests. These symptoms must be present in the early developmental period and must cause significant impairment in social and occupational functionality [32]. Accompanying the behavioural symptoms, many effected individuals also experience physical symptoms such as seizures, sleep problems, metabolic conditions, and gastrointestinal (GI) disorders [32]. Initially, it was believed that GI symptoms were just comorbidities occurring alongside ASD, but more recent studies have indicated that it may also be a causative mechanism [33].

One study used maternal immune activation (MIA) gnotobiotic mice to understand the endophenotypic connections between behavioural traits of individuals with ASD and GI pathology. Pregnant mice were injected with the viral mimic poly (I:C) that activates an immune response and is known to result in offspring expressing both behavioral and physical traits associated with ASD: core communicative, social, and stereotyped impairments, and a localized deficiency in

cerebellar Purkinje cells [33]. The study observed additional changes to the offspring's GI permeability, commensal gut microbiota, and serum metabolites. It was noted that some of these alterations are like endophenotypes observed in human individuals affected by ASD, as reviewed in [33]. The mice were treated with the normal commensal *Bacteroides fragilis* that are native to the human gut. This treatment corrected gut permeability defects and modulated levels of tight junction proteins and the high levels of cytokines that are typically associated with ASD individuals [33]. The main cytokine of interest was interleukin-6 (IL-6).

In previous studies involving MIA mice models, high levels of IL-6 were discovered to be contributing to the behavioral changes associated with ASD. This was demonstrated through injecting a pregnant mouse with IL-6, observing the behaviors of the offspring, and treating with an anti-IL-6 antibody [34]. The ASD like behavioral traits of the offspring were reversed after the antibody treatment. To further solidify their findings, MIA IL-6 knockout mice were bred and several of the key behavioral characteristics of ASD were not observed [34]. The *B. fragilis* study observed that treatment with the microbe significantly lowered IL-6 levels in MIA mice, successfully ameliorating the decreased gut permeability [33]. It was also noted that in human children with ASD that only one species of Bacteriodaceae appeared less frequently than the others, and that was the one most closely related to *B. fragilis* [33].

Beyond gut physical integrity, *B. fragilis* was also noted to correct changes in gut serum metabolites associated with MIA. Several normal *Clostridium* species are believed to be responsible for producing 4-ethylphenylsulfate (4EPS) precursors [33]. High levels of the metabolite 4EPS have been linked to inducing anxiety-like behavior in mice, and high numbers of both the responsible *Clostridium* species as well as 4EPS have been observed in MIA mice. Treatment with *B. fragilis* lowered both numbers [33]. This evidence suggests the lack of *B. fragilis*, in MIA mice and children with ASD, could be contributing to dysbiosis resulting in an imbalance of metabolites and disease states [33].

These studies are still relatively new and involve complex multifaceted interactions, but as each aspect is dissected, potential preventative or therapeutic treatments could come to light.

Conclusion

While this field of study has yet to reach its adolescence, mounting evidence indicates that throughout our evolutionary history, bacteria have been with us every step of the way guiding our developmental process through cell signaling, the contribution of metabolites, and horizontal gene transfer. These interactions refine and train our immune system, which subsequently facilitates the development of a proper functioning neural network. Within this neural network, the gut-brain axis works as a direct line of communication between the external environment, the enteric microbial populations, and the host, which are constantly working in sync to maintain systemic homeostasis. The perturbation of the enteric microbial ecosystem has been shown to coincide with a wide range of functional abnormalities and is thought to be a contributing causal mechanism of widespread conditions such as irritable bowel syndrome [15], autism spectrum disorders [32], depression, and anxiety [24].

Some studies have gone one step further and found that augmentation of the gut microbiome through probiotic treatment, fecal microbial transplant, or colonization of specific microbes, has led to the amelioration of certain conditions. These findings provide enough evidence to motivate novel studies using bacteria for therapeutic treatments.

Due to the field still being in its infancy there are still many unknowns and questions that need to be answered. Due to the complex nature of the interactions of all organisms involved, it is difficult to pinpoint exactly what interactions or metabolites are responsible for the observed effects. In addition, most studies to date have primarily been performed on mouse models which may or may not directly translate to human physiology.

As technology continues to develop and approaches are refined, this dynamic field of study has the potential to unlock new layers of understanding regarding the complex interactions and history that we share with our microbial counterparts, and could potentially lead to novel therapeutic treatments as well as early life innovations greatly improving one's overall health and quality of life.

