VOLUME 14, 2022-2023

Catalyst

RICE UNDERGRADUATE SCIENCE RESEARCH JOURNAL

DISARMING MICROBES

A Novel Approach in the Fight Against Anti-microbial Resistance

IN THIS ISSUE:
+ Consequences of Topical Retinoid Application
+ Possible Treatment for Type I Diabetes

from the editors

Dear reader,

We are ecstatic to bring you the 14th issue of Rice Catalyst, Rice's own Undergraduate Science Research Journal! Even though COVID-19 prevented us from publishing new issues of Rice Catalyst from 2019 to 2022, we have compiled the contributions from our writers during those years, which we published as the Omnibus Edition on our website, ricecatalyst.com. Despite setbacks in our publication schedule, we remain committed to providing our peers with a platform to exhibit interdisciplinary perspectives on scientific dialogue as a peer-reviewed journal written, edited, and designed by undergraduate students, as well as encouraging scientific dialogue both on and off the Rice University campus. Hopefully, regardless of your academic background or affiliations, Rice Catalyst can continue to bring you exciting scientific literature in an engaging and accessible manner. As the first issue to be published in print since the start of the pandemic, this year's publication presents articles on every thing from vaccine development, to treatments for Type I Diabetes, to hypothyroidism.

It goes without saying that pausing publication for several years meant that we had taken several steps back as an organization. However, surviving the decline in engagement and outreach that we suffered as a result of COVID-19 was our foremost priority in our gradual return to normalcy. We began the year with several goals in mind: to ensure our past writers' works come into fruition by publishing the Omnibus Edition, to return to our regular annual publication schedule, and to revise our internal infrastructure in order to create a solid groundwork for future Catalyst leaders to make the journal more successful than ever. We not only achieved these goals, but also expanded remarkably compared to our pandemic years, and we are optimistic about the future of our publication. Furthermore, in pursuit of bringing quality scientific communication on a national level, Rice Catalyst has joined the National Undergraduate Consortium for Science Journalism as a participating member, and we will help organize the annual NUCSJ conference to facilitate the creation of a collaborative network for quality undergraduate scientific journalism. In the coming years, we hope to bring back familiar initiatives like the Eureka, Fusion, and the Annual Catalyst Fall Research Panel to promote scientific engagement on the Rice campus. Please stay on the lookout for different ways to engage with Rice Catalyst throughout the upcoming school year!

Our return to regular annual publication could not have been done without the responsibility and diligence of all Catalyst members, and we would like to acknowledge their indispensable contribution to our journal. Furthermore, Rice Catalysts' return could not have been made possible without assistance from external collaborators. We would like to acknowledge Rice Design for helping Catalyst recruit designers for our beautiful journal, the Student Association's Blanket Tax Committee for sponsoring Catalyst's renewal, and the Student Activities/ President's Programing Fund for continuing to support us financially and making the printed publication possible. Last but certainly not least, we remain incredibly grateful for our advisor, Dr. Daniel Wager, who has guided Catalyst through its highs and lows for over a decade with invaluable advice.

We are proud of Catalysts' rebound in the post-pandemic world, and we are more excited than ever for our growth in the coming years. From the entire Catalyst staff, we hope you enjoy our latest issue as much as we enjoyed making it!

Best,

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ALL ABOUT Catalyst

Catalyst is Rice University's Undergraduate Science Research Journal. Catalyst's goal is to showcasesstudent perspectives on popular science topics and undergraduate research. We're committed to fostering interdisciplinary interest in scientific writing and dialogue about science at Rice. We invest in our writers to represent our organization, our university, and the power of writing in this context to educate and engage a broad audience. Catalyst was founded over a decade ago and the current faculty advisor is Dr. Daniel Wagner, a professor in the Biosciences department. Typically Catalyst publishes one issue per year; however, due to the COVID-19 pandemic this cycle was disrupted.

A

Attractions

Attraction's articles are 1-2 pages and focus on various science-related topics. They are written with a conversational tone so that anyone can read and understand them.

B

Breakthroughs

Breakthrough's articles are 1-2 pages and focus on current research by interviewing rice faculty as well as other researchers. They are geared towards being understandable by anyone.

C

Connections

Connection's articles are 2-4 pages and focus on original personal research or a research review. These articles are more complex and modelled after peer-reviewed scientific articles, and thus are geared towards an audience with some scientific background.

D

Discoveries

Discoveries's blog posts can be found on the Catalyst website. The blog posts are are short and about any topic in science. They are written in a conversational tone and geared towards the general public.
Website: catalyst.rice.edu

design

The Catalyst design team works to create visual graphics and page design for the articles in the final publication. The design team works to make articles easier to understand and pleasing to look at. The design team also works to compile and format all articles into one magazine.

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Vaccine Development

A Window into Revolutionary Progress with Alzheimer's Disease

By Abby McKellop

The human brain is a fascinating masterpiece: a complex network of components working together harmoniously to allow us to think, learn, and feel. With neurological disorders, one small malfunction can bring attention to the importance of each piece of the puzzle. Many of these neurological disorders still do not have a cure, which inspires scientists to push the boundaries in search of solutions to improve quality of life. One major neurological disorder is Alzheimer's disease: a neurological condition that is characterized by the loss of neural functioning.

"For around two decades, neurological disorders like Alzheimer's disease have been a major focus of clinical research... Recently, the efforts of this research have begun to come to fruition"

For around two decades, neurological disorders like Alzheimer's disease have been a major focus of clinical research. This research has led to a strong foundational understanding of the biological processes that cause Alzheimer's symptoms. Recently, the efforts of this research have begun to come to fruition, with many new, promising treatment options in clinical trials. One of the major treatment options seeing recent progress is different types of vaccines.

In order to develop these treatments, scientists first needed a strong understanding of the potential underlying causes and common risk factors found in Alzheimer's patients. Symptoms of Alzheimer's can include confusion, memory loss, mood or personality changes, and even impaired motor coordination.1 Risk onset of the disease significantly increases with age.

Other potential risk factors include family history of the disease and family history of cardiovascular disease, as well as other lifestyle factors associated with cardiovascular diseases.2,3

Although many risk factors can be identified, scientists are unable to distinguish any direct causes of the harmful changes in the brain that lead to the development of Alzheimer's.
What scientists do know is that the underlying biological processes that can lead to Alzheimer's disease are related to the misfolding of specific proteins in the brain. It is important to understand that the structure of a protein, which is made up of an amino acid backbone, directly correlates to its function. The process of protein synthesis can be divided into two major parts: transcription and translation.
First, the gene—a specific region of DNA that contains the information to create a given protein—is transcribed into a messenger RNA (mRNA) molecule. Then, this mRNA molecule is modified in important ways, and then translated into an amino acid sequence. This chain of amino acids folds into a particular structure, based on chemical properties of the amino acids in the chain. As the information is transferred from DNA, then to RNA, and finally to an amino acid sequence, each step of the process must transmit the correct information. When there is an error in one of these steps, the amino acid sequence for a protein will likely end up incorrect, and thus negatively alter the resulting shape and function of the protein.4

With Alzheimer's disease, there are two specific protein types that fold incorrectly in neurons. These two proteins are an amyloid protein and a tau protein. The amyloid protein spans the plasma membrane and assists with repair of damage to the neuron,5 and the tau protein maintains the shape and stability of the neuron.6 In both cases, misfolded versions of these proteins are unable to carry out their correct function and cause harmful effects for the cell. The misfolded amyloid proteins will clump together and form beta-amyloid aggregates, and the misfolded tau proteins group together in tangles. The term tangles is used specifically to describe abnormal clumps of misfolded tau proteins. These clumped structures of misfolded proteins will create blockages, and interfere with communication between neurons.6

Further, these blockages are not localized to one part of the brain.

Evidence has shown that the misfolding of these proteins can spread to other neurons, and inhibit the functioning of those cells as well.7 Neighboring cells malfunction, producing even more harmful protein clumps. In addition, there are high amounts of inflammation in the brain and spinal cord that are found in Alzheimer's patients, and this inflammation tends to exacerbate problems with neurological communication. It is the existence of these beta-amyloid plaques, tau protein tangles, and high levels of inflammation that are responsible for the hallmark symptoms of Alzheimer's. As these problems spread to different areas of the brain, patients may experience a wider range of symptoms, because brain areas correspond with different functions.

With dedicated research efforts to learn more about the disease, scientists have attempted to explore new solutions that could help the more than six million Americans with Alzehimer's.1 Many new treatments for Alzheimer's are being developed, including vaccines that are currently in the clinical trials phase. Vaccines are a versatile tool, and their use to treat Alzheimer's displays their multifaceted nature as a treatment for disease.

The development of these vaccines has the potential to revolutionize treatment possibilities for Alzheimer's disease.

These vaccines tend to target one of three specific elements: beta-amyloid plaques, tau tangles, or the immune system.7,8 Vaccines targeting beta-amyloid plaques seek to train the immune system to recognize and break down these harmful protein aggregates. On the other hand, vaccines targeting tau tangles look to inhibit the misfolding of tau proteins.

And finally, vaccines that target the immune system have the widest range of variation, as they can activate or inhibit the immune system in different ways to counteract the negative effects of the disease, one of those being neuroinflammation.9

One of the vaccines being developed for Alzheimer's is notable for being the first nasal vaccine. This nasal vaccine, called Protillion, targets the immune system. Protillion is currently in the first phase of clinical trials, and its development is based on over 20 years of research.10 The vaccine is an adjuvant, an immune-system activator, and it triggers the innate immune response, which is the body's first line of defense.10 This vaccine operates on a similar technology used to create influenza vaccines, and stimulates the production of various immune cells through activation of receptors that recognize specific pathogenic characteristics.10,11The activation of the innate immune system is able to trigger the adaptive immune response, which has the ability to remember the specific antigen to fight off a future encounter. After extensive data collection and testing, scientists are able to see that stimulating the immune system in this manner clears up beta-amyloid plaques.10,12 As the testing of the vaccine progresses, scientists will monitor the vaccine's effectiveness and look out for any adverse effects.10,12

Although this vaccine and many others have not yet completed the entirety of clinical trials nor the FDA approval process, progress has been hopeful. The development of these vaccines has the potential to revolutionize treatment possibilities for Alzheimer's disease. But more importantly, the applications of this biotechnology do not end here. The usage of vaccines for Alzheimer's could open the door to treatment possibilities for other neurological disorders, many of which are currently deemed incurable. This technology could just be the beginning, and could inspire further scientific work that will lead us to a brighter tomorrow.

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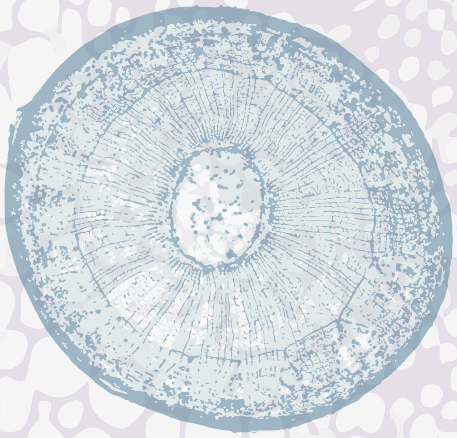

SEEING THE UNSEEN: OPTICAL MICROSCOPY

By Carlson Nguyen

Beneath our fingertips, in the air we breathe, and on virtually every surface imaginable, there are countless microbes living just like you and me. The study of such microbes, invisible to the human eye, can aid scientists in unraveling mysteries regarding biological mechanisms. This, in turn, facilitates our understanding of many physiological conditions, such as precancerous lesions and malignant tumors, and the potential pathways for treating them. In order to study these hidden microbial worlds, we need to develop specific tools for their imaging and observation. For many scientists, visualization often comes in the form of optical microscopy techniques. However, as with any instrument, using microscopy techniques requires an understanding of their purpose, capabilities, and limitations. Here, we'll take a peek into the world of these microscopy techniques and how they enable the progression of science.

Microscopes are not new inventions by any means; they have existed since the early 17th century when the first simple (one lens) and compound (two or more lenses) microscopes were created—allowing for up to a hundredfold magnification of objects.[1] These early compound models functioned by using a lens, close to the sample, to collect sunlight and magnify images. These images could be further magnified by adding another lens near the eyepiece. As one might imagine, these images contained many imperfections, such as color distortions, and were nowhere near the level of quality achievable through modern microscopes. Today, optical microscopy techniques have been refined to achieve greater magnification, serve specialized functions and minimize image aberrations—allowing for much higher resolution and detail in a variety of applications.

Today, optical microscopy techniques have been refined to achieve greater magnification, serve specialized functions and minimize image aberrations—allowing for much higher resolution and detail in a variety of applications.

Optical microscopy techniques can generally be divided into two categories: *transmitted light* and *epi-fluorescence light*. Both are techniques that allow for the visualization of cells and their components but differ in their capabilities and uses.

Although they are separate categories, these techniques are often used in conjunction with one another to provide a better interpretation of a biological sample. In the first category, *transmitted light microscopy* is the general term used for any type of microscopy where the light is transmitted from a source on the opposite side of the specimen to the objective lens—including the stereotypical microscope you might find in a high school classroom.[2] This group of techniques is based on the differences in a sample's refractive index, which determines how much a path of light is bent when it enters the sample. Within this category, *Brightfield microscopy* is the most basic form of transmitted light microscopy where incident light, or light that falls onto the sample, transmits through a sample and creates contrast through denser areas.[2,3] However, this contrast is lower than that of other techniques; therefore, brightfield requires thick samples of high refractive indices or chemical staining for the best results. This is important because staining of biological samples will often kill cells and prevent live-cell imaging, thus making other visualization techniques necessary. On the other hand, *phase contrast microscopy* makes up for the brightfield limited contrast by converting 'phase shifts' to a change in the image's light intensity. In this case, light passes through a phase condenser, which concentrates and defocuses light before it reaches the sample. The diffracted and background light passes through a phase plate/objective lens, which slows

down background light and allows for destructive interference between the two lights, resulting in increased contrast and visibility. In other words, phase shifts are being converted to differences in brightness that make structures in an image more visible.[2,3]

In order to generate transmitted light images with more accurate 3D representations, *differential interference contrast microscopy* (DIC) creates contrast in a specimen by creating a high-resolution image of a thin optical section. With DIC, two closely spaced parallel rays are generated and made to interfere after passing through an unstained sample. The background is made dark and the interference pattern is particularly sharp at boundaries, allowing specimens to appear really bright in contrast. Finally, *darkfield microscopy* is similar to brightfield microscopy but results in a dark background and a bright sample. Darkfield microscopes use a special aperture to focus incident light, so the background stays dark. The light does not pass directly through the specimen but is reflected off it, causing the specimen to appear as if it is emitting light. The resulting image reveals much more minute details and has an appearance similar to that of an x-ray.[2,4]

The second category, *fluorescence microscopy*, uses luminescence caused by an object's (i.e. living cells or proteins) absorption of radiation at a certain excitation wavelength to generate an image. More specifically, electrons of a fluorophore (fluorescent chemical) absorb photons of light from an excitation source, raising the fluorophore electron's energy level. As the electron returns to its original energy level or ground state, it emits a photon of light at a higher wavelength; this is the light we see.[5] *Fluorescence microscopy* produces vibrant and colorful images that aid scientists in tasks such as labeling biomolecules or super-resolution imaging. However, this technique does have a drawback in that excessive excitation of a sample can lead to photobleaching (damage to fluorophores) of the cells

Fluorescence can be observed through two general techniques: *wide-field epi-fluorescence* and *confocal*. During *wide-field epi-fluorescence*, a high-powered mercury light or LED passes through an excitation filter (to select for an excitation wavelength), through a mirror, the objective lens, then through the sample. The sample then emits light of a higher wavelength that is reflected by the mirror to an emission filter and to the eyepiece. Finally, in *confocal microscopy*, a laser set at the excitation wavelength, rather than a wide-field light, is moved through each part of the sample. The sample will return an emission in a similar process, but the presence of a pinhole will block out-of-focus light, allowing only the in-focus light to participate in image formation. Compared to wide fields, confocal microscopy allows for greater control of depth-of-field and reduction of background noise, but is more time-consuming because lasers concentrate on one point at a time.[2,3]

Optical microscopy is just a small taste of what the field has to offer. With new and growing technologies, we are able to study objects at the near-atomic level, decipher unresolved structures of proteins, and unveil biological mechanisms.

We have now seen how transmitted light techniques can generate images with varying brightness or contrast, and how fluorescence techniques can be used to excite cells to produce spectacular color images. Despite all their differences, these techniques are the same at their core: they provide insight into how the microscopic world functions. This insight, in turn, can build the foundation for groundbreaking advancements in our understanding of diseases and treatments. Optical microscopy is just a small taste of what the field has to offer. With new and growing technologies, we are able to study objects at the near-atomic level, decipher unresolved structures of proteins, and unveil biological mechanisms. Whether it be as simple as a high school frog dissection, or as complex as live-cell imaging, microscopy empowers scientists of all backgrounds to understand a world of unimaginable complexity—a world that you, too, can access if you choose to do so.

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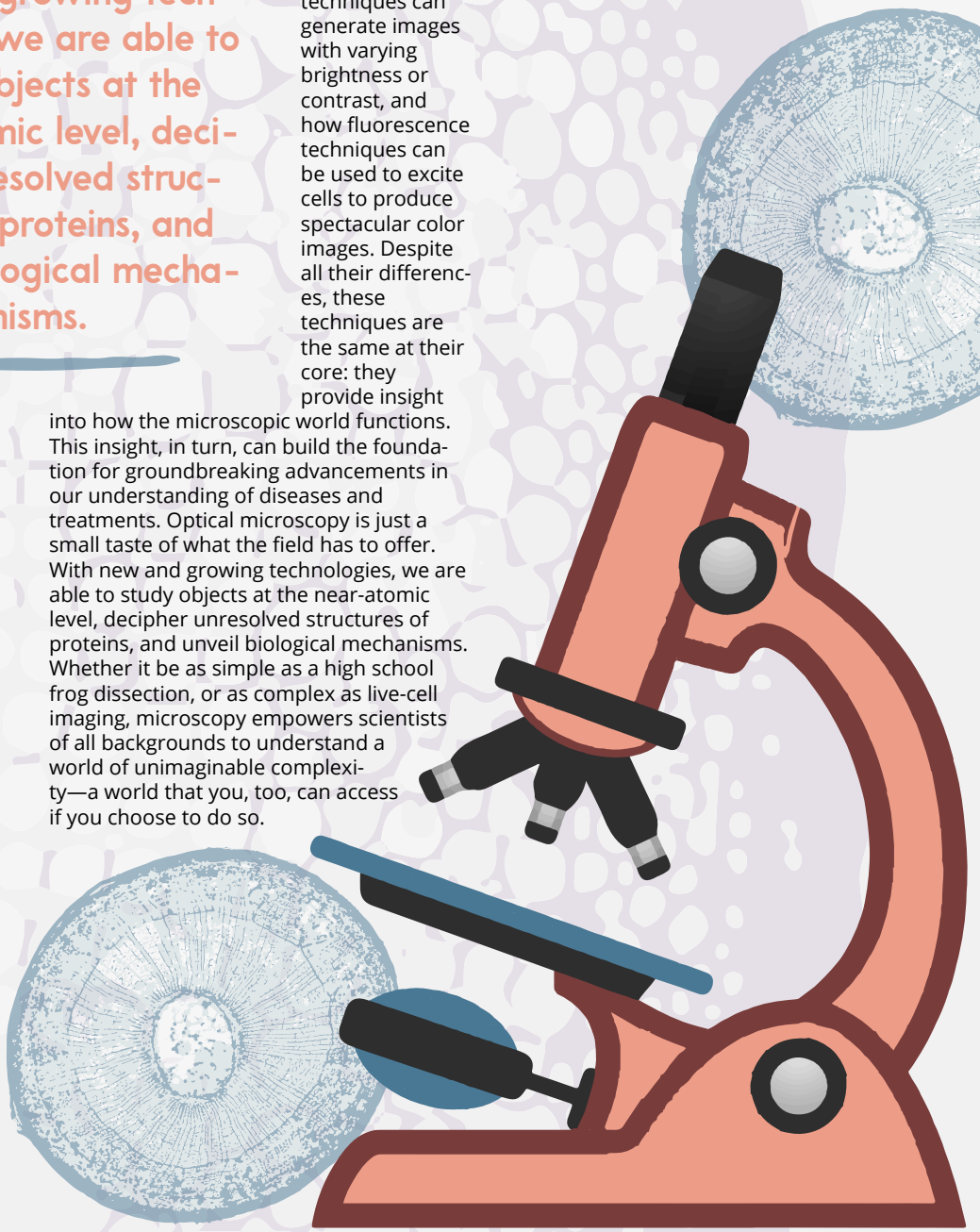
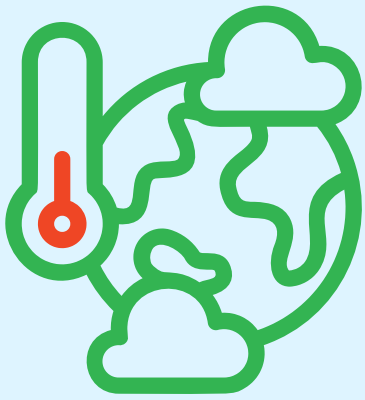


HOW CLIMATE CHANGE IS WORSENING NEUROLOGICAL DISEASES

WRITTEN BY Anika Sonig

From recent hurricanes such as Hurricane Ian in Florida to wildfires in California, we cannot deny the fact that climate change is already exerting disastrous effects on our communities. Further, climate change has very real effects on human health and disease, especially neurological diseases such as epilepsy, sleep deprivation, and viral diseases such as malaria.

Not only does climate change impact our physical communities by destroying infrastructure, it also worsens complex neurological diseases that depend on a patient's environment. Global warming contributes to extreme weather conditions by inducing an average long-term increase in global temperatures, affecting neurological diseases that are exacerbated by temperature, humidity, and heat. With epilepsy, for example, low temperatures, high humidity, or low atmospheric pressure may trigger seizures. Patients with some severe epilepsies, such as Dravet syndrome, have reported increased seizures during the unusually hot summers in recent years. In a survey conducted by Epilepsy Climate Change[1], among 1000 patients with epilepsy, 62% of patients saw an increase in seizure frequency or severity during hot summers. Dravet syndrome is also known to play a role in Sudden Unexpected Death in Epilepsy (SUDEP), which is fever-sensitive, and increases in temperature can lead to higher SUDEP rates and a higher mortality rate. Therefore, climate change leads to increased seizure frequency, seizure severity, and higher mortality rates.



In addition to epilepsy, climate change also impacts a factor that is relevant for everyone: sleep. Weather variations exacerbate the effects of fatigue and sleep deprivation and alterations in climate due to the impacts of climate change have already been shown to cause large changes in an individual's sleep patterns. A large-scale US survey indicated that a deviation of one degree celsius in night-time temperatures was associated with an increase of three nights of self-reported insufficient sleep per 100 people per month[2]. Poor sleep is bad for one's health, and moreover, the elevation of night-time temperatures even more negatively affects poor sleepers as they tend to feel fatigued. The stress caused by climate change-induced wildfires, floods, and hurricanes may result in the displacement of communities, increased viral infections, and food insecurity, which in turn triggers stress and decreases sleep quality. This is how climate change both combines and exacerbates stress, sleep deprivation, and fatigue, which are incredibly relevant for college students experiencing many of these symptoms as well for patients with epilepsy as these factors increase seizure frequency.

> "... WE CAN FUEL OUR ENERGY TOWARD SERVICE AND ACTIVISM IN WHATEVER WAY WE CHOOSE - BIG OR SMALL - EVERY EFFORT MATTERS."

Further, in addition to neurological diseases, climate change also increases the spread and prevalence of infectious diseases such as malaria and the West Nile virus. Malaria is a deadly, tropical, mosquito-borne disease that kills almost one million people and affects almost one billion people around the world. Increases in rainfall, humidity, and temperature may cause a proliferation of the malaria-carrying mosquitoes at higher altitudes and drier climates, resulting in an increase in malaria transmission in areas in which it was not reported earlier[3]. Changes in climate also affect West Nile viral transmission rates, which mosquitoes spread as the vectors for this virus.

Since weather conditions have direct influences on the ability to acquire and transmit the viral vector, and this affects the viral replication rate within the mosquito, with higher temperatures accelerating replication rate and viral transmission efficacy[4] . The Intergovernmental Panel on Climate Change lists vector-borne diseases among the consequences most likely to change due to global warming, so we should focus on creating solutions that would help reduce the spread of viral diseases. It's extremely important to minimize the spread of these illnesses by mitigating the effects of climate change, as they are both deadly.

If we do not improve upon the current progression of climate change and create policies to limit its effects, then the rates of seizures in epilepsy, rates of stress and sleep deprivation, and rates of viral-borne infections will only continue to skyrocket. Climate change effects will also be distributed differently across nations worldwide and exacerbate health disparities that already exist within and between communities. International collaboration and public health efforts will be imperative for studying and mitigating the impacts of climate change and creating creative solutions. Like so many people, I wonder what to do at this time. This summer, I could not only visibly see, but also hear, the glaciers in Iceland melting and this further instilled the urgency of the situation in me. Whether it be through adopting renewable energy such as electric vehicles or installing solar panels, not supporting fast fashion methods, composting at the serveries, or supporting the climate movement through activism - there are many ways in which we can contribute and make a difference. It's sometimes hard to escape the sense of pain and heartbreak for the state of our planet, but while acknowledging this grief, we can fuel our energy toward service and activism in whatever way we choose - big or small - every effort matters.

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Somatic Gene Therapy: An Innovative Approach for Spinal Muscular Atrophy Treatment

BY: NIMAH HAIDAR

In the first few weeks of his life, Nico Nieves, a patient at the Children's Hospital of Richmond, was diagnosed with spinal muscular atrophy (SMA), a destructive and rapidly progressive neurodegenerative disease. [1] Infants diagnosed with type 1 SMA typically exhibit a life expectancy of two years and present symptoms of difficulty walking or sitting, tremors, scoliosis, dyspnea (difficulty breathing), dysphagia (difficulty swallowing), and muscular weakness. [2] Remarkably, Nico celebrated his second birthday recently, and continues demonstrating major developmental milestones, such as gaining the ability to walk, talk, and play with his siblings. [1] His success can be attributed to the continuous advances in somatic gene therapy, a medical intervention providing a definitive pathway for addressing genetic abnormalities.

Somatic gene therapy, or SGT, permits the insertion of specific genes into an organism and the subsequent translation of those genes to modulate gene expression. Among applications associated with SGT, spinal muscular atrophy has garnered substantial attention in recent years. SMA occurs most frequently due to the Survival Motor Neuron 1 gene (SMN1) experiencing mutations. [3] This gene encodes the survival motor neuron protein, an essential protein for the maintenance and functionality of motor neurons. [3] Motor neurons send nerve impulses from the central nervous system - including the brain and spinal cord - to the muscles, thereby facilitating movement. When the SMN1 gene is mutated, SMN protein production is compromised, leading to the degeneration of motor neurons within the spinal cord and brainstem. Consequently, affected children gradually lose their ability to function autonomously, initially presenting with muscular atrophy and mortality by age two. [2] Notably, SMA is hereditary, and because SMN1 irregularities are detectable in the early stages of gestation through genomic testing, treatment postnatally can ensure the patient's survival. [1]

Despite the progressive nature of SMA, efficient and rapid treatment has been made possible by the new drug Zolgonesma. With Zolgonesma, infants diagnosed with the condition can receive treatment before significant motor neuron degradation has occurred, allowing them to function independently and live through adulthood. [3] Zolgonesma introduces the SMN gene artificially via an adeno-associated virus serotype 9 (AAV9) vector into cells. [3] This virus replaces the patient's malfunctioning or absent SMN1 gene with a correct and functioning version of the gene. [3] Currently, Zolgonesma is administered intravenously in pediatric patients, but administration requires a cerebrospinal fluid injection for older children or adults, a process currently undergoing testing. [3]

Although the SMN gene is currently only integrated through postnatal treatment, SMA is a candidate for prenatal treatment. The incomplete development of the blood-brain barrier in fetuses enhances the ability of inserted genes to reach the brain.4 Moreover, the immune response prior to birth is limited, resulting in decreased likelihood of treatment rejection. [4] By utilizing somatic gene therapy (SGT) prenatally, adverse effects of the disease are halted immediately before significant damage occurs.

SMA treatment is currently one of the most prominent examples of gene therapy success. Furthermore, research concerning gene therapy continues to expand, enabling infinite possibilities of application. For instance, at Boston Children's Hospital, a treatment center currently leading the way in somatic gene therapy treatment, doctors are utilizing somatic gene therapy to treat children with SMA, retinal conditions, sickle cell disease, immunodeficiencies, leukemia, lymphoma, and various other conditions. Although gene therapy appears to be a tremendous therapeutic breakthrough, unyielding costs, and ethical considerations warrant further research and investigation. The scientific community continues to debate the appropriate contexts for gene therapy and what constitutes an emergency or disability that requires such interventions. Critics have expressed concern that gene therapy may exacerbate existing socioeconomic disparities, where only the wealthier population can afford prohibitively expensive treatments left uncovered by insurance companies. As of 2021, a single dosage of Zolgonesma was priced at over two million dollars, making it the most costly drug approved in the United States. [6] Although efforts are underway to create a more affordable technique for drug production, the current cost is impractical and inaccessible for uninsured or low-income families. [6] While more efficient production methods may eventually reduce costs, advocacy for affordable SGT is essential for ensuring economic inequality is not further exacerbated within the healthcare sector.

Ultimately, SGT is an invaluable tool that will continue to be developed and optimized in the coming years. As seen in the case of Nico Nieves, the effect of SGT is life-changing for families and loved ones, and the possibilities are vast. Yet, careful addressing of ethical questions and financial considerations is essential in ensuring such innovation is not abused or misused. Somatic gene therapy has the ingenuity and adaptability to alter medical care, resulting in a vastly efficient and revolutionary medical sector in the coming years. No longer will doctors have to fight to alleviate symptoms as diseases progress, but instead be able to eliminate the problem at its source, saving and improving countless lives worldwide.

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Edited By: Carlson Nguyen
Designed By: Lillian He, Meghan Lim

Investigating The Potential Negative Consequences of Topical Retinoid Application

by Samah Haidar

Retinol, a derivative of Vitamin A, is a prized ingredient in the field of dermatology. Various forms of topical and oral prescriptions of retinol are used to treat a variety of skin concerns such as natural aging of the skin, acne, hyperpigmentation, and psoriasis. So, how does this multi-tasking, seemingly perfect ingredient work? It is actually fairly simple: retinol is a fat soluble molecule that can work beneath the top layer of the skin—the epidermis—and reach the dermis. Within the dermis, retinol fights free radicals, agents that contribute to aging of the skin, and stimulates the production of collagen and elastin, which are fibrous proteins that support skin strength. Retinol can degrade free radicals, reducing their presence in the dermis. It is important to limit free radicals in the skin because free radicals are unstable molecules that often damage heathy skin cells. Furthermore, retinol is able to increase collagen and elastin in the skin by promoting cell growth [1]. However, while retinol has been proven to improve numerous skin conditions, many medical professionals claim the use of retinol can contribute to skin thinning which can make the skin more susceptible to sun damage. In addition, there are professionals who believe the topical use of retinol during pregnancy can harm an unborn child. In this paper, I will explore the existing literature to determine the efficacy of these claims.

Despite the propagation of the rumor that retinol causes skin thinning and increased susceptibility to sun damage, there is no clinical proof that retinol causes such side effects. On the contraryBy contrast, in a clinical study on the role of retinol in antiaging, it was determined that "a low dose of retinol...induces epidermal thickening ... and alleviates skin aging signs, without any significant adverse reaction" [2]. While skin thinning is not a result of retinol application, it is unclear whether increased photosensitivity is associated with the use of retinol. Photosensitivity is when a certain factor increases the likelihood of an individual developing a sunburn after exposure to the sun. Many dermatologists claim retinol may cause photosensitivity because retinol increases the rate of cell turnover, which exposes fresh skin cells to sunlight, given new cells are more likely to burn without a protective layer of dead skin cells. While this theory could be true, there is no clinical evidence to prove retinol increases the risk of sunburns and sun damage to the skin. However, one important reason as to why retinol has been blamed for causing sun damage is because the symptoms of redness, dryness, itching, and sensitivity that result from the use of retinol are similar to the symptoms people face after getting a sunburn. As a resultIn many cases, people may misinterpret the natural side effects of retinol as a sunburn.

Another common claim on the use of retinol is the idea that a pregnant mother's use of topical retinol may cause harm to her unborn child. In a study conducted by the European Network of Teratology, 235 pregnant women were exposed to various samples of topical retinoid [3]. The study found that there was no significant difference in termination of the pregnancy, birth defects, or other adverse effects between women who had no topical retinol use and women that did use topical retinoid during their pregnancy. However, despite the lack of proof that retinoids can harm an unborn baby, the researchers still can not promote the use of retinol during pregnancy because there is not enough evidence to prove it is entirely safe. Few credible studies have been done that expose women to topical retinoids for obvious ethical concerns. In general, there are simply not enough studies on the effects of retinol use during pregnancy to recommend its use during pregnancy. Given the limited number of studies we have to work with, dermatologists and obstetricians recommend pregnant women stop the use of topical retinoids during pregnancy out of caution.

Ultimately, this review paper concludes that there is limited clinical proof that retinoid application causes skin to be more susceptible to sun damage. Most individuals are well aware of the extensive research that has shown how increased sun exposure contributes to accelerated aging of skin, hyperpigmentation, and the development of skin cancer. In fear of causing their skin to be more sensitive to sun damage, people have refrained from using topical retinoids. This paper confirms there is no reason to avoid topical retinoid application due to fear of increased likelihood of sunburns and further encourages all individuals, those who use retinol and those who do not, to regularly use sun protection. Furthermore, while there is little to no clinical or research proof that use of topical retinoids while pregnant will harm an unborn baby, the lack of research on the use of retinoids during pregnancy cannot fully rule out all possible concerns. Therefore, this paper does not encourage the use of topical retinoids during pregnancy to protect an unborn baby from possible negative implications of retinol exposure that have yet to be discovered.