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In Vitro Assessment of Drug-Eluting Stents as Novel Chemotherapeutic Devices: Proof-of-Concept Study

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Reese is a third-year undergraduate student at McGill University, in the Biological, Biomedical and Life Sciences department. He is currently majoring in Microbiology and Immunology. Since the beginning of high school, Reese has had a strong passion for cancer research, specifically innovative cancer treatments. Reese couldn't wait to get started working in the Mitha lab when he heard that Dr. Mitha was trying to utilize drug-eluting stents to treat cancer. Reese looks forward to continuing to work on new, innovative cancer treatments and aspires to one day make a positive global impact on cancer patients.

Abstract

Although effective, current forms of chemotherapy pose risks to cancer patients. Intravenous chemotherapy is a standard form of treatment and causes numerous side effects due to its systemic nature. Despite its use to combat cancerous cells, healthy cells are also destroyed because it lacks specific targeting mechanisms within the treatment. Intra-arterial chemotherapy is an example of a more targeted therapy but still has its risks due to the high dosages administered to patients during a short duration. Drug-eluting stents, which utilize a biodegradable coating to release drug, are a much safer alternative not only because of the lower dosages administered, but also due to the continuous treatment with minimal disruptions in the daily lives of patients. To determine if drug-eluting stents are viable for such an application, three critical properties of the coating were assessed: smoothness/uniformity of the coating, ability to endure the delivery process, and efficacy of elution from the stent. To assess smoothness and uniformity, stents were coated and subsequently analyzed by SEM. For durability, the coating was analyzed by SEM before and after the stents underwent mock deployment conditions. To determine how efficiently the coating is released from the stent, the coated stents were placed in a physiological flow model and the concentration of drug in the system was measured, which is indicative of how much coating has been released. The amount of drug released was measured through high-performance liquid chromatography. Results showed the majority of the stent can be coated in a smooth and uniform fashion, with exception to its bifurcations. The durability test showed that in some regions the coating was retained as desired, while the majority of the stent had a very disrupted coating. The release-profile of the stents showed that the drug concentration in the eluent consistently increased over the course of the trial. From these results, drug-eluting stents have shown promise as a new and novel delivery method for chemotherapy, a function of stents that has not yet been explored. Future directions involve obtaining more uniform and durable coatings on the entire stent, and performing *in vivo* trials to determine the efficacy of drug release and target organ drug concentrations.

Keywords: Cancer, intra-arterial chemotherapy, drug-eluting stents, coating properties

Introduction

Chemotherapy is typically administered intravenously (IV). Although effective, it poses many risks for the patient due to its systemic nature [1]. Alternatively, intra-arterial (IA) chemotherapy is a more targeted route that utilizes catheters to administer doses. IA chemotherapy requires the administration of high dosages to the patient over a short period also rendering the treatment a health risk [1]. If drug-eluting stents (DES) were used to deliver IA chemotherapy, the coating on the stent need contain only as much drug as would be required to reach the target end organ, which is lower than what would typically be administered IV or IA. In addition, as the coating is continuously released from the implanted stent, patients would be able to carry on with daily activities while the drug is being administered. The objective of this study was to examine the viability of DESs to deliver chemotherapy, by assessing three crucial properties of the coating: its smoothness and uniformity, its ability to endure the delivery process, and efficacy of elution from the stent.

Methods

To assess the smoothness and uniformity of the coating, coating solutions were prepared by dissolving 100mg of the chemotherapy drug 5-fluorouracil (5-FU) and 900mg of the bioabsorbable polymer poly (lactic-co-glycolic acid) (PLGA) into 70mL of acetone. After coating the stents by a process known as dip-coating for 3 hours and allowing for the coating to solidify for 72 hours, the coating on the stents was analyzed by scanning electron microscopy (SEM). To evaluate coating durability, coated stents were deployed by an endovascular surgeon, mimicking clinical conditions. The appearance of the coating on pre-deployed and post-deployed stents was then compared. To determine the elution profile, coated stents were placed in an *in vitro* physiological flow model for 21 days and the concentration of drug in the eluent was measured daily by high-performance liquid chromatography (HPLC).