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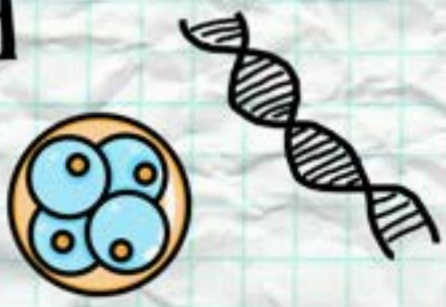
DESIGN BY Jessica Huang
EDITED BY Supraja Kadagandla

GENOMIC IMPRINTING—A UNIVERSAL PATTERN

BY KALINA TSUNG



What does patterning have to do with you, me, and basically everyone else? Let's take a look at patterning — the organization of cells in an embryo into different functions, depending on their location within the embryo—and see how important it is for our understanding of human development. That's what Dr. Aryeh Warmflash, an Associate Professor of Biosciences at Rice University and CPRIT scholar in cancer research, focuses on. Inspired by his early research with frogs as a model, he switched to researching the embryonic developmental processes in humans. In his current research, he aims to understand the processes that happen in the early human embryo, including how cells within these embryos communicate and integrate different decisions for the patterning process.

Unique differences in human development that may result in birth defects or infertility, as well as pattern formation during these stages, form a new field of scientific research that aims to understand the complexity behind the formation of these complex organisms. With access to embryonic stem cells in a culture dish, which are equivalent to the cells of a two-week embryo, the Warmflash lab recreates the early developmental process in humans. Cells make their first choice in differentiation between the germ layers. The ectoderm forms the nervous system and skin, the mesoderm forms the heart and skeletal muscles, and the endoderm forms the digestive and respiratory tube.

As the developmental process continues, there are specific steps in determining the final state of cells. These cells communicate with each other in a manner that is not yet well understood. However, there are particular cellular proteins that are formed and are responsible for cellular signaling, which have patterns set up in space and time. And that forms the crux of Dr. Warmflash's research—spatial pattern formation and signaling in embryonic development. Cells are sensitive to their environment, and Dr. Warmflash concludes that the embryonic development process is heavily influenced by neighboring cells. As a way to visualize these pattern formations, micropatterning is used, which creates cell surfaces that confine cells to specific regions. It is a technique to create groups of cells which are reproducible in size, shape, and pattern to mimic that of the human embryo.

> LIKE A GAME OF TELEPHONE, THE CELL PASSES THE SIGNAL TO ITS ADJACENT CELL, WHICH CONTINUES TO SPREAD AND ACTIVATE THE SIGNAL

What approach does the Warmflash lab take to visualize these time dependent signals? This is another area of the lab's research—figuring out the dynamics of protein signals to understand how these patterns are developed. To visualize time-dependent signals, he uses fluorescent cell lines modified with CRISPR that light up in specific ways when certain signals are received by cells. By recording the development of these cells, the Warmflash laboratory can visualize the cells communicating with each other in real time. Dr. Warmflash says these "waves of signaling sweep through the colony." The sensitivity to which these signals are received produces a "code [that] plays out in time as much as in space."

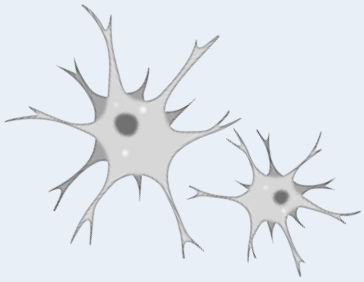
The Warmflash laboratory has discovered how the Nodal protein, which is critical for signaling in early development, is used to spread signals between cells. When that protein is knocked out in mice, development fails at an early stage. The Warmflash lab attached a fluorescent protein to the Nodal protein, which he was able to visualize during the developmental process. On a microscopic level, cells that make Nodal only "pass" it to their immediate neighboring cells. Like a game of telephone, the cell passes the signal to its adjacent cell, which continues to spread and activate the signal. One cell makes Nodal protein that is passed to the next cell and activates the signal there, which will then produce its own Nodal protein. What's so special about this signal propagation is that it "does not rely on the signals moving in the extracellular space," but instead on activating one neighboring cell at a time.

In a video published at the Warmflash Laboratory about the Nodal signaling pathway, the fluorescence of cells with the Nodal protein are only moving to their immediate neighboring cells. In another experiment, the Warmflash lab deleted Nodal, and the cell received the signal and was activated, but the signal was not passed onto the adjacent cell because it could not synthesize Nodal. Similarly, in the game of telephone, one player can receive the message but cannot tell their neighbor what they received, as they cannot make the signal. With the new discovery of combinatorial interpretation, which is the reception and integration of multiple signals at once by a cell, the laboratory is currently interested in finding how the cell integrates multiple occurring signals and how these interactions work. This continued work further aims to understand the complexities underlying differentiation in embryonic cells.

The Warmflash Laboratory is currently studying the tissue interactions of the embryo and extraembryonic tissue, which supplies patterning signals to the embryo. With these exciting discoveries and ongoing research in the Warmflash Laboratory, the scientific community is progressing toward grasping the embryonic patterning formation that will help us understand our own development. The entire process, from a system that starts with no pattern but structurally forms one in an organized fashion, is a fascinating area of research that will potentially help us understand birth defects or infertility. The next time you think about the people around you, remember the complex patterning they once went through!

DESIGNED BY CAROLYN TENG

Myelin Really Gets On My Nerves



UNDERSTANDING MYELINATION TO TREAT NEURODEGENERATIVE DISORDERS

BY SEOJIN KWON

Neurons are the cells within our brains and nerves that allow us to interact with and understand the world around us. From the dendrites of the cell body that receive signals, to the axons that carry them, and finally, the axon terminals that send them further, neurons are the messengers that send information all throughout our bodies. This allows us to breathe, talk, eat, and even think. However, there are billions of cells in our brain that are often forgotten and overlooked but equally as vital to the function of our nervous system: glial cells. [1]

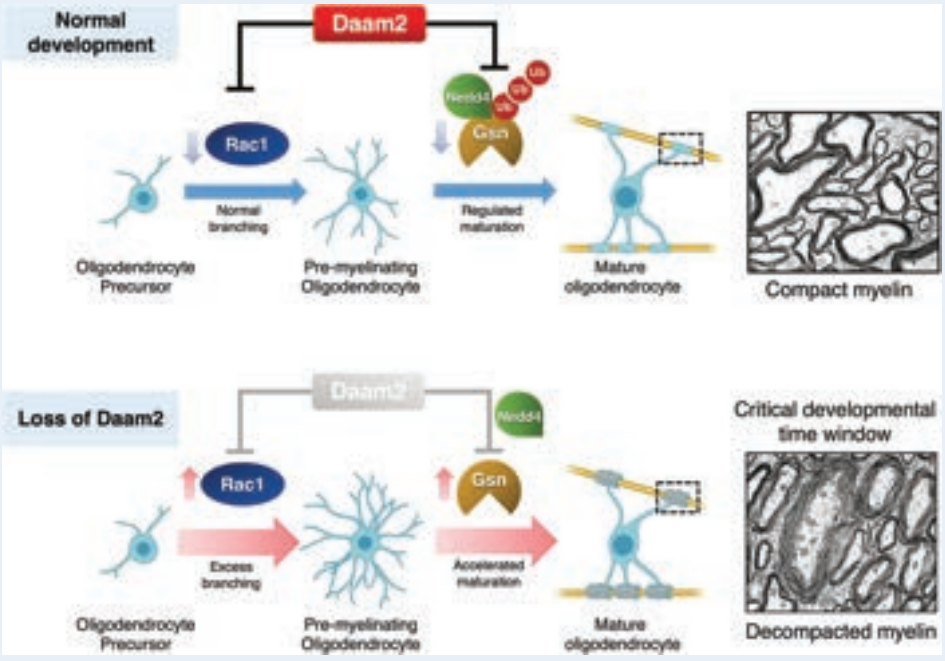
Also known as the glue of the nervous system, glial cells have a number of functions that help support, maintain, and develop the neurons of both the central (CNS) and peripheral nervous systems (PNS). The oligodendrocyte, in particular, is a type of glial cell that plays the crucial role of creating myelin sheaths around neurons of the CNS. Myelin sheaths are layers of proteins and lipids wrapped around axons that insulate the electrical signals neurons send to one another for communication, allowing for the efficient transmission of signals. Due to the significance of the myelination process, there are disastrous effects when it is disrupted, one possibility being the autoimmune disease, multiple sclerosis (MS). MS causes one's body to attack their own myelin sheaths, disrupting the communication between the body and brain. Despite its pivotal importance, the mechanism for myelination has yet to be fully understood.

Dr. Hyun Kyoung Lee, an associate professor at the Baylor College of Medicine and principal investigator at the Jan and Dan Duncan Neurological Research Institute, is building the foundation for treating demyelinating and neurodegenerative disorders by discovering the molecular mechanisms and regulatory pathways for glial cell development and function. Amidst the dominance of neurons in the field of neuroscience and the treatment of neurodegenerative disorders, Dr. Lee and her lab are working on understanding "glial-specific function in human health." By understanding these complex yet fundamental processes, her lab helps create new strategies to combat these diseases.

In spite of its complexity, we know that the myelination mechanism has three distinct phases of development: (1) oligodendrocyte progenitor cells (OPC), (2) premyelinating oligodendrocytes, and (3) myelinating oligodendrocytes. [2] Initially, OPCs exist while the oligodendrocyte begins to extend its branches. After extension occurs, premyelinating oligodendrocytes begin to attach to the axons of neurons. Finally, after fully maturing, myelinating oligodendrocytes start wrapping the myelin sheaths around axons.

However, the complex process of myelination involves several intricate signaling pathways, which are interactions between the proteins of a cell that regulates its functions. Initiating these pathways requires an environmental stimulus, such as when a ligand binds to its receptor. Of these pathways involved in myelination, the Wnt signaling pathway is one of the main regulators for oligodendrocyte development and repair. [3] The activation of the Wnt signaling pathway has been found to inhibit the development of oligodendrocyte differentiation, thus hindering myelin repair after injury. As such, Dr. Lee believes that "targeting Wnt pathways will be beneficial for promoting remyelination in neurodegenerative disorders."

To investigate this phenomenon, the Lee Lab rigorously studied this pathway

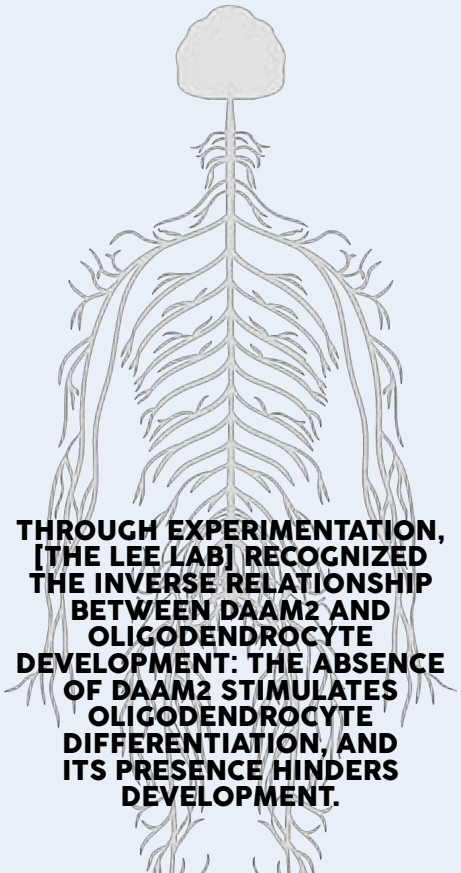


WORKING MODEL ILLUSTRATING THE ROLE OF DAAM2 IN REGULATING THE OLIGODENDROCYTE CYTOSKELETON. OLIGODENDROCYTES ARE A TYPE OF GLIAL CELL THAT PLAYS A CRUCIAL ROLE OF CREATING MYELIN SHEATHS AROUND NEURONS OF THE CENTRAL NERVOUS SYSTEM. [5]

and discovered the significant role of the Daam2 (Dishevelled-Associated Activator of Morphogenesis 2) protein. Through experimentation, they recognized the inverse relationship between Daam2 and oligodendrocyte development: the absence of Daam2 stimulates oligodendrocyte differentiation, and its presence hinders development. [3] This is due to the substantial role of Daam2 in the promotion of the Wnt signaling cascade. [3]

Previous studies from the Lee Lab suggest the crucial role of Daam2 in the structural regulation of myelination development and repair. Her lab used mice to model gain-of-function, and loss-of-function experiments, which is when a specific protein is amplified or deleted to identify its function, respectively, specifically with the Daam2 protein. Initially, Dr. Lee hypothesized that "because oligodendrocyte differentiation is promoted in the absence of Daam2 and loss of Daam2 really encourages oligodendrocyte differentiation, we just assumed that axons would have thicker myelin in the absence of Daam2." However, after analyzing their results, they found this was not the case. The data from these initial trials led to the discovery of the dynamic and stage-dependent nature of myelination. Of the many different downstream proteins, the main ones related to Daam2 that impacted the cytoskeleton structure of myelin sheaths were identified as Rac1 and Gelsolin. [5]

The compaction of myelin, which ensures that the layers of myelin are dense and closely packed, is crucial for normal maturation and neuronal function. [4] Rac1 and Gelsolin are the proteins responsible for ensuring precise compaction through the coordination of Daam2. However, they are most active in different stages of myelin development. Rac1 is involved with the process extension stage of myelination when the OPCs begin to branch (phase 1). Gelsolin, on the other hand, instigates maturation from premyelinating to myelinating oligodendrocytes (phase 2 to 3). This can lead to problems with the structural integrity of the myelin sheath down the road if these proteins are not clearly regulated. As the prime regulator for Rac1 and Gelsolin,



THROUGH EXPERIMENTATION, [THE LEE LAB] RECOGNIZED THE INVERSE RELATIONSHIP BETWEEN DAAM2 AND OLIGODENDROCYTE DEVELOPMENT: THE ABSENCE OF DAAM2 STIMULATES OLIGODENDROCYTE DIFFERENTIATION, AND ITS PRESENCE HINDERS DEVELOPMENT.

Daam2 ensures the compaction of myelin. Therefore, in the absence of Daam2, there is a lack of proper myelination due to the excess branching from malfunctioning Rac1 and premature growth of the myelin by dysfunctional Gelsolin regulation, leading to the myelin sheath being neither compact nor functional. [5]

In the words of Dr. Lee, we can regard "Daam2 as the major motherboard" for myelination control. Its pivotal importance as a regulator of myelin development and repair can be seen through its interactions with Rac1, Gelsolin, and even the Wnt signaling cascade. Unlike transcription factors, which are hard to target due to their location within the nucleus and the inability of the host to survive without them, and ligand-receptors, which are hard to control for, the Daam2 protein is relatively accessible to manipulate into inducing re-myelination promotion in diseases such as MS and injuries to the white matter, which are areas of the nervous system consisting of axons.

Dr. Lee's lab focuses on the long-underappreciated underdog of the nervous system. Due to the difficulty of studying and identifying glial cells, they have gone ignored for far too long. However, now, by focusing on the fundamental signaling processes and mechanistic interactions of the glial cells, Dr. Lee's lab has brought them into the spotlight and is building the foundation to treat these elusive diseases by understanding how they work on molecular levels.

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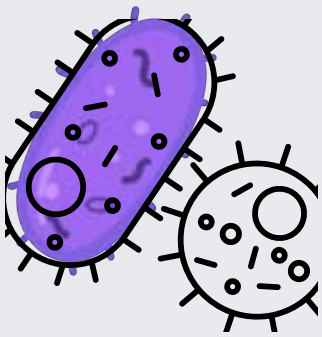
Edited By ARIEL MA
Designed By LILLIAN HE

WHAT DOESN'T KILL YOU MUTATES YOU

WRITTEN BY Jina Park

What comes to mind when you think of bacteria? Perhaps you remembered the last time you had a bad sinus infection and spent the entire evening coughing up mucus in the sink. Or maybe you pondered how elementary school taught you the habit of washing your hands after using the bathroom. More often than not, bacteria have a bad reputation for being the root of common human infections. While some bacterial species do fit this description, many more serve as our invisible allies. For example, have you ever enjoyed a glass of wine during Thanksgiving or grabbed some Chobani yogurt from Walmart? These foods are fermented with the help of bacteria–without them, we wouldn't be able to enjoy these delicacies.



Bacteria are all around us, whether we notice them or not. Like humans, bacteria live in communities where they interact and exchange resources. Depending on environmental conditions, these interactions can either be mutually beneficial (mutualistic) or self-serving (antagonistic). In other words, bacteria can either cooperate to gather resources or target competitors to protect themselves and their community.

Dr. Marcos H. de Moraes, an assistant professor at Rice University's BioSciences department, is one of the leading researchers investigating bacterial interactions. His previous research examined interbacterial antagonisms and how pathogens establish their niche in different environments. What stood out to Dr. de Moraes, however, was how bacteria could infect other bacterial cells through the release of antibiotic toxins. Cells that were intoxicated by the toxin died, while those that survived had their DNA permanently mutated. "We call it: what doesn't kill you mutates you," Dr. de Moraes said with a laugh.

> "Cells that were intoxicated by the toxin died, while those that survived had their DNA permanently mutated."

The unique bacterial ability to mutate DNA caught Dr. de Moraes' attention; he began to wonder if scientists could utilize bacterial toxins for biomedical purposes. While CRISPR-CAS9 and other genome editing technologies can correct mutations in human nuclear DNA, attempts to utilize them in other parts of the cell have proved challenging.[1]

This posed a problem for the potential editing of mitochondrial DNA (mtDNA). The mitochondria, most commonly known as the powerhouse of the cell, generates the chemical energy that drives all biochemical processes within the cell. Although most of the DNA in a cell is contained within the nucleus, the mitochondria also houses its own bit of DNA that it passes on to mitochondria within daughter cells.[2] Mutations in human mtDNA can lead to a variety of issues, including metabolic diseases and even cancer, but the lack of a functional gene editing tool for mtDNA has prevented scientists from addressing these problems.

This motivated the de Moraes lab to create their own novel mtDNA editing tool through the use of bacterial toxins, which have their own versatile properties. Through their research, the de Moraes lab discovered an interbacterial toxin called DddA that can change an RNA component called cytidine to aid in DNA modification.

However, previous attempts to edit mitochondrial DNA have been unsuccessful. The challenge was that guide RNAs that were in charge of directing editing tools like CRISPR-CAS9 to the right position were unable to permeate the mitochondria. It quickly became clear that DddA would require a non-RNA guide to reach the mitochondria and edit the mtDNA.

Collaboration with the David Liu Lab at Harvard University allowed the de Moraes lab to find a solution. They built an RNA-free delivery system by combining various components like lego pieces. This system used signal peptides and TALE proteins to guide DddA to the targeted mtDNA site for editing.

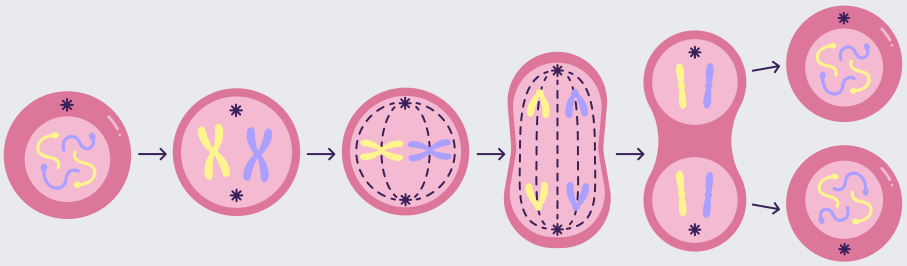


Figure 1. Antibacterial Toxin DddA.

Key

Grey: Immunity protein: blocks DddI

Purple: DddA

Red: Catalytic residue involved in the activity of DddA

Another problem loomed on the horizon: DddA in its complete, unbroken form proved too toxic for cells to handle, so introducing DddA into the mitochondria triggered cell death. A loophole method found that breaking DddA into smaller pieces would remove its toxicity and allow entry into the mitochondria, but would simultaneously render DddA unable to edit mtDNA. The solution? Split DddA into halves, allow for it to safely enter the mitochondria, and reassemble DddA at the editing site. Recombining the two non-toxic halves at the editing site allowed for DddA to become active and edit mtDNA[3]. This groundbreaking discovery opened up new possibilities for mitochondrial DNA editing.

But Dr. de Moraes has no intention of stopping here. When asked about his next projects, Dr. de Moraes said that he wanted to explore the functional diversity of deaminases, or enzymes that transform molecules through the removal of amino groups.

"[This research] was just the tip of the iceberg," Dr. de Moraes said. "There's so much more that we don't know [about deaminases]. It's interesting [as] a foundational biological aspect, and we can learn a lot about how enzymes work and evolve, [as well as] finding new resources for biotechnological applications." These new discoveries can lay the groundwork for improving gene therapy for genetic disorders, modifying agricultural crops, improving waste treatment and pollution, as well as many other fields.

" I'm also interested in [discovering] what happens to recipient cells that [become] intoxicated; not just how they are [affected], but how their intoxication [also] impacts the dynamics of the [bacterial] community," Dr. de Moraes said. Diving deeper into the mutualistic and antagonistic interactions within the bacterial community not only grants scientists insight into the unknown microorganisms around us, but also allows us to create more biotechnological applications. So the next time you wash your hands or eat a cup of yogurt, think about the several ways bacteria have helped us survive, as well as the possibilities they can create for us.

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EDITED BY Nikitha Kota

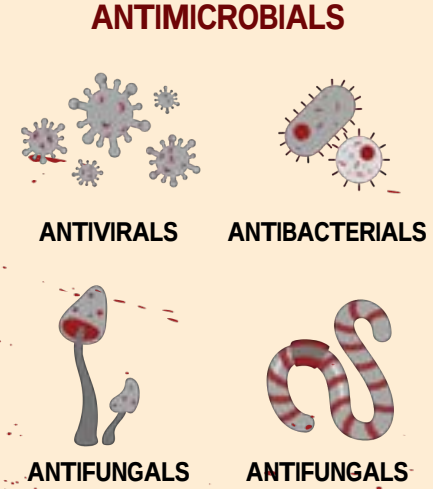
DESIGNED BY Abby McKellop

DISARMING MICROBES: A NOVEL APPROACH IN THE FIGHT AGAINST ANTIMICROBIAL RESISTANCE

BY ERICA LIN

I'm sure many of us, including myself, have heard the all too common recommendation from our doctors and pharmacists to "Finish the course of antibiotics!" As a kid, I may have rolled my eyes and allowed that piece of advice to go in one ear and out the other. Now, however, I have a greater understanding of the true ramifications of choosing to flush my antibiotics down the toilet instead of completing the course. Through this misuse, any surviving bacteria may develop properties allowing them to resist antibiotics that are meant to kill them. Although antibiotic resistance can be caused by several naturally occurring factors, antibiotic misuse and overuse is one significant factor that can contribute to this ever-growing public health problem.

While the term "antibiotics" only refers to products that specifically target bacterial infections, antimicrobials is a broader term that refers to products that treat infections caused by all microbes, including bacteria, fungi, protozoa, viruses, etc. According to the CDC, antimicrobial resistance occurs when "germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them" and is one of the most significant issues in the modern world. In 2019, it was reported that antimicrobial resistance killed "at least 1.27 million people worldwide and [is] associated with nearly 5 million deaths."1 Accordingly, the WHO has declared antimicrobial resistance as one of the top 10 global health threats facing humanity. Specifically, the spread of superbugs, which are strains of bacteria, viruses, parasites, and fungi that are resistant to most existing antimicrobial treatments, is particularly alarming.



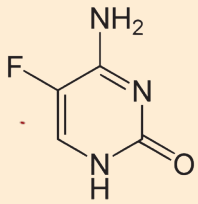
Dr. Natasha Kirienko, an Associate professor at Rice University in the Biosciences Department, has focused much of her research on identifying "novel treatments for bacterial infections that exhibit resistance to antimicrobials." Dr. Kirienko describes how, to date, any antimicrobial that has been introduced into market and clinical practice has resulted in the development of resistance against it. In addition, even combinations of antimicrobials are no longer effective against certain infections since microbes may develop resistance against multiple classes of antimicrobials. Therefore, it comes as no surprise that there is currently a lot of research devoted to developing solutions to this issue. Researchers have explored several avenues, including phage treatments and antimicrobial peptides. Specifically, Dr. Kirienko's lab focuses on understanding host-pathogen interactions and, instead of killing the pathogen, developing a way to block the pathogen's ability to harm the host.

The human body contains a multitude of bacteria that are adapted to being inside our bodies. We also have a commensal relationship with many bacteria, meaning that the bacteria are able to reside inside the human body while benefiting, or at least without harming, the human. Hence, as long as the bacteria are commensal rather than harmful and pathogenic, having bacteria inside the human body is acceptable. Therefore, Dr. Kirienko's research is based on the idea that bacteria do not need to leave the body or be killed and, instead, just need to be "disarmed," losing their ability to harm the host. A way of "disarming" a pathogen is to inhibit its virulence factors, which are "bacteria-associated molecules that are required for a bacterium to cause disease while infecting eukaryotic hosts such as humans."2 If the bacteria are simply disarmed rather than killed, the bacteria will not behave as if their survival is threatened. Therefore, the bacteria will experience less selective pressure to mutate and develop resistance against the antimicrobial. With this logic, drugs that inhibit the virulence factors of bacteria will be able to last longer on the pharmaceutical market without bacteria developing resistance against them.

DRUGS THAT INHIBIT THE VIRULENCE FACTORS OF BACTERIA WILL BE ABLE TO LAST LONGER ON THE PHARMACEUTICAL MARKET WITHOUT BACTERIA DEVELOPING RESISTANCE AGAINST THEM.

To achieve this, the lab focuses on gaining a deep understanding of host-pathogen interaction to determine which virulence factors need to be inhibited. Bacteria often have several virulence factors that play different roles in helping the bacteria infect and damage the host cells. Therefore, it is a challenge to determine whether there is any particularly important virulence factor whose inhibition would result in a significant decrease in the bacteria's ability to cause damage to the host. Dr. Kirienko's lab works with the Caenorhabditis elegans (C. elegans) roundworm and the pathogenic bacteria Pseudomonas aeruginosa. By mixing these two organisms together in 384-well plates and adding molecules to each well, the researchers observed which molecules allowed for the highest survival rate of the C.elegans worms.

Over 84,000 molecules were tested in these experiments. According to Dr. Kirienko, there are three main mechanisms of action



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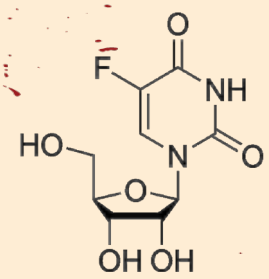

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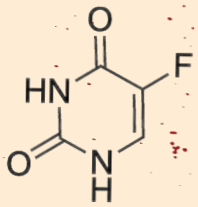

5-FLUOROURACIL

that are observable. First, the added molecule could act as an antimicrobial agent, which would kill the bacteria but not the worm. Second, the molecule could inhibit bacterial virulence. Third, the molecule could stimulate the worm's immune response, enabling it to fight the bacteria off on its own. According to Dr. Kirienko, their lab found representations of all three of these classes. Out of the molecules that inhibit bacterial virulence, Dr. Kirienko's lab is specifically interested in the class of drugs called fluoropyrimidines, which is typically used in cancer treatment. The lab is particularly interested in 5-fluorouracil, which is one type of fluoropyrimidine.
In their screens, Dr. Kirienko's lab discovered that 5-fluorouracil is a molecule that can cause significant inhibition of bacterial virulence. 5-fluorouracil is known to metabolize into 5-fluorodeoxyuridine, which inhibits enzymes involved in DNA replication. Therefore, the researchers initially thought that 5-fluorouracil killed bacteria by inhibiting their growth. However, they found that the bacteria's growth was unaffected. Instead, 5-fluorouracil was simply making the bacteria less dangerous and reducing its ability to harm the host through an alternate pathway. This pathway involves the conversion of 5-fluorouracil into 5-fluorouridine, which then inhibits the synthesis of one of the bacteria's virulence factors called pyoverdine.

THREE POSSIBLE ANTIBACTERIAL MECHANISMS OF ACTION

1. ADDED MOLECULE COULD ACT AS AN ANTIMICROBRIAL AGENT

2. MOLECULE COULD INHIBIT BACTERIAL VIRULENCE

3. MOLECULE COULD STIMULATE THE WORM'S IMMUNE RESPONSE

5-FLUOROCYTOSINE IS RELATIVELY HARMLESS TO HUMANS BUT QUITE DANGEROUS TO BACTERIA, MAKING IT POSSIBLE TO USE AS A MOLECULE THAT WOULD TARGET AND DISARM THE BACTERIA WITHOUT HARMING THE HUMAN.

Pyoverdine is a type of siderophore, also called an iron-chelating compound, meaning that it binds to iron and helps the organism accumulate iron.3 Bacteria need iron in order to grow and regulate their virulence factors and, specifically, bacteria need a pyoverdine-iron complex in order to produce virulence factors. Hence, inhibiting pyoverdine will have a "global disarming effect on bacteria," according to Dr. Kirinko, since the bacteria will no longer have the ability to produce the virulence factors they need to harm the host.

Another compound important to consider is 5-fluorocytosine. 5-fluorocytosine can also be converted into 5-fluorouridine - the compound that is dangerous to bacteria since it reduces the bacteria's ability to harm the host, as mentioned above. While bacteria can convert 5-fluorocytosine into the harmful 5-fluorouridine, humans cannot perform this conversion. Therefore, 5-fluorocytosine is relatively harmless to humans but quite dangerous to bacteria, making it possible to use as a molecule that would target and disarm the bacteria without harming the human.

Dr. Kirienko performed these experiments in-vitro, yet there was another lab that performed similar experiments on mice using a smaller library of drugs and came across the same molecules that have the same effects, which further confirms Dr. Kirienko's findings. In addition, outside of 5-fluorocytosine, Dr. Kirienko's lab also found other novel molecules that need to be tested in mice to hopefully produce the desired effect to act as treatments for antimicrobial-resistant bacteria. Currently, Dr. Kirenko has submitted grants to perform more pre-clinical and clinical experiments. The applications of her work have the potential to really make a difference in this fight against antimicrobial resistance, and there is certainly a promising future ahead for the Kirieinko Lab.

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TOWARDS AN IMPLANTABLE IPSC DERIVED TREATMENT FOR TYPE 1 DIABETES

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Dr. Aryeh Warmflash December 15, 2022

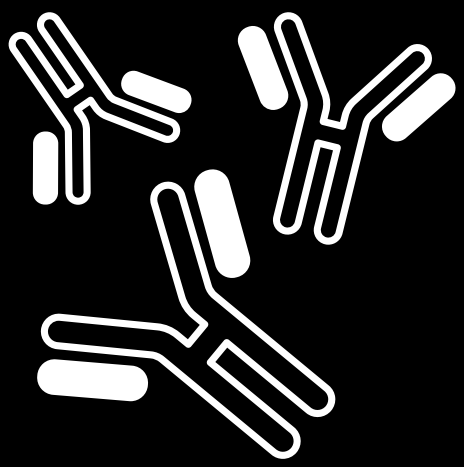
ABSTRACT

Type 1 diabetes (T1D) is a common autoimmune disorder that progressively destroys pancreatic β-cells. Left untreated, T1D leads to progressive pancreatic destruction, hyperglycemia, vascular disease, and eventual death. Current treatments of insulin and lifestyle modification have evolved but have remained relatively unchanged for decades, requiring complex healthcare management and frequent insulin doses. While various novel treatments have been recently proposed, induced pluripotent stem cells (iPSCs) may provide a very promising, future treatment for T1D. Still under research, transplanted iPSC - derived β-cells may provide glycemic control without the immune rejection risks associated with cadaveric or ESC - derived β-cells. This review explores the current status of iPSC - derived β-cells research, the strengths and weakness of iPSC - derived β-cells, and future research directions necessary for clinical application.

TOWARDS AN IMPLANTABLE IPSC DERIVED TREATMENT FOR TYPE 1 DIABETES: AN INTRODUCTION TO TYPE 1 DIABETES

Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease that progressively destroys pancreatic β-cells, reducing insulin production and inducing life-threatening hyperglycemia (van Belle et al., 2011; Pugliese, 2013; DiMeglio, 2018). The Eisenberg Model of T1D plots decreasing β-cell mass against age, portraying a sequence of events that begins with genetic predisposition, followed by T-cell activation from a triggering environmental event, leading to progressive β-cell destruction and causing eventual T1D symptoms and death (DiMeglio, 2018). While increasing research has revealed that T1D pathogenesis is far more complicated than the Eisenberg Model suggests, the model remains relevant due to its utility in explaining the loss of β-cell mass over time.

The existence of polygenic risk factors and environmental risk factors to T1D are readily apparent. Regarding genetic risk factors, T1D has an identical twin risk of 30-70%, non-twin sibling risk of 6-7%, and risk of 1-9% for children who have one parent with T1D (Redondo, 2008; Pociot, 2016). Furthermore, two HLA class 2 haplotypes, HLA DRB1*0301-DQA1*0501-DQ*B10201 (DR3) and HLA DRB1*0401-DQA1*0301-DQB1*0301 (DR4-DQ8), are collectively
linked to approximately 50% of disease heritability (Noble, 2015). However, evidence of environmental factors is also readily apparent. Although T1D is traditionally considered to emerge during childhood, up to 50% of T1D cases emerge during adulthood (Thomas et al., 2018). In fact, up to 50% of adults diagnosed with type 2 diabetes (T2D) may have misdiagnosed T1D (Hope et al., 2016). Furthermore, the rates of T1D appears to be increasing across children, adolescents, and adults, which imply unknown environmental factors play a role in disease onset (DiMeglio, 2018). T1D is now understood as complex interactions between genetic, microbiome, immune, and environmental factors (DiMeglio, 2018).