Results

Compared to an uncoated stent (Figure 1A), the struts on the coated stent (Figure 1B) appear to have no edges and looked circular, indicating the presence of a coating. Unlike the bifurcations, struts were coated smoothly and uniformly as seen in Figure 1C.

Compared to the stent's coating pre-deployment (Figure 2A), the post-deployment coating (Figure 2B) is less dense and apparent. Some regions retained a smooth and uniform coating in Figure 2B.Measured concentration of 5-FU in the eluent of the physiological flow model increased throughout the experiment (Figure 3) ($p<0.1$ is sufficient, given the proof-of-concept nature of the study) [2].

Discussion & Conclusion

Based on the results, it is apparent that not only can stents be coated in a smooth and uniform manner, but the coating can also withstand deployment to a sufficient extent. At the same time, the coating can release drug in detectable amounts in physiological flow conditions. Hence, DESs may represent a novel route of chemotherapeutic drug delivery. This is the first time that DESs have been tested as a device for downstream drug delivery. Other innovative treatments, such as CAR T cell therapy, are limited in the array of cancers they can treat [3]. DES chemotherapy is more versatile as stents can be placed in a variety of arteries that lead to different organs, thus addressing numerous types of cancer. Future directions involve modifying the coating technique to obtain a more uniform and durable coating, and to perform *in vivo* studies to evaluate target organ drug concentrations.

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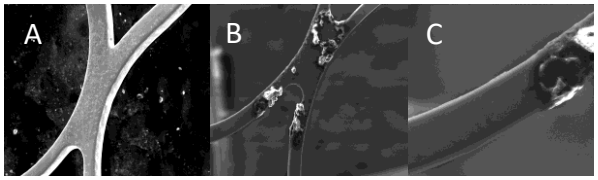

Figure 1: (A) Uncoated stent. **(B)** Coated Stent. **(C)** Enhanced bottom-left image of (B).

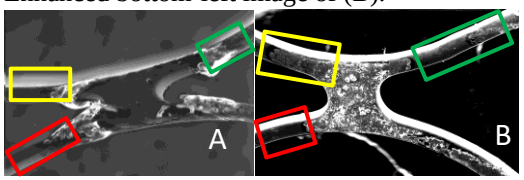

Figure 2: (A) Pre-deployed stent, **(B)** Post-deployed stent. Different colors correspond to same regions

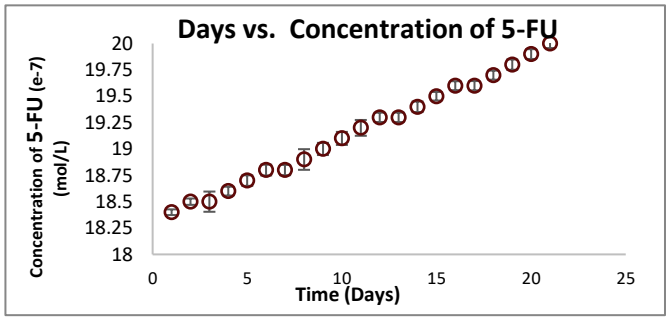

Figure 3: Drug release profile of coated stents. $p<0.1$ between Day = 1 and Day = 21. Sample size is n=3, one tailed t-test.

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Differences in Subchondral Bone Fat Content of Young and Adult Rats Fed a High-Fat High- Sucrose Diet

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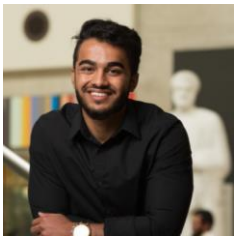

Muzammil Nasir is 4th year Bachelor of Science student at the University of Calgary majoring in Kinesiology. He started working as a summer research student in the Human Performance lab in 2019 under the supervision of Dr. Walter Herzog. His work in the lab in the past 2 years has focused on studying the development of knee osteoarthritis and the effects of Botox on the properties of a rabbit spine. Furthermore, Muzammil is a University of Calgary Dinos Track & Field athlete who hopes to pursue an academic career in sports medicine. He hopes to use his contribution in research to influence current and future medical studies.