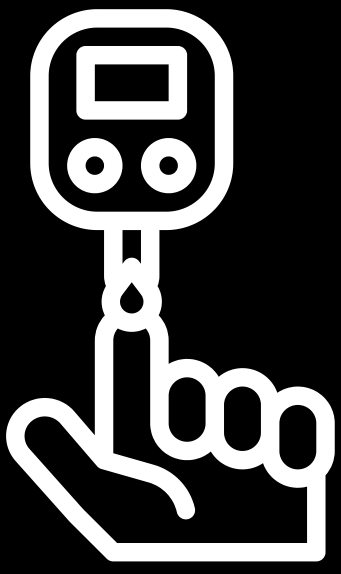
Despite various possible mechanisms of pathogenesis, the final T1D disease process is largely driven by T-cells. While the exact signaling mechanism is not known, autoreactive T-cells target β-cells following periods of β-cell ER stress (Engin, 2016). Since β-cell ER-stress is associated with alterations in mRNA splicing and overexpression of class 1 HLA, it is possible that the stressed β-cells are displaying novel surface antigens that upregulate the immune system (Ezerik et al., 2012). In addition, over 90% of T1D patients have serum detectable antibodies against β-cell related antigens, such as insulin, islet antigen 2, glutamate decarboxylase, zinc transporter 8, and tetraspanin-7 (McLaughlin et al., 2016). In fact, the presence of merely two serum detectable antibodies is associated with 84% risk of T1D symptoms by 18 years of age (Ziegler et al, 2013). Over time, the T-cell mediated attack leads to the progressive destruction of pancreatic β-islets, lowering insulin levels and causing the onset of T1D symptoms.

In addition to chronic hyperglycemia, T1D causes various symptoms that significantly increase morbidity and mortality. As the disease progresses, the pancreas reduces in size and β- cell mass continues to decrease (Virostko et al, 2016). Left untreated, the chronic hyperglycemia leads to various microvascular-related pathologies, such as alterations in attention, visual attention, memory, retinopathy, neuropathy, nephropathy, and cardiovascular disease (Caroline, 2013). Thus, T1D can lead to significant deterioration in quality of life. Even with modern medical treatments, T1D patients can still die 8-13 years younger than people without T1D (Huo et al, 2016; Livingstone et al., 2015).

CURRENT TREATMENTS FOR TYPE 1 DIABETES:

INSULIN THERAPY

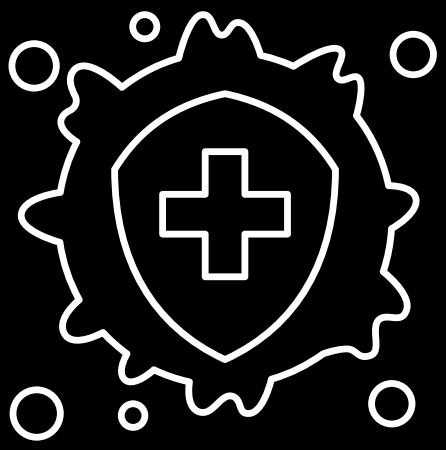
Since glucose is necessary cellular metabolism, type 1 diabetes was historically fatal until the discovery of insulin (Banting et al., 1922). Even in the 21st century, insulin remains the primary treatment for T1D (Caroline, 2013). However, dosing insulin properly remains difficult for many T1D patients. Optimal blood glucose control requires mimicking the timing of body's natural release patterns, such as basal release during the night, slow release between meals, and larger releases to control complex carbohydrates during mealtimes. Furthermore, the dosing must be altered for levels of physical activity, stress or other preexisting healthcare conditions (DiMeglio, 2018). Although various mechanisms of insulin delivery have been developed, such as intramuscular injections, aerosol insulin, and implanted insulin pumps, all of the methods have weaknesses (DiMeglio, 2018). Currently, the development of an artificial pancreas (combination of continuous glucose monitoring (GCM) device and implanted insulin pump) would provide the most promising method to control blood glucose levels (Kovatchev, 2017).



IMMUNE THERAPY

Since T1D is an autoimmune disorder, hope exists for an immune therapy that would prevent T-cell mediated attacks on β-cells. For example, the immunosuppressant ciclosporin temporarily inhibited T-cell activation, but it was unable to induce permanent disease remission (Feutren et al., 1986). Newer approaches have examined the efficacy of monoclonal antibodies that target either B-cell receptors (rituximab) or T-Cell receptors (teplizumab and otelixizumab), however these treatments failed to progress to stage three trials (DiMeglio, 2018). Future

immunotherapies could be implemented during the early part of the disease process to slow the rate of β-cell mass (DiMeglio, 2018).



CADAVERIC β-ISLETS TRANSPLANT + IMMUNE THERAPY

Cadaveric β-islet implantation is an emerging treatment for advanced T1D, which must be coupled with immunosuppressants to prevent allogeneic immune responses (Shapiro et al., 2000; Sibley et al., 1985). The β-islets are surgically placed into the hepatic portal vein of the recipient. Strikingly, this procedure is low risk and can lead to high rates of insulin independence. However, due to the low availability of donors, and the complications from necessary immunosuppressants, the majority of T1D patients are not eligible for this islet transplantation despite the efficacy of the procedure (Shaprio et al., 2000, DiMeglio, 2018). However, cadaveric β-islet implantation set the stage for stem cell-based therapies, and the theoretical use of induced pluripotent stem cell (iPSC) derived β-cells may provide solutions to such limitations.

CREATING IPSC β-CELLS FOR TREATING TYPE 1 DIABETES

Over the last decade, various stem cell therapies have gained attention as possible treatment for T1D. While there are many candidate types of stem cells, iPSCs have the capacity for self-renewal, differentiation, and will not trigger an allogenic response (Leite et al., 2020). These factors make iPSCs an ideal candidate for creating β-cells for implantation. If successful, the implantation of iPSC derived β-cells would simultaneously lower blood glucose levels and avoid complications from allogeneic immune responses associated with cadaveric β-islets or human embryonic stem cells (ESCs). Of course, the new β-cells would be vulnerable to attack from the T1D patient's immune system. However, since T1D is largely mediated by T-cells, the implanted β-cells could be protected in two different ways. First, the new β-cells could be genetically modified to reduce the expression of class 1 HLA, which has been correlated with T- cell activation (Leite et al., 2020). Alternatively, the implanted iPSC derived β-cells could be placed in a semipermeable device that permits the release of insulin, but prevents T-cells from contacting the β-cells, eliminating the need for immunosuppression (Leite et al., 2020; Ellis et al., 2017). The following sections outline the progress made towards an implantable, iPSC derived treatment for T1D.

DIFFERENTIATION AND CHARACTERISTICS OF IPSC DERIVED β-CELLS

The mere transplantation of pancreatic progenitor cells into mice can cause minor spontaneous differentiation into β-cells; however, specifically creating in vitro β-cells for transplantation is a far more efficient strategy to treat T1D. As reviewed by Millman & Pagliuca (2017) several successful protocols create functional β-cells from iPSCs (see Fig. 1).

Report	SC Type Used	Culture Format	Percent of β-cells	In-Vivo reversal of diabetes?
Rezania et al., 2014	iPSC	Air-liquid interface	~40% (NS+/NKX6-1+)	Yes (40 days)
Pagliuca et al., 2014	iPSC	Spinner flask	33 +/- 3% (CP+/NKX6-1+)	Yes (18 days)
Millman et al., 2016	iPSC (T1D+)	Spinner flask	24 +/- 2% (CP+/NKX6-1+)	Yes
Millman et al., 2016	iPSC (ND)	Spinner flask	27 +/- 2% (CP+ /NKX6-1+)	Yes

Fig 1. Adapted from Millman & Pagliuca (2017)

The various protocols involve adding or restricting various signaling molecules to induce differentiation, causing undifferentiated iPSCs into mature iPSC derived β-cells. For example, Pagliuca et al., (2014) demonstrated that exposing the iPSCs (OCT4+ and NANOG+) to Nodal and Wnt caused differentiation into definitive endoderm (SOX17+ and FOXA2+). Then, exposing definitive endoderm cells to RA, FGF, and PKC, while removing BMP and SHH, caused differentiation into pancreatic progenitors (PDX1+ and NKX6-1+). Then, exposing pancreatic progenitors to EGF, triiodothyronine, RA, while removing Alk5, SHH, y-secretase, and BMP, caused differentiation into endocrine progenitors (CHGA+ and NKX6-1+). Finally, exposing endocrine progenitors to further triiodothyronine and vitamin E, while removing ALK5 and AXL, caused differentiation into iPSC derived β-cells (Pagliuca et al., 2014; Millman & Pagliuca, 2017).

Matured iPSC derived β-cells are often characterized by expression of NKX6-1, PDX1, MAFA, GLIS3, and MNX1. However, most iPSC derived β-cells do not express MAFA and GLIS3, which is expressed by normal adult β-cells. Thus, it is important to remember that stem cell derived β- cells, from both iPSC and ESC sources, are not actually normal adult β-cells (Millman & Paglucia, 2017; Sipioine et al., 2004).

Despite the differences, iPSC derived β-cells mimic normal adult β-cells, in both in-vitro and in-vivo settings. iPSC derived β-cells respond to elevated glucose levels with (1) increased intracellular Ca2+ concentrations, and (2) release of insulin and c-peptide. The insulin is even secreted in secretory granules, however the concentrations of glucose contained within are lower than their normal adult β-cells counterparts (Pagliuca et al, 2014; Rezania et al., 2014; Millman et al, 2016).

Despite the lower insulin concentration, transplanted iPSC derived β-cells demonstrate reliable insulin secretion in response to sequentially induced hyperglycemia in immunosuppressed mice models. Furthermore, both iPSC derived β-cells from T1D patients and non-diabetics responded equally well to glucose challenges in vitro and in mice models. This provides evidence that iPSCs from T1D patients would be viable sources of β-cells for autologous transplant, meaning that future T1D β-islet transplant candidates would not need to receive β- cells from non-diabetics, avoiding the risk of allogenic rejection (Millman et al, 2016).

Furthermore, it provides evidence that T1D patients' β-cells would be functional in the absence of an overactive immune system.

Due to these results, Bar Nur et al., (2011) even suggest specifically sourcing β-cells for creating iPSCs, rather than using cells from elsewhere in the body. While this review focuses on iPSCs derived β-cells, many researchers focus on ESC derived β-cells, which would potentially offer many of the same benefits. However, differentiating ESCs into β-cells is often difficult, creating cell mixtures that contain both endocrine progenitors and relatively few β-cells. Furthermore, many of the supposed ESC derived β-cells are mis-identified glucose-sensitive neural progenitors that produce insulin (Sipioine et al., 2004). However, iPSCs sourced from β- cells maintain their epigenetic memory, which predisposes the iPSCs to differentiate back into β-cells. Theoretically, this skewed differentiation potential would increase the yield of derived β-cells and minimize the yield of unwanted progenitors (Bar Nur et al., 2011).

CURRENT LIMITATIONS OF IPSC DERIVED β-CELL RESEARCH

The future promise of iPSC derived β-cells is kept in check by several limitations. First, immunocompromised mouse models do not have a long lifespan, which makes evaluating the long-term efficacy of the treatment difficult. Second, the impurity of the implanted iPSC derived β-islets can create life threatening teratomas, indicating the need for improved protocols and bioengineered safeguards.

IMMUNOCOMPROMISED MICE MODELS

Thus far, all studies examining efficacy of iPSC derived β-cells have been tested in immunocompromised mouse models with induced hyperglycemia. Since the transplanted iPSC derived β-cells are of human origin, experimental mice must be an immune compromised strain or given immunosuppressants (Millman & Paglucia, 2017). Although Pagliuca et al (2014) were able to demonstrate iPSC derived β-cell glucose control for 40 days, no published study has successfully lasted longer. Since the mice are immunocompromised, their lifespans are shortened, which makes testing the longitudinal efficacy of the iPSC derived β-cell therapy difficult. However, in unpublished work, Millman & Paglucia (2017) allege that an exceedingly small portion of iPSC derived β-cells survived over a year in their immunocompromised mice, demonstrating the possibility of long-term efficacy.

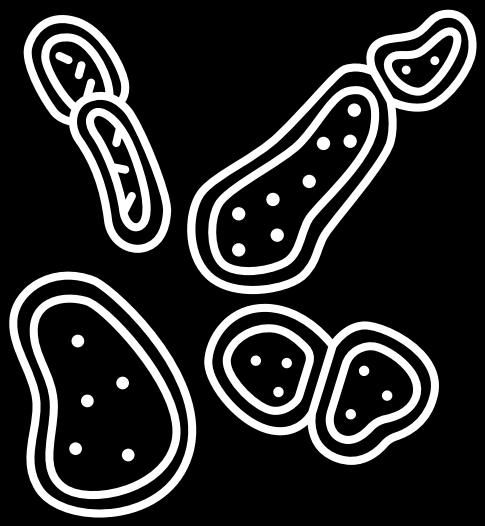
TERATOMA FORMATION

When iPSC derived β-cells are transplanted into the host, the transplanted β-islet is not purely composed of β-cells. Rather, the iPSC derived β-islet contains a variety of endocrine cells, partially differentiated endocrine progenitors, undifferentiated endocrine progenitors, and even the possibility of undifferentiated iPSCs. In contrast, transplanted cadaveric β-islets do not contain undifferentiated cells, and their proportion of non β-cells is much smaller than the iPSC derived β-islets. Thus, transplanted iPSC derived β-islets carry risk of teratoma formation in mice (Halme, 2016). This problem is not unique to the iPSC approach. In mice transplanted with ESC derived β-islets, the mice experienced remission from T1D for three weeks, until teratomas developed and killed the mice (Fujikawa et al., 2005).

Fortunately, there are several methods to lower the risk of accidental teratoma formation. First, differentiation protocols can be improved to increase the ratio of iPSC derived β-cells to non-β-cells. This could be accomplished by (1) developing a medium that will selectively kills the undifferentiated progenitor cells, or (2) selectively removing cells that display progenitor surface markers (Kelly et al, 2011; Tang et al., 2011). Second, a kill-switch gene could be incorporated into the iPSCs, which would trigger self-destruction of the entire β-islet, if mutations are detected (Li et al., 2013). Finally, the β-islet could be placed inside of a bioengineered capsule prior to transplantation (Millman & Paglucia, 2017).

FUTURE DIRECTIONS FOR IPSC DERIVED β-CELL RESEARCH

As mentioned previously in the review, iPSC derived β-cells provide immunological advantages over cadaveric β-islets and ESC derived β-islets. Since the iPSCs are sourced from the T1D patient's own body, there is theoretically no concern for allogenic rejection (Rezania et al., 2014). However, since the patient has T1D, the transplanted iPSC derived β-cells would also experience T-cell mediated autoimmune attacks, just like the β-cells they were intended to replace. While immunosuppressants could theoretically solve this issue, there would major drawbacks for the patient's quality of life, as seen in cadaveric β-islet transplants (DiMeglio, 2018). In order to address this predicament in the iPSC approach, researchers must focus on mechanisms to hide the iPSC derived β-cells from the immune system.



IPSC DERIVED β-CELL IMMUNE INVISIBILITY

Since T1D is largely mediated by T-cell interaction with β-cells' class 1 HLA complexes, genetic modification of the iPSC derived β-cells could render them invisible to the adaptive immune system. In an in-vitro model of T1D, Leite et al., (2020) transduced a B2M guide RNA and Cas9 into differentiated iPSC-derived β-cells. The cells subsequently decreased expression of class 1 HLA, and T-cell activation decreased compared to controls. Although this experiment was performed to demonstrate the necessity of T-cell interaction with class 1 HLA to start an autoimmune attack, the results show the potential promise of class 1 HLA modification on iPSC derived β-cells. However, since this experiment was done in vitro, the iPSC-derived β-cells were not exposed to the entire adaptive immune system, so in-vivo efficacy of this approach remains untested.

Although the immune invisibility approach holds potential, this approach also creates greater risk of oncogenesis. Should the transplanted iPSC-derived β-cells become cancerous or infected with a virus, the immune system would have difficulty recognizing any abnormality in the transplanted cells (Millman & Paglucia, 2017). If researchers pursue this approach, the iPSC-derived β-islet must be able to regulate itself, possibly through previously proposed incorporation of kill-switch genes (Li et al., 2013).

IPSC DERIVED β-CELL ENCAPSULATION

Since T1D is largely mediated by T-cell interaction with β-cells' class 1 HLA complexes, physically preventing the cells from touching would significantly decrease autoimmune response. Bioengineered microcapsules aim to protect iPSC-derived β-cells from T-cell attack, while simultaneously allowing for the secretion of insulin (Scharp, 2014). While such encapsulation techniques have been attempted in various human clinical trials, the capsules usually fail due to poor vascularization and pericapsular fibrosis (Millman & Paglucia, 2017). However, newer approaches using have succeeded in mouse models. Capsules made of alginate derivatives have successfully protected human ESC derived β-cells for 174 days inside immune competent mice (Vegas et al, 2016). In addition, capsules made of alginate with CXCL12 polymers have successfully protected human ESC derived β-cells for 150 days inside immune competent mice (Alagpulinsa et al, 2016). In both experiments, the capsules evaded the expected pericapsular fibrosis, and the internalized ESC derived β-cells provided glycemic control successfully. These techniques may be adapted in the future for iPSC-derived β-cell transplants in humans.

CONCLUSIONS

T1D is a common autoimmune disorder that progressively destroys pancreatic β-cells, causing increased morbidity and mortality when left untreated. Currently, insulin remains the standard treatment for T1D, but iPSC-based therapies pose promise for future T1D treatment. iPSCs are ideal candidates for pancreatic therapy over other types of stem cells, because iPSCs have the capacity for self-renewal, differentiation, and will not trigger an allogenic response. Although current iPSC derived β-cells are different than normal adult β-cells, iPSC derived β-cells still demonstrate clinical effectiveness in lowering blood glucose in hyperglycemic mice models. However, before human trials can reasonably progress, further research should determine the longitudinal efficacy of transplanted iPSC derived β-cells, and how to prevent autoimmune activation after transplantation, without increasing risk of further pathogenesis.