Abstract

Previous work from our lab shows that when young and adult male Sprague-Dawley rats are fed a high-fat high-sucrose diet, the adults develop OA-like damage in their knee joints while the young rats do not. We investigated this occurrence by feeding both young and adult rats a high-fat high-sucrose diet for 12-14 weeks and then analyzed their knee joints using histology. We found that the damage in the adult knee joints is likely attributed to the differences in how fat is stored in the body. We found that the adult rats showed a significantly higher fat cell size while the young rats did not, although they had the same percentage of body fat. Therefore, it led us to believe that the differences in knee joint damage between the two groups is likely related to the size of their fat cells (hypertrophy) and likely not body fat percentage or the amount of fat cells they have (hyperplasia). However, to fully explain our results, full metabolic profiles of these rats need to be performed and related to the observed knee joint damage.

Keywords: Osteoarthritis, subchondral bone, adipocyte

Introduction

Obesity and metabolic syndrome have been identified as primary risk factors for the development of knee osteoarthritis (OA). Previous work from our lab has demonstrated that adult male Sprague-Dawley rats exposed to a high-fat high-sucrose (HFS) diet become obese and develop OA-like damage in the knee that manifests itself primarily in the subchondral bone (Rios et al, 2019). Studies have indicated that increased bone marrow adipocyte (fat cell) infiltration can impair bone remodelling, potentially helping to explain the changes seen in the subchondral bone of adult rats (Duque et al, 2014). Contrary to adult rats, unpublished work from our lab suggests that young male Sprague-Dawley rats exposed to a HFS diet for 14-weeks post-weaning do not develop OA-like damage in the knee. Therefore, the purpose of this study was to assess differences in bone marrow adipocyte infiltration in young and adult rats exposed to a HFS diet. We hypothesized that adult rats have increased adipocyte infiltration compared to young rats.

Methods

Twenty-one male Sprague Dawley rats were assessed based on age (3 or 12 weeks of age). All animals were subject to a HFS diet for 12-14 weeks. Upon completion of the dietary intervention, body mass and body composition (DXA) were determined and knee joints were harvested, fixed, processed, stained, and imaged. Five 100,000 μm^2 regions of bone marrow in the subchondral bone of the femur and tibia that showed the highest density of adipocytes were chosen for manual analysis of the primary outcome variables using cellSens software *(Figure 1).* Outcome measures included adipocyte cell number, size, and density within each region. Independent t-tests were performed to determine differences between young and adult rats.

Results

Adult rats had significantly greater body mass than the young rats (Adult: 725 ± 65 g; Young: 650 ± 51 g; $p < .05$), but there was no difference in percent body fat (Adult: 30 ± 7 %; Young: 28 ± 3 %).

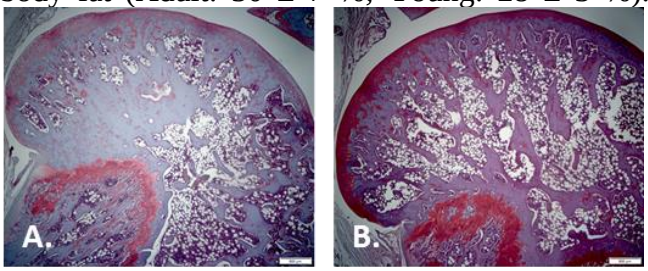

Figure 1. Knee Joints. Representative images used to quantify adipocyte infiltration in bone marrow. The white dots represent adipocytes. **A.** Young rat. **B.** Adult rat. Scale bar = 500 µm

When compared to the young rats, adipocyte cell density in the subchondral bone marrow of adult rats was 18% greater in the medial femur and 21% greater in the lateral tibia (*Figure 2*).

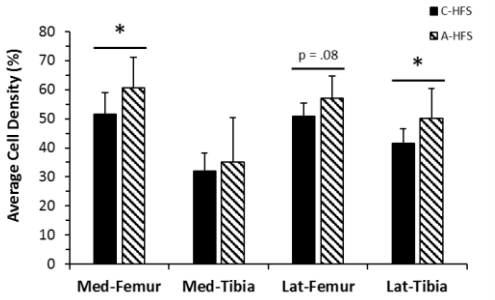

Figure 2. Bone Marrow Adipocyte Infiltration. Average adipocyte cell density in the subchondral bone for the medial (Med) and lateral (Lat) compartments of the knee joint. Data bars represents means ± SD. Asterisks indicate statistical significance for $p < .05$. C-HFS represents the young animals while A-HFS represents the adult animals. N=9 (young). N= 12 (adult).