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Comparing Electrode and Optogenetic Solutions for Artificial Sight

KATHERINE WU

ABSTRACT

Electrode-based systems and optogenetics are two distinct methods that address blindness by developing artificial vision. Optogenetics is a rapidly evolving field that involves the use of light to control the activity of cells in living tissue, typically neurons. Some current areas of research include designing smaller and more efficient optogenetic devices, developing new ways to target specific cell types, and using optogenetics to study and treat a wider range of brain disorders. By comparison, electrode-based solutions for restoring vision involve implanting an array of electrodes to bypass a damaged retina and stimulate remaining cells in the visual pathway, allowing people with certain retinal diseases to regain some level of visual perception. This review discusses the effectiveness of both electrodes and optogenetics in producing artificial vision.

INTRODUCTION

Vision loss can have a profound impact on an individual's quality of life, affecting their ability to engage in daily activities, maintain independence, and participate in society. Aging populations around the world are beginning to experience greater rates of age-related vision reduction, presenting as macular degeneration, glaucoma, and cataracts. Although an important public health issue, the issue at hand necessitates costly assistive devices and long-term care. Therefore, continued research and development in technologies to restore and improve vision is an important area for future investment.

OPTOGENETIC SOLUTIONS

Optogenetics is a technique that uses light to control and study the activity of cells in the brain and other tissues, allowing researchers to better understand and potentially treat neurological disorders. Optogenetics uses a genetic tool called the LoxP system to selectively control the expression of specific genes in cells. LoxP is a specific DNA sequence recognized by a protein called Cre recombinase, which is commonly used to manipulate the expression of genes in cells. Cell genomes can be edited by light-activated enzymes called Cre recombinases that localize to flanking LoxP sites at specific locations to either activate or silence gene expression in a precise and reversible manner. By selectively activating or silencing gene expression optogenetics can achieve gain-of-function or loss-of-function of specific cells of living tissue [1].

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Optogenetics uses light to control specific neurons by introducing a protein into the neurons that responds to light; when light is shone on these neurons, the protein activates or deactivates the neuron, allowing researchers to study the effects of specific neural activity on behavior or physiology. In optogenetics, two proteins are commonly introduced into neurons. The first are opsins. Opsins are light-sensitive proteins that respond to different wavelengths of light, and they can be used to activate or inhibit neural activity depending on the type of opsin used. The second protein is enzymes. Enzymes are proteins that can be activated by light to perform specific chemical reactions, and they can be used to manipulate various signaling pathways or biochemical processes within neurons. By introducing these proteins into neurons, researchers can control the activity of the neurons with light, allowing them to better understand how neural activity contributes to behavior and disease [Figure 1].

Manipulating gene expression of specific cells using proteins and light allows individuals to experience a phosphene, which is a sensation of light without any light actually entering their eyes. These

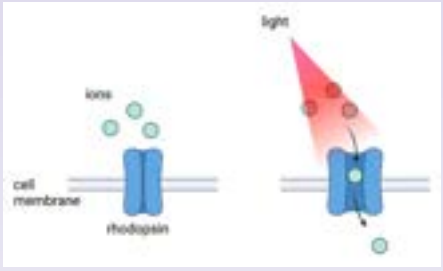

FIGURE 1: **Shining intense red (635nm) light opens the rhodopsin, allowing ions to flow into the neuron. The result is neuronal activation and specific behavior. In optogenetics, rhodopsins are used to activate neurons by allowing the influx of positively charged ions into the cell in response to light. This causes the neuron to depolarize and fire an action potential, allowing researchers to control the activity of the neuron with light.**

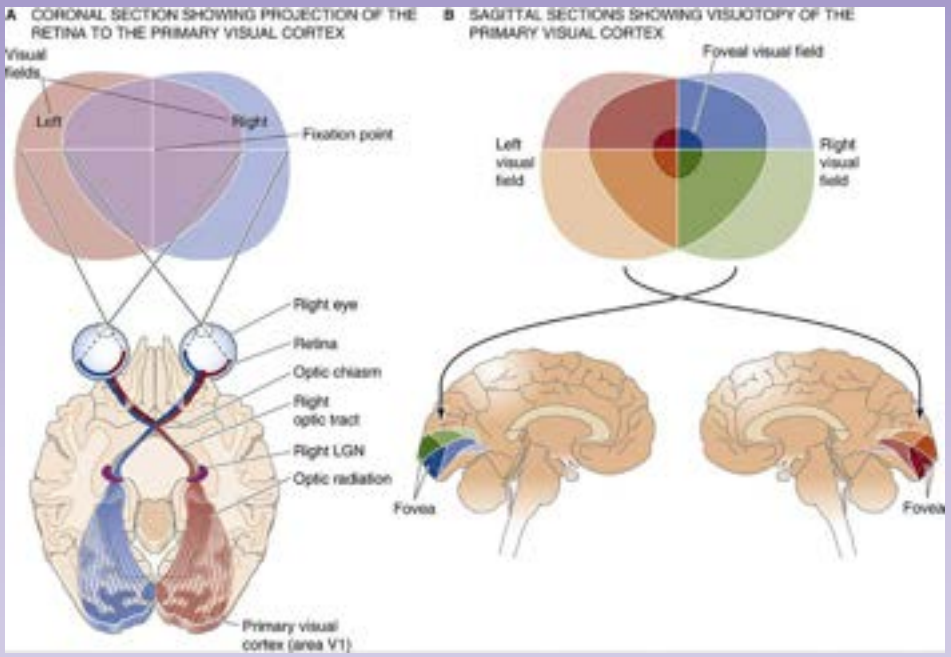

FIGURE 2: **Visual fields and visual maps. A, The right sides of both retinas project to the left lateral geniculate nucleus (LGN), which in turn projects to the left primary visual cortex (area V1). B, The upper parts of the visual fields project to lower parts of the contralateral visual cortex [3].**

phosphenes, or sensations, can be caused by physical pressure on the eye, electrical stimulation of the visual system, or other types of sensory stimulation [2]. In the case of electrical stimulation, optogenetics uses light to stimulate relevant neurons and create the sensation of light, even in the absence of true visual stimulation. Specifically, this sensation is created by taking advantage of the retinotopic organization of the primary visual area V1 of the cerebral cortex, where the first stage of cortical information processing occurs and a complete map of the visual field is generated [Figure 2]. Precise perturbations in the targeted neurons of this area lead to the creation of these light percepts.

One of the main advantages of optogenetics is that it allows for high-resolution and selective stimulation of neurons due to the ability to target specific genetic sites with the LoxP system. Because optogenetics has a high specificity, it activates only the targeted neurons, resulting in a high degree of specificity and spatial control, and a more accurate and detailed visual perception. Research has shown that optogenetics can provide reliable and targetable light sensation in the absence of visual stimulation [4]. While optogenetics is still in the early stages of development and testing, early clinical trials have shown effective restoration of vision in retinal degeneration [5].

However, there are some challenges with optogenetics, specifically the limitations of current light delivery methods and neuron structure itself. In order to activate photoreceptors, a substantial amount of energy must be conferred to surpass the action potential threshold [6]. Furthermore, the limitation of current light delivery methods prevent stimulation of deeper nervous tissue. Activation of underlying brain tissue requires higher energy pulses, which can lead to thermal injury, causing damage or destruction of cells and tissues [7].

ELECTRODE-BASED SOLUTIONS

Electrodes can be inserted through shuttle microwires [Figure 3], which then form a bidirectional connection between neural implants and the controlling computer. This connection allows control of the neural implant through both receiving and transmitting information by the electrode. Electrode solutions involve five components: an amplifier, filter, analog-to-digital converter (ADC), stimulator, and communication interface [8]. One strategy to induce sight using electrodes is called visual cortical prosthesis (VCP), which uses electrical current to stimulate the visual cortex [9]. Previous studies have taken advantage of the retinotopic organization of the visual cortex to produce form vision in both sighted and blind humans. In these studies, an array of multiple electrodes are implanted in different locations to create multiple phosphenes. In an electrode array, each stimulated electrode contributed to a phosphene at one predictable visual field location, resulting in a pattern of artificial vision that could be used to help blind individuals navigate their environment or perform other visual tasks [10].

In contrast to optogenetic-based solutions, solutions involving electrodes have a minimal energy loss. Llectrode-based solutions directly stimulate the retina or the visual cortex using electrical signals. One of the main advantages of electrode-based solutions is that they can be highly efficient, as electrical signals can be delivered directly to cells. However, one of the main challenges associated with electrode-based solutions is that it can be less selective than optogenetics due to the size limit of individual electrodes. This size limit results in less accurate and detailed visual perception. Another challenge is that electrode-based solutions can trigger the body's immune response. When a foreign object, such as an electrode, is implanted in the brain, the brain's immune system may recognize it as a potential threat and attempt to remove it. When it occurs in response to an implanted electrode, it can lead to a process known as the foreign body reaction, during which the immune system releases a variety of inflammatory molecules that can cause damage to the tissues surrounding the electrode. This can lead to the formation of scar tissue and the buildup of immune cells, which can interfere with the function of the electrode and reduce its effectiveness over time.

Current research has been conducted to evaluate the safety of electrodes in creating artificial vision. One study used a direct optic nerve electrode (AV-DONE) in a blind patient with retinitis pigmentosa (RP) [13], which is a genetic eye disease that affects the retina and results in the inability to perceive light [14]. The AV-DONE consists of three wire electrodes, which were implanted into the optic disc of a patient. The researchers then induced visual sensations by electrical stimulation through each elec-

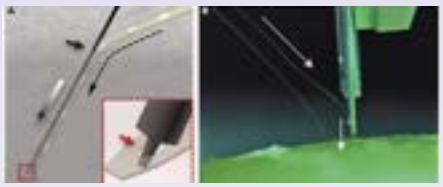

FIGURE 3: **A, Schematic showing the needle-thread temporary engaging mechanism [11]. B, Photograph of the insertion process. Arrows indicate a needle penetrating tissue proxy, advancing the thread to the desired depth [12].**

Pros and Cons of Existing Solutions

OPTOGENICS	ELECTRODE-BASED
• Non-invasive • High-resolution • Selective stimulation • Less well-established	• Highly efficient • More advanced • Numerous trials • Used for decades to treat variety of conditions

trode. The phosphenes, which ranged in size from that of a match head to an apple, were round, oval, or linear, primarily yellow, and focally distributed [15]. The area of the phosphenes changed when the electrical stimulation was supplied from different electrodes. It is worth comparing the surgical accessibility of visual prosthesis in brain structures that are deep in the brain. One example of such a structure is the lateral geniculate nucleus of the thalamus (LGN), which links the retina to the primary visual cortex. A current area of desearch for electrode-based solutions is the mobility of the patient. Because of the invasive nature of electrodes, this method was originally performed while attaching electrodes to the computer through wires. However, recent advances have shown that electrodes can communicate with the computer wirelessly. The lack of a wire gives the patient greater mobility and allows electrodes to be a more attractive treatment option in a clinical setting.

CONCLUSION AND FUTURE WORK

Both optogenetics and electrode-based solutions have potential for creating artificial vision, but each has their own advantages and challenges. Optogenetics is relatively non-invasive and allows for high-resolution and selective stimulation, while electrode-based solutions can be highly efficient but may be more invasive. In addition, it is worth comparing the stages of clinical trials that these solutions are in. Both electrode and optogenetic solutions have been tested in human trials, but electrode-based approaches are more advanced and have been used in a greater number of patients than optogenetics. Electrode-based approaches, such as deep brain stimulation (DBS), have been used for several decades to treat a variety of neurological and psychiatric conditions, including Parkinson's disease, essential tremor, and depression. There have been numerous clinical trials of DBS, and it is now an FDA-approved therapy for several indications. Optogenetic approaches are newer and less well-established in humans. While there have been a number of promising preclinical studies in animal models, there have only been a few small clinical trials of optogenetics in humans to date. These trials have typically focused on safety and feasibility, and more research will be needed to determine whether optogenetics can be used effectively to treat neurological and psychiatric conditions in humans. Another factor to consider is the ethics of optogenetics. If we are able to overcome the implantation risks, there could come a day where optogenetics is implanted in nearly everyone. If someone were to hack the control system, they would be able to precisely control the mental processes and behaviors of thousands of people.

Future work for optogenetics is focused on improving the precision and specificity of optogenetic tools and methods, as well as developing new ways to deliver light to targeted cells. Other areas that need to be addressed are implantation issues and immune response issues, which are major barriers to the implantation of optogenetics. Future goals for electrode-based solutions include developing more advanced electrode arrays that can provide higher-resolution vision and improved reliability. Another area of focus for electrode-based solutions is to develop ways of stimulating the eye wirelessly and in a miniaturized form. Since these electrodes reside in the body for long periods of time, an area of development is to achieve long-term stability and safety. To minimize the risk of a foreign body reaction, researchers and clinicians may use a variety of techniques to reduce the electrode's foreignness and promote tissue integration. These techniques may include using materials that are less likely to be recognized as foreign by the immune system, coating the electrode with molecules that promote tissue integration, or stimulating the brain in a way that reduces the immune response.

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UNDERSTANDING UNCONVENTIONAL CAUSES OF CHALAZIA

BY BRIAN LEE

ABSTRACT

A chalazion is an inflammation of the meibomian glands, which lubricate the eyes. This inflammation is generally painless and usually results from poor eye hygiene, but certain variations can develop complications if left untreated. Chalazia have a well-researched treatment protocol of warm compresses, antibiotics, and surgical removal. However, there is significantly less research on the causes of chalazion formation, leading to issues with diagnosis and detection. Most ophthalmologists do not usually perform biopsies on chalazia, and this lack of chalazia specimens limits further research into the area. This oversight has negative repercussions on patient health outcomes, as chalazia can arise from diseases such as tuberculosis or respiratory viruses. Even relatively harmless chalazia can eventually endanger the entire eye ecosystem if left undetected. In an effort to better understand and distinguish the causes of chalazia, this review examines unconventional biotic and abiotic drivers of chalazia. This review relies on case studies, pulling observations from non-traditional chalazia patients around the world, as well as extensive hospital patient datasets. Specific biological data was derived through biopsies and biochemical assays. This review reveals biological, viral, oral-pathogenic, and chemotherapeutic methods of chalazia formation, each utilizing unique pathways to inflame the meibomian glands. Every pathway is difficult to detect and research without invasive biopsies and as such, little is known about the molecular mechanisms that cause the disease. Future research should focus on developing a comprehensive understanding of chalazia etiology and finding less invasive methods to diagnose different types of chalazia.

INTRODUCTION

The eye is a sensitive organ that is very prone to infection and inflammation. Common problem areas include the oil glands, the eyelids, and the conjunctiva, which is the thin membrane coating the eye. A chalazion forms when the meibomian glands become inflamed [1]. These glands run down each eyelid and produce meibum, the lubricating lipid portion of tears. The inflamed meibomian gland leads to swelling in either the upper or lower eyelid, which can cause discomfort and pressure against the eyeball. The swelling tends to grow over a week, and generally heals on its own within a few weeks [1]. Treatment typically consists of warm compresses, eyedrops, and antibiotics; however, more persistent chalazia require surgical intervention through curettage, where the chalazion is sliced open and drained [2].

> However, there is significantly less research on the causes of chalazion formation, leading to issues with diagnosis and detection.

While the treatments of chalazia are well known, less is known about their etiology. The meibomian glands are complex tissues that interface with a variety of ducts and muscles, presenting several potential sources of inflammation. As a result, chalazia lack a single established cause. Prior research has demonstrated that viruses [2], mask-wearing [3], or poor hygiene [1] can all lead to chalazion formation. Since relatively few cases of chalazia are physically examined by ophthalmologists, the primary origin is difficult to deduce. Additionally, chalazia are similar in appearance to a variety of other eye conditions, complicating diagnosis and treatment. For example, chalazia can closely resemble harmless styes, which are bacterial infections of the eyelash or eyelid. Yet, they can also mimic sebaceous carcinomas, a rare but aggressive form of skin cancer [4]. Chalazia can sometimes be linked to more malignant conditions, such as tuberculosis [5]. All of these studies demonstrate the diverse etiology and appearances of chalazia. The lack of research comparing different causes of chalazia makes it difficult to accurately discriminate between benign and malignant causes.

Effective treatment of chalazia depends on accurate diagnosis, which in turn depends on strong distinguishability. For example, if a patient's cancerous chalazion is misdiagnosed as a normal, poor-hygiene chalazion, the patient will not be provided with valuable cancer therapies. These diagnoses depend on a comprehensive and comparative understanding of chalazion etiology, as different pathogens that cause chalazia can be detected with their own distinct methods. For example, viruses can be detected through immunoassays [2]. This literature review organizes the many origins of chalazia and examines them to better understand how this meibomian inflammation begins. The analyzed papers draw from large hospital patient datasets and smaller case studies conducted by private clinics, both international and domestic. Specific pathogens were obtained through biopsies and analyzed through biochemical methods, including meibographies [4] and electron micrography [2].

The literature review will first discuss unconventional biotic causes of chalazia, including *Mycobacterium tuberculosis* (TB), oral pathogens, and viruses and parasites. The review will then discuss chemotherapy, which is a notable abiotic cause of chalazia. The conclusion will tie together all discussed causes and examine future areas of study.