Discussion & Conclusion

Increases in bone marrow adipocyte infiltration has been linked to abnormal bone remodeling through the suppression of osteoblasts, potentially helping to explain the OA-like changes seen in the knee joints of adult rats (Duque et al, 2014). Considering the body fat percentage in the two groups of rats was similar, it is hard to argue that body fat might have led to the differences in the knee joint damage, rather we believe it may be due to the increase in adipocyte size, leading to knee joint damage in the adult rats. We have also previously shown that similar percentages of body fat, i.e., obesity, can be associated with vastly differing metabolic profiles for Sprague-Dawley rats (Rios et al, 2019). Therefore, in order to fully understand the current results, a full metabolic profile of the young and adult rats needs to be performed and related to the observed knee joint damage. Ongoing work will look to perform full metabolic profiles of these animals along with using more time- efficient software to quantify fat levels to attain quick and complete results regarding the relationship between knee joint damage and bone marrow adipocyte infiltration in the two groups.

Acknowledgements

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Investigating the Structural Implications of Obesity on Skeletal Muscle in Adult and Young Rats

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Taylor is a current undergraduate student at the University of Calgary, taking a combined academic program with a Bachelor of Science (Honours) in Biological Sciences, and a Bachelor of Science in Psychology. She completed a summer studentship in 2019 under the supervision of Dr. Walter Herzog and Dr. Venus Joumaa, during which she contributed to ongoing research on the association between obesity and musculoskeletal disorders. Taylor's research in the Herzog group has contributed to her interest in health and the musculoskeletal system, and has further encouraged her to pursue a Master of Physical Therapy, following completion of her Bachelor's degrees.

Abstract

Obesity is classified as a global epidemic disease and is increasing dramatically. Obesity has been associated with musculoskeletal disorders, such as osteoarthritis, as well as muscle weakness. It is hypothesized that obesity negatively affects muscle health because it compromises its structural integrity. The purpose of this study was to test this prediction by investigating muscle integrity in a Sprague-Dawley rat model of obesity, using young and adult rats fed either a high-fat/sucrose or chow diet. Myofibril protein and contractile protein (actin and myosin) content, myosin heavy chain isoforms, and triglyceride content were assessed in the vastus lateralis muscle. It was hypothesized that obesity decreases myofibril protein content and amounts of myosin and actin, transforms slower MHC isoforms to faster isoforms, and increases triglyceride content in young and adult animals. The study showed, however, that obese rats had no difference in myofibril content, myosin and actin content, or MHC isoforms, compared to lean animals, with the exception of MHC IIb which was higher in adult animals compared to young animals. Adult obese rats had more triglycerides, compared to young and adult lean animals. It was concluded that the vastus lateralis was able to cope, to some extent, with the negative molecular effects of obesity, by maintaining protein concentration relative to muscle mass. However, the study is limited, as the length of this diet intervention may not have been long enough to provide detailed information separating the effects of age and obesity.

Keywords: Diet-induced obesity, fat infiltration, protein content, triglyceride

Introduction

Obesity is classified as a global epidemic disease (Hurt et al., 2011), and is associated with musculoskeletal disorders, such as osteoarthritis (OA) (van der Schouw et al., 2016; King et al., 2013), as well as muscle weakness (Rios et al., 2019). OA-like symptoms have been observed systematically in diet-induced rat models of obesity (Rios et al., 2019; Collins et al., 2015). Furthermore, maximal absolute skeletal muscle strength is higher in obese compared to non-obese individuals, but lower when normalized to muscle and body mass (Lafortuna et al., Tomlinson et al., 2014). The mechanism by which obesity leads to muscle weakness is not well understood. It is hypothesized that obesity compromises muscle structural integrity by reducing contractile protein content, increasing fat content, relative to muscle mass, and by inducing inadequate transitions in myosin isoforms.

The purpose of this study was to examine this prediction by investigating muscle integrity in a CD Sprague-Dawley diet-induced rat model of obesity in young and adult males. We hypothesized that obesity is associated with compromised muscle integrity and that its detrimental effect is more severe in adult compared to young animals.