> The meibomian glands are complex tissues that interface with a variety of ducts and muscles, presenting several potential sources of inflammation.

BIOTIC ORIGINS OF CHALAZIA

Tuberculosis is primarily a respiratory disease caused by *Mycobacterium tuberculosis* (both the pathogen and disease will hereafter be referred to as TB). TB aggressively attacks the lungs and other organs, but it can also lay relatively dormant and slowly grow over the course of months [6]. While most TB research focuses on its impacts on the respiratory system, TB can also affect eye health. In certain cases, TB can infect eye tissue and directly lead to the formation of a chalazion [5][7]. In both case studies, a young patient developed TB-induced chalazia from a previous, unrelated surgical procedure. The surgical removal of the TB-induced chalazion was complicated by its invasion and inflammation of surrounding tissue. The presence of TB was confirmed through PCR from a biopsy (Figure 1), but it was undetectable through any other diagnostic methods.

Figure 1: Gel Electrophoresis Confirms TB Presence in Chalazion Biopsy. A biopsy from a patient chalazion was used in a PCR specific for TB, the results of which were visualized on a gel. Lane 1 contains the reference ladder, with DNA base pair (bp) lengths shown on the left. Lane 2 is the positive control (mimics TB length ~300bp), lanes 3-5 and 7 are negative controls (no TB present). Lane 6 is the biopsy of the TB-induced chalazion, and demonstrates that the chalazion contains TB [5].

TB represents an unexpected and problematic cause of chalazia. TB's role in generating chalazia is often overlooked, as primary TB infection does not typically occur in eye tissue. As demonstrated by the case study, TB can infect prior eye wounds and become endemic in the body [5]. The first case study patient contracted the pathogen while recovering from her previous surgical removal of a chalazion [5]. The second case study patient contracted TB after undergoing cosmetic surgery on her upper eyelid [7]. Eye tissue can be left vulnerable by surgeries or even vigorous itching, opening up a pathway for TB infection and potential chalazion formation. Such infections are nearly impossible to detect without a biopsy and further physical examination [5]. TB's ability to slowly grow can allow untreated and/or misdiagnosed chalazia to grow into surrounding tissues, risking significant damage to the overall eye. For the second case study patient, the initial removal of the chalazion was unsuccessful, and nearly a year of further surgeries and therapy was necessary to fully treat the chalazion [7]. Earlier detection of the chalazion etiology may have slowed down the TB progression and improved patient outcomes.

Other unexpected causes of chalazia include respiratory pathogens and masks [3]. The oral microbiome is rich with bacteria that thrive in the moist, nutrient-rich environment. The COVID-19 pandemic introduced public masking, leading to a novel chalazia risk factor. Improper mask wearing may lead to the upflow of exhaled air over the eyes, which dries out the eyes, diminishing the anti-pathogenic effects of tears and hardening the meibomian glands [3]. This two-fold effect may directly increase the risk of developing chalazia. Moreover, the exhaled air can potentially carry oral pathogens and introduce them to the sensitive tissues of the eye. In order to explore this unconventional chalazia source, Silkiss et al. studied the number of new COVID-19 cases and prevalence of chalazia cases. They noticed that the two statistics were strongly correlated. The California-based ophthalmologists noted that a rapid rise in COVID-19 cases in April 2020 was closely preceded by a two-fold increase in chalazia incidence (Figure 2). This increase in chalazia incidence was well above seasonal variations and greatly surpassed any value over the two previous years (Blue and Orange lines).

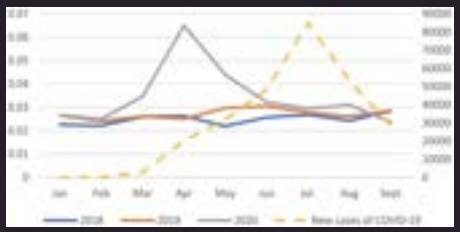

Figure 2: Chalazion Incidence in UCLA and New Cases of COVID-19 in LA County: The monthly prevalence of chalazia cases at University of California Los Angeles (UCLA) is compared with new COVID-19 cases in Los Angeles (LA) county, California. 2018-2020 Chalazia data is expressed as a proportion of all ophthalmology cases on the left column, while new COVID-19 cases are displayed through 2020 on the right column. No COVID-19 data is available before 2020. All data was sourced from clinic and UCLA medical records [3].

Oral pathogens are a complex cause of chalazia. The global pandemic necessitates frequent mask-wearing to mitigate community spread of the virus, yet most people do not properly wear a mask or maintain adequate oral hygiene [8]. As a result, increased mask-wearing facilitates more frequent oral-eye interactions, which has potentially driven an increase in chalazia prevalence. Though COVID-19 can be detected relatively easily through PCR or antigen-based testing, oral pathogens can be harder to detect in the eye without dedicated biopsy. Similar to TB, oral pathogens are rarely tested for and may be an underrepresented cause of chalazia [3].

TB, respiratory viruses, and oral pathogens can all find ways to attack the eye's meibomian glands.

Viruses, including respiratory infections, could also play a direct role in chalazion formation [2]. A seven-year study of first time chalazia patients found that nearly half of studied patients (n=27) reported some sort of respiratory infection occurring before the emergence of a chalazion [2]. To rule out confounding variables, the study did not include any patients with a medical history of eye disease or surgeries. Viral presence was confirmed by patient biopsies; electron micrography revealed numerous viral particles within somatic cells. Nearly all viral infections were associated with both chalazia and conjunctivitis (inflammation of the conjunctiva) of one or both eyes, strengthening the connection between viral respiratory infections and eye disease. Interestingly, the study suggests that chalazia may be partially contagious [2]. Patient One of the study initially developed a chalazion and conjunctivitis. Within a week, most of her immediate family, who had no prior medical history of eye complication, had also developed the same diseases. The likely mechanism of spread was viral propagation through touch and/or respiratory droplets. This case study weakened the historical belief that chalazia are an individual, non-contagious disease [2]. Viruses can spread extremely easily throughout populations and do not readily respond to antibiotics and other common medications, hindering chalazion treatment.

AN ABIOTIC ORIGIN OF CHALAZIA

Not all cases of chalazia stem from pathogens and other biotic sources. In rare cases, chemotherapy can lead to chalazion development [9]. Certain aggressive cancers of the breast and prostate are treated with Docetaxel, a chemotherapeutic drug that severely hinders mitosis. Though it improves patient prognosis and promotes tumor reduction, the drug negatively impacts fluid retention, neuromuscular health, and nail and hair health [9]. In the Gupta et al. case study, an elderly patient with metastatic prostate cancer and no previous medical history of eye issues developed a large chalazion as a side effect of taking Docetaxel (Figure 3) [9]. Unlike conventional chalazia, this mass caused significant irritation, lacrimation (frequent tear secretion), and hemorrhage. Even after surgical curettage and biopsy, the patient continued to exhibit irritation and lacrimation. The authors of the study theorized that Docetaxel secretion in the tears and meibum led to inflammation of the meibomian gland area and the subsequent chalazion [9]. Disrupted cell division hampered effective tear production, causing irritation and lacrimation.

Figure 3: Chalazion of Patient with Prostate Cancer. A 71-year old patient with metastatic prostate cancer developed a chalazion on the lower right eyelid following treatment with Docetaxel. Chalazion was accompanied by lacrimation and conjunctivitis [9].

Cancer is one of the leading causes of death in developed countries [9]. Treatments almost always include radiation therapy and chemotherapy. The numerous associated side effects have seen little research into how they may affect eye health. Chemotherapy drugs, such as Docetaxel, can wreak havoc on the delicate eye environment. Other common cancer drugs, such as carboplatin and decarbazine, also attack the cell cycle and may have similar side effects [10] [11]. Though chalazia are rarely life-threatening, early detection and proper treatment can greatly increase the quality of life of chemotherapy patients.

CONCLUSIONS

Chalazia are unique in their variety of origins. TB, respiratory viruses, and oral pathogens can all find ways to attack the eye's meibomian glands. Administered medication can even lead to chalazia formation. The eye's membranous nature, constant motion, and multitude of glands and ducts all leave it vulnerable to these vectors. All discussed etiologies can appear outwardly similar, requiring more tests and/or biopsies (Figure 1), which are more

Future research should explore how eye tissues respond to chalazion causes at a molecular level. Only then can more effective drugs be developed to treat persistent chalazia.

expensive and invasive than most patients are willing to accept.

Infrequent biopsies reduce the accuracy of chalazia detection. Many patients either allow the chalazia to recede over time or do not seek medical treatment. Even when surgical curettage is performed on larger chalazia, physicians rarely retain the excised chalazion for biopsy and later study [5]. As a result, there is a distinct lack of data on the different causes of chalazia. It is simpler to prescribe antibiotics or excise a chalazion, thus few physicians will perform PCR, immunoassays, and micrography to learn more about the causes behind chalazion formation. All of these reviewed papers posit a question: on a molecular level, how do these pathogens and drugs lead to chalazion formation? The reviewed papers noted symptoms, causes, and identified pathogens. Yet, each paper could only theorize a molecular mechanism. Future research should explore how eye tissues respond to chalazion causes at a molecular level. Only then can more effective drugs be developed to treat persistent chalazia. In the meantime, ophthalmologists would greatly benefit from performing biopsies more frequently. Research would benefit from chalazia biopsies when the patient has any sort of medical history of eye damage, or if the chalazia is of abnormal size or location. Though ophthalmologists do not yet comprehensively understand chalazion etiology, early detection will significantly mitigate harmful complications.

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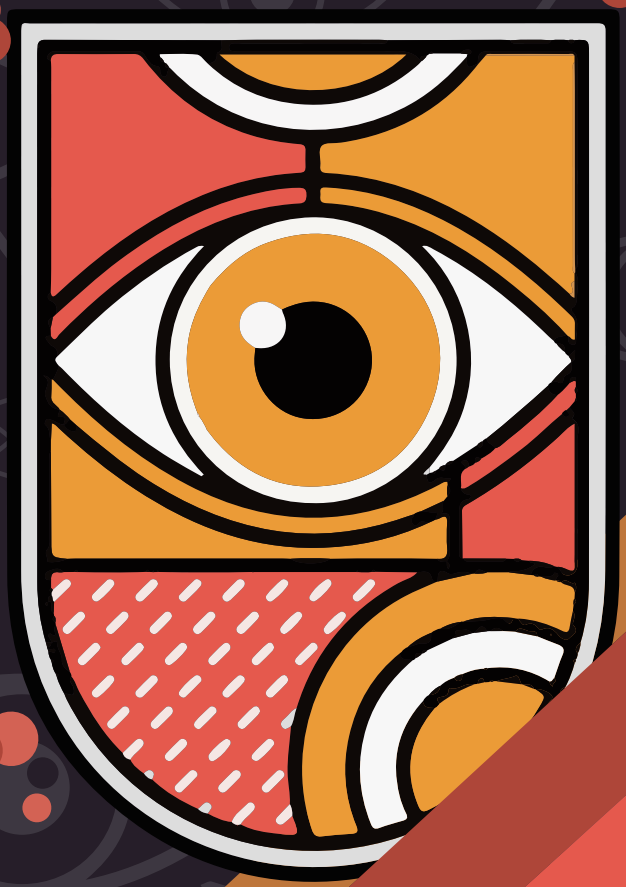

Neurocognitive Implicat

Nancy Jo

Abstract

Individuals suffering from hypothyroidism, characterized by unusually low T3 and T4 hormone levels, frequently report symptoms such as brain frog, fatigue, and lack of concentration. Studies show that hypothyroidism can decrease IQ, lower hippocampal volume, and negatively impact cognitive and motor functions. Currently, research demonstrates that the severity of these symptoms are dependent on the onset, severity, and duration of T3 and T4 imbalance. Though most symptoms of hypothyroidism have been identified and understood by researchers, the biochemical mechanisms which cause the neurological symptoms associated with hypothyroidism have not been thoroughly understood yet. Currently, emerging studies suggest that these neurological symptoms are partially caused by disturbances in parvalbumin levels, improper myelination, and impaired cellular metabolic homeostasis. Hence, further research in these areas will help identify the underlying biochemical causes of the neurological symptoms associated with hypothyroidism, opening the pathway to better mitigation and treatment of neurocognitive problems caused by the disease.

Introduction

Thyroid disorders, such as hypothyroidism, affect an individual's daily life through symptoms such as weight fluctuation, depression, fatigue, and cognitive impairments which are caused by thyroid hormone imbalances. [1] In hypothyroidism, the body produces insufficient amounts of T3 (thyroxine) and T4 (triiodothyronine), both of which are critical for cell differentiation and fostering cellular metabolic homeostasis uniquely in each organ system. [2-3] Though significant progress has been made in understanding the neurological symptoms of hypothyroidism, the biochemical mechanisms through which these symptoms manifest remain elusive. One of the most prevalent neurological symptoms of hypothyroidism is brain fog, a condition characterized by fatigue, forgetfulness, difficulty concentrating, and lack of mental clarity. [4] Current research on brain fog is associated with diseases such as cancer where studies suggests that damage to myelin cells contributes to brain fog; however, few studies have touched on the biochemical mechanisms that causes brain fog in hypothyroidism patients, so the relationship between low T3 and T4 levels and brain fog remains unclear. [4-5] Additional research which links changes in biochemical cascades caused by low thyroid hormones (THs) and neurocognitive symptoms will assist researchers in developing treatments to mitigate, prevent, and alleviate neurological manifestations, opening a pathway for better treatment outcomes in the future. Particularly, further research which focuses on disrupted parvalbumin levels, myelination perturbations, and lack of cellular metabolic homeostasis in relation to low THs will be especially beneficial.

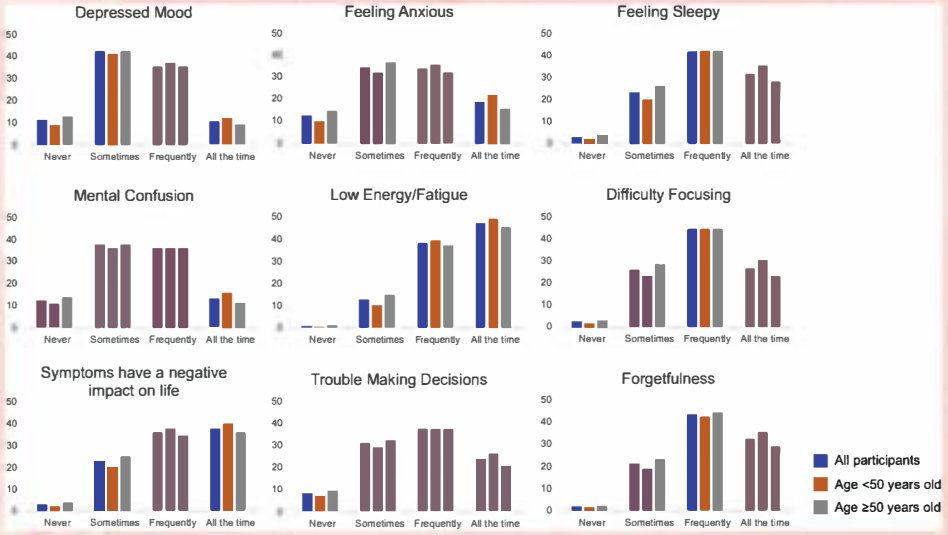

Figure 1. Neurocognitive impacts felt by 5,170 participants answering Likert-Style questions. In the study, participants rated how often they associated a specific symptom with brain fog. [4]

Discussion

I. Maternal Hyperthyroidism

Hypothyroidism has the capacity to affect individuals from all stages of life, but human fetuses are particularly sensitive to the low TH levels of their mothers because fetuses only develop the ability to independently produce their own THs at around four to five months after conception. [6] This is relevant because research by Sahay and Nagesh (2012), Haddow et al. (1999), and Man et al. (1971) establishes a connection between maternal hypothyroidism and decreased neurological function in the growing fetus. [7-9] Beyond the womb, lower neurocognitive skills are observed well beyond infancy in human fetuses that developed with inadequate TH levels; specifically, a study conducted by Haddow et al. (1999) found that 48 children of woman who were not treated for hypothyroidism during pregnancy averaged 7 points lower in full-scale IQ scores compared to 124 control children (P=0.005). [8] Related studies by Schroeder and Privalsky (2014) and Prezioso et al. (2018) identify that THs hold critical roles in fetal nervous system development, connecting maternal hypothyroidism with an increased likelihood for abnormal fetal brain development and function. [10-11] This connection could also

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explain the findings by Haddow et al. (1999) on IQ score discrepancies. [11-12] These IQ discrepancies are further explained by research performed by Moog et al. (2015) which demonstrates that THs are fundamental in the proper development of the fetal cortex which is involved in memory, thinking, learning, and reasoning [6, 22]. Additionally, Moog et al. (2015) also identifies that both T3 and T4 have significant effects on neuronal proliferation, migration, differentiation, synaptogenesis, and myelination. [6] With so many biochemical cascades building off of each other during fetal development, even subtle changes in TH levels can accumulate and begin to manifest as neurocognitive problems, likely amounting to a lower IQ in human fetuses in later stages of life. [6] Altogether, the fetal consequences of maternal hypothyroidism observed through lower IQ scores establishes a near definite link between T3 and T4 levels and neurocognitive function.