Methods

Young (3 weeks old, n=10) and adult (12 weeks old, n=12) male CD Sprague-Dawley rats were fed a high-fat/sucrose (HFS) diet, for 14 weeks and 12 weeks, respectively. Young (n=10) and adult (n=8) control animals were fed a standard chow diet for the same timeline. The vastus lateralis (VL) was harvested and its integrity was assessed by investigating: myofibril protein content using a colorimetric bicinchoninic protein assay, myosin and actin content using 12% SDS-PAGE gel electrophoresis, myosin heavy chain (MHC) isoforms using 8% SDS-PAGE gel electrophoresis, and triglyceride (TG) content using a colorimetric assay. All animals were included in analysis, and Kruskal-Wallis testing on SPSS Statistics (SPSS version 25, IBM®, Chicago, IL, USA) was used to assess statistical differences between groups. For significant ($p<0.05$) results, a post hoc Mann-Whitney U-Test with a Bonferroni correction was performed to determine the groups that differed.

Results

There was no statistically significant difference in myofibril content or myosin and actin levels, nor the proportions of MHC isoforms (except MHC IIb, which was higher in adults than young animals), between obese and lean animals. However, adult rats fed the HFS diet had statistically more triglycerides (52.8 ug/mg dry muscle) when compared to young ($p=0.008$) and adult ($p=0.005$) lean animals (34.4 and 32.7 ug/mg dry muscle, respectively) (Figure 1).

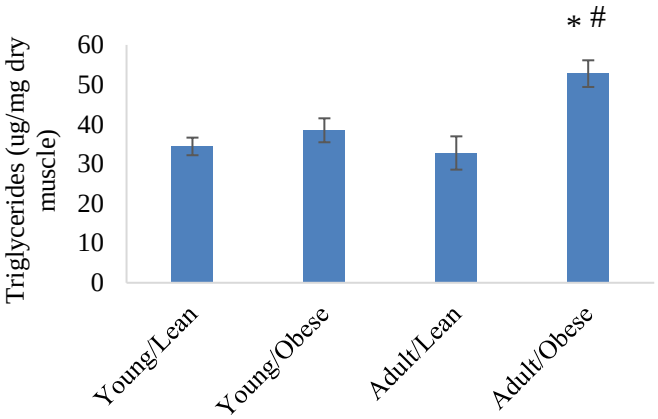

Figure 1. Mean TG concentration. Bars indicate ± SD. * significant difference compared to adult lean animals, # significant difference compared to young lean animals.

Discussion

The VL was able to cope with obesity, to some extent, and retain myofibril content and myosin and actin levels, relative to muscle mass. Moreover, there was no maladaptive progression from slower to faster MHC isoforms, nor compensatory measures to produce slower isoforms, which are more efficient for loads associated with obesity. The increase in triglycerides in adult obese rats may indicate a threat to muscle integrity, but it could be interpreted as a positive adaptive response of the muscle to cope with the HFS diet by storing fat without compromising the amount of proteins.

Conclusion

It seems that VL protein content, and thus contractile capacity, are not compromised in this model of obesity. This model does indicate an increase in triglyceride content associated with a high-fat/sucrose diet. However, the study is limited, as the mechanical properties of VL were not tested and the length of diet intervention may have been insufficient to compromise muscle protein content, making these steps of interest for future studies.

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Cell vibrational profiling (CVP) of skin cancer carcinoma using optical tweezers: Continuation of a novel approach

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AUTHOR BIO

Jared Topham is a recent graduate of the University of Calgary's Bachelor of Science program with a major in Kinesiology (with Distinction). He has been working under the guidance of Dr. Amrein since the summer of 2016 where his work has mainly focused on using atomic force microscopy to study the vibrational spectra of healthy cells, brain cancers, and most recently, skin cancers. Throughout his work, Jared has developed a keen interest studying cellular processes. As of Fall 2019, Jared has undertaken a Master of Medical Science specializing in Cancer Biology at the University of Calgary.

Abstract

Previous research using Atomic Force Microscopy (AFM) has been able to differentiate cells based on their unique sounds. These sounds are thought to be due to metabolic processes inherent to a particular cell type. Optical tweezers (OT) have recently become an improved instrument for detecting these sounds, now termed vibrations, and provide higher resolution recordings. While cell and tissue vibrations have been observed, the exact frequencies and signals emitted have never or only vaguely been recorded. As such, the purpose of this project was to determine if skin cancer carcinoma cell lines, emit unique vibrational spectra that permits cell differentiation. Our study discovered that skin cancer carcinoma cell lines and healthy cells can be visibly, and more importantly, statistically differentiated based on their signal intensities and frequency spectra. While future directions are currently focused on improving vibrational detection and accuracy via improved statistical analysis, signal filtering, and machine learning, in the long-term, we envision that this novel discovery may prove useful in pre- and intra-operative methods within the clinical setting.

Keywords: Optical tweezers, skin cancer carcinoma, vibrational profiling

Introduction

Current treatments often incompletely resect larger basal and squamous cell carcinomas (BCC & SCC), permitting reoccurrence. This stems from the inaccurate determination of pre-operative margins leading to increased operating time, patient morbidity, and expense on the health care system. This study explored the feasibility of using frequencies to differentiate cancerous margins in the clinical environment. The presence of unique cellular frequencies within cells has been previously demonstrated without instrumental sensitivity or proper statistical analysis (Nelson et al., 2017). Our current study combined the highly sensitive optical tweezers (OT) and software in a novel manner to determine vibrational differences between SCC, BCC, and human epidermal keratinocytes (HEKa). We hypothesized that each cell type has a specific frequency profile.

Methods

All cell vibrations were measured at resting state in 2mL of cell-media solution at 37°C. Concentrations of 20,000-30,000 suspended cells minimized interference to optimize environments. Recordings were achieved using the OT. The 3D gradient/scattering force interactions trapped, manipulated, and recorded cells. Cell displacement (nm) due to Brownian fluctuations, was converted into force (N). Cells were recorded three times over 15 seconds. We designed novel software in R-studio® to record signal and frequency spectra up to 2000Hz and performed multivariate statistical analysis (MVSA) to cell line likeness.

Results

Deflection over time depicted a maximum value of around $2.0x10^{-12}$N, $0.5x10^{-12}$N, and $0.25x10^{-12}$N for SCC, BCC, and HEKa cells respectively (Figure 1, A). Three spectral regions allowed for the differentiation of cell lines. The 300-330Hz region to the left displayed no oscillations for HEKa cells, the 520-600Hz middle region is similar among all cell types; HEKa cells had the lowest amplitude, and the region on the right from 1470-1500Hz displayed visibly different clusters of peaks for all cells (Figure 1, B).

Multivariate interpretation using orthogonal partial least squares discriminatory analysis (OPLS-DA), used the frequency spectra of HEKa and SCC to cluster data based on peak likeness. Two unique groups were shown (Figure 2).

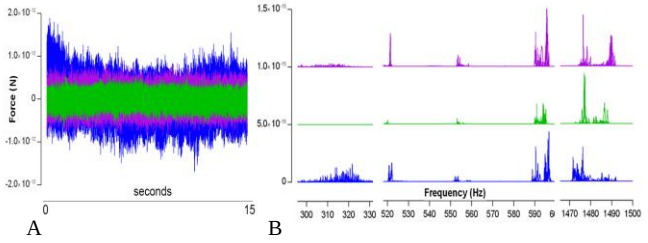

Figure 1. A) Deflection over time for normal skin cells (HEKa, n=6, green), and cancerous skin cells (SCC, n=10, blue & BCC, n=7, purple), n: number of different cells recorded. B) Three spectral regions of HEKa, SCC, and BCC cells illustrating similarities and differences.

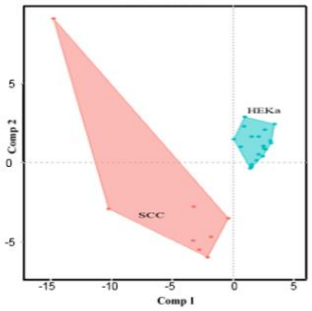

Figure 2. OPLS-DA normal skin cells (HEKA, n=6, blue) and cancerous skin cells (SCC, n=3, red) providing statistically distinct frequency clusters indicative of unique vibrational profiles.

Discussion & Conclusion

In this study, skin cancer carcinoma cell lines were differentiated, shared similarities in vibrational profiles, and displayed unique spectra (Figures 1 & 2). In line with the literature, the most aggressive cancerous cell line (SCC) displayed the most intense signal followed by BCC and then HEKa (Tanese, Nakamura, Hirai, Funakoshi, 2019) (Figure 1, A). Given these vibrational spectra, frequencies may prove instrumental as frequency biomarkers to differentiate within and between cancerous cells. Further research including BCC in MVSA will further determine spectra differentiability. Amplifying profiles and limiting interference from magnetic and electric fields will improve this method's clinical viability.

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