[I]ndividuals untreated for clinical hypothyroidism present with explicit symptoms, including, but not limited to, menstrual disturbances, abnormal weight gain, hair loss, and excessive fatigue.

Beyond research focusing solely on connections between THs and IQ scores, studies unrelated to hypothyroidism by Oommen (2014) and Marsman et al. (2017) identify that lower IQ is associated with abnormalities in total brain gray matter, cortex thickness, magnitude of blood and biochemical activity, frontal lobe integrity, and neurological excitatory-inhibitory balance. [12-13] Hence, it is possible that low T3 and T4 levels during pregnancy can result in any one or a combination of the following neurological abnormalities due to the synergistic nature of neurological activity. Ultimately, such perturbations in brain activity on a biochemical level could be a leading factor in the lower IQ scores observed in human fetuses by Haddow et al. (1999). [8]

Severity of neurocognitive implications, such as lowered IQ scores, are believed to be dependent on the extent of TH imbalance. [14-17] Specifically, though a study conducted by Akintola et al. (2015) identified potential cognitive impairments linked to subclinical hypothyroidism, characterized by moderately elevated TSH levels of 4.6–8.0 mIU/mL and normal T4 levels, through a systematic literature search, the study acknowledged that the data was conflicting and could not confidently establish a connection between altered neurocognition and subclinical hypothyroidism. [14-15] However, the same is not upheld with clinical hypothyroidism, classified with raised TSH serum levels greater than 12mU/L and low T4 levels, where researchers have established a clear connection between clinical hypothyroidism and disturbances to neurocognitive function. [16-17] These studies demonstrate that the extent of deviation from normal THs and TSH serum levels corresponds to the severity of clinical symptoms because while individuals suffering from subclinical hypothyroidism are often asymptomatic and present with nonspecific symptoms suggestive of early abnormal thyroid function, individuals untreated for clinical hypothyroidism present with explicit symptoms, including, but not limited to, menstrual disturbances, abnormal weight gain, hair loss, and excessive fatigue. [1,18] Studies by Stasiolek (2015) and Akintola et al. (2015) similarly suggest that severity of TH deficiency correlates to severity of symptoms because subclinical hypothyroidism is not correlated with significant neurocognitive decline, but clinical hypothyroidism is strongly associated with mood and cognitive disturbances and in severe cases, myxoedema coma. [14,16] This trend is also reflected in fetuses whose mothers had varying severities of

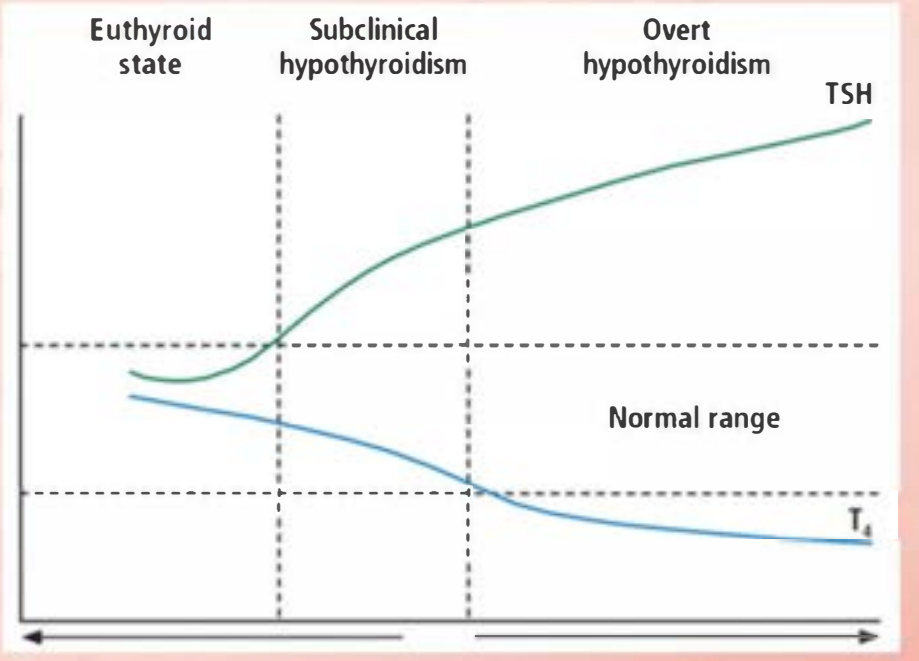


Figure 2. Illustrates differences between the Euthyroid state, Subclinical hypothyroidism, and Overt hypothyroidism. T4 levels are still in the normal range in subclinical hypothyroidism, and below normal T4 levels is a defining criteria for overt hypothyroidism. [18]

hypothyroidism during pregnancy. [6] Specifically, Moog et al. (2015) establishes that mothers with severe hypothyroidism during pregnancy are more likely to birth babies with cognitive and motor defects, developmental delay, speech defects, and hearing difficulties; these irreversible symptoms are collectively known as neurological cretinism, a condition unique to severely deficient hypothyroid babies. [6] Despite the large possible range of severity in TH deficiency, since early fetal developmental stages are characterized by neuronal proliferation, even moderate TH imbalances are likely to compound into more severe disturbances in brain anatomy, neuronal connectivity, and brain function during the first trimester of pregnancy compared to the second and third trimester. [6] Illustrating the crucial role THs play in fetal brain development, the severity of fetal cognitive function deficits is dependent on the onset, severity, and duration of maternal TH deficiency. [6]

Early detection and treatment of hypothyroidism during pregnancy is widely effective in preventing abnormal fetal neurocognitive functions. [19] However, the vague and non-specific symptoms of hypothyroidism such as unusual weight gain, fatigue, and cold intolerance are often mistaken for pregnancy symptoms. [7,20] Hence, the relatively asymptomatic nature of maternal hypothyroidism makes it difficult to identify without professional medical consultation and biochemical thyroid testing. [7] Today, there are mixed opinions in the medical community on screening for thyroid disorders during pregnancy. [21] Currently, mothers being treated for hypothyroidism during pregnancy are recommended to be screened via thyroid-stimulating hormone (TSH) concentration every four weeks for the first half of pregnancy. [21, 23] Frequent testing as such is ideal since a woman's body requires 50% more iodine intake during pregnancy, and women with insufficient iodine levels are more likely to develop hypothyroidism during pregnancy. [24] In addition to screening predisposed expecting mothers periodically, it is also recommended that pregnant women who are at high risk for iodine deficiency due to geographical location and lack of adequate dietary intake be screened for iodine deficiency during their first prenatal visit. [25] Despite the importance of maintaining ideal TH levels throughout pregnancy for all expectant mothers, the guidelines for screening high-risk mothers are more clear than the guidelines for low-risk mothers because there is no strict protocol for low-risk mothers. [21] Since there is controversy between some medical professionals who doubt if early thyroxine treatment is effective during pregnancy, there is debate on if universal vs targeted screening for TH deficiency will be beneficial for low-risk expectant mothers due to fears of overdiagnosing. [21, 26] Despite this controversy in the medical community, it is agreed that establishing a concrete protocol for screening low-risk pregnant mothers is a balancing act which must do more good than harm in the most effective manner possible.

II. Congenital Hyperthyroidism

Beyond the womb, babies can also be born with hypothyroidism, independent of their mothers' endocrine health. [27] Congenital hypothyroidism (CH) is a form of hypothyroidism where newborns are unable to make an adequate amount of THs. [27] CH is most commonly caused by an underactive thyroid gland, thyroid agenesis, or a hypoplastic thyroid gland. [27] Babies with CH are more likely to develop auditory problems which can lead to impairments in language, literary, and cognitive development. [28] On a biochemical level, research by Uchida and Nagesh (2012) identifies that untreated CH causes synapse malformations, defective neuronal migrations, and impaired myelinations, similar to the effects of maternal hypothyroidism. [29] This is relevant in understanding symptoms of hypothyroidism such as brain fog because neuronal health is critical for optimal brain signaling and ideal cognitive conditions. [30] Subsequently, the decline in overall neuronal health caused by CH is one of the foundational causes of neurocognitive symptoms associated with hypothyroidism.

In addition to an increase in defective neuronal activity, Uchida and Suzuki (2021) utilized animal models–specifically rats– to demonstrate that individuals with low TH levels exhibit decreased parvalbumin expression in the brain. [29] Identifying a potential connection between THs and parvalbumin is important in understanding hypothyroidism on a more biochemical basis, potentially leading to more targeted treatments. Most promisingly, a molecular psychiatry study by Nahar et al. (2021) found that decreased parvalbumin expression alters cognitive behavior in humans. [22] In addition to this, research from Uchida and Suzuki (2021) also found that hypothyroidism in rats resulted in microstructural differences in parvalbumin neurons and a decrease in density of parvalbumin-positive terminals in the hippocampus specifically. [29] This is relevant because Wöhr et al. (2015) indicates that the downregulation of parvalbumin can cause neuroanatomical changes that can change the efficiency of synaptic transmissions in the brains of mice. [31] Hence, if low parvalbumin levels interferes with neuronal communication, which is required for optimal cognitive function, then it is possible that the downregulation of parvalbumin is a contributing factor to the atypical neurocognitive function associated with hypothyroidism. [22, 31] Moreover, parvalbumin is involved in neurological processes in the cortex and hippocampus – which are heavily involved in memory, thinking, learning, and reasoning – and the striatum. [22] Thus, it is possible that abnormal parvalbumin expression can negatively disrupt the cortex, hippocampus, striatum, and their related functions. Specifically, a disruption in the striatum, which assists in memory retrieval, could correspond to the brain fog associated with hypothyroidism. [22] With previous scientific research demonstrating that abnormally low levels of parvalbumin disrupt the brain's synergistic interactions, it is possible that the cognitive and neurological problems due to CH can be partially attributed to low parvalbumin levels since the cells play important roles in areas of the brain associated with memory, hinking, and learning processes. [32] Currently, there are no medications or treatments which aim to maintain parvalbumin levels in regards to endocrine disorders, thus it is desirable for more research to be done on how parvalbumin-related interventions might improve CH symptoms.

III. Adult Hyperthyroidism

Beyond an individual's formative years, the development of hypothyroidism in adulthood seemingly brings upon similar but relatively more moderate symptoms when compared to the fetal and neonatal stages, since brain development is most crucial in the first few years of an individual's life. [33] Nonetheless, hypothyroidism in adulthood still has neurological consequences partially due to TH involvement in adult neurogenesis and neuroplasticity. [34-36] Studies utilizing rodent brains by Cooke et al. (2014) and Chamas et al. (2022) shows that adult hypothyroid rodents have smaller hippocampal volumes than rodents without hypothyroidism. [36] Specifically, Cooke et al. (2014) found that the brain scans of rats with low TH levels had considerable volume reductions in the right

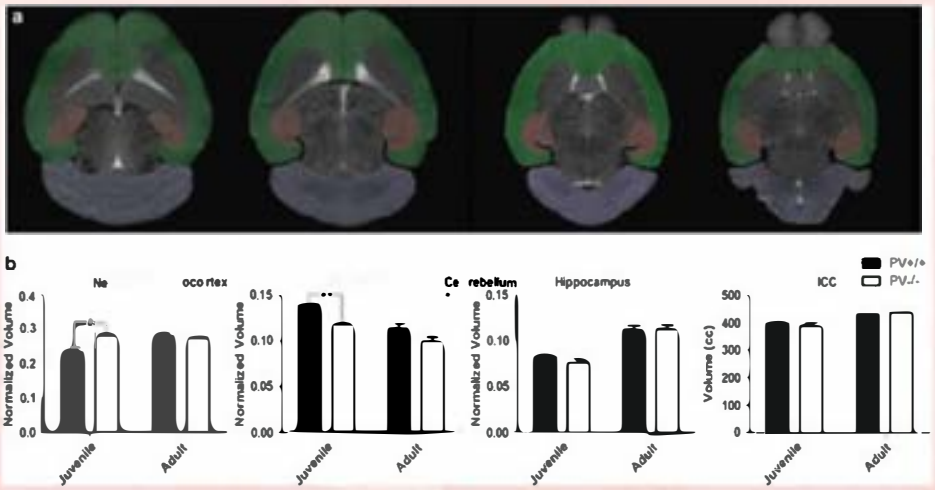


Figure 3. Imaging of male mice brains demonstrates the neuroanatomical changes associated with the downregulation of parvalbumin expression. Specifically, a decrease in parvalbumin appears to cause neocortical hypertrophy and cerebellar hypoplasia. [31]

hippocampus. [36] This is relevant because research by Ezzati et al. (2016) indicates that the right hippocampus is linked with spatial memory. [37] Though problems with spatial memory are not specifically associated with hypothyroidism, problems with spatial memory are likely more commonly reported by patients as brain fog and difficulty in concentrating in studies. Focusing on both hemispheres, the hippocampus is important in short and long term memory, so a reduction in hippocampal volume – which leads to an overall structural deficit in the brain – can cause the neurological clinical symptoms observed in hypothyroid patients such as brain fog and difficulty concentrating. [38]

Additionally, an emerging study by Thvilum et al. (2021) suspects that hypothyroidism is associated with an increased likelihood for dementia in older patients. [39] Specifically, the study observed that for every 6 months of elevated TSH levels above 4.0 IU/L, the risk for developing dementia increased by 12 percent. [39] However, Wieland et al. (2022) suggests that this is only the case for patients older than 65 years old. [40] Though substantial research is yet to be done in the connection between hypothyroidism and dementia, current research indicates that lower TH levels disrupt synaptic connections since dementia is characterized by dysfunctional synaptic transmissions which is suspected to contribute to symptoms of brain fog as well. [41]

Most neurological symptoms of hypothyroidism in adults have been identified, but the biochemical mechanisms that induce such symptoms remain understudied. Considering that the vast amount of neurological problems associated with hypothyroidism are affected by duration, onset, and severity of TH deficiency demonstrates that T3 and T4 play a vital role in the brain. Though T3 and T4 are both thyroid hormones, their significance to organ systems and their contribution to neurological function differ significantly. [42] Previously, T4 and T3 were believed to play similar roles in the brain. However, recent studies show that deiodinase-deficient mice have no observable problems in brain development and neurological function. [42] These findings are relevant because T4 is converted into T3 by deiodinase 2. [42] Considering that without deiodinase, the mice would not have been able to convert T4 into T3 sufficiently, researchers now believe that T4 is more active than T3 due to the lack of significant neurological implications caused by decreased T3 levels. [42] In addition to this, T3 and T4 are primarily able to function in the body by binding to thyroid hormone receptors. [42] The most common receptors are TRα1, and TRβ1 and TRα1 is more responsive to T4 than it is to T3. Additionally, TRα1 makes up around 70 to 80% of the brain's thyroid hormone receptors. [42] Hence, the findings from Schroeder and Privalsky (2014) on T4 being more involved in biochemical processes in the body possibly explains the corresponding difference in percent composition of TRα1 and TRβ1 receptors in the brain where TRα1 is more abundant. [42] Currently, thyroid research focuses on both T3 and T4 together. However, these findings suggest that individual research on T3 and T4 will be beneficial in better understanding the biochemical cascades involved. Specifically, a focus on solely the mechanisms of T4 in the human brain will be beneficial in understanding how neurological problems arise in hypothyroid patients.

A decrease in myelinated axon levels can be caused by low TH levels, ultimately impairing proper myelination in the brain and decreasing optimal neurocognitive function

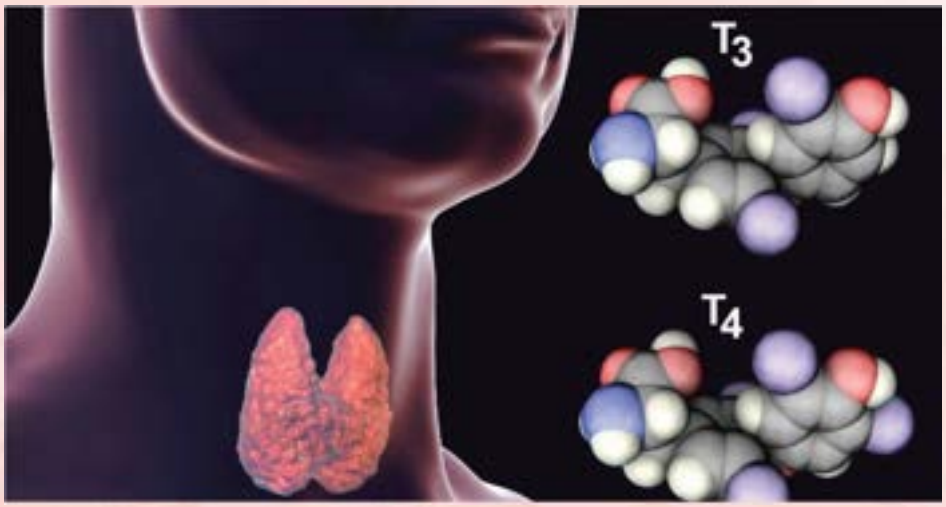
In addition to identifying a difference in activity between THs, researchers have also found strong connections between hypothyroidism and abnormal myelin cells. [43] Specifically, through cell microscopy, Bernal (2022) found that adult rats that had prolonged neonatal hypothyroidism (CH) have less myelinated axons in adulthood compared to adult rats that did not have hypothyroidism in early developmental stages. [43] This suggests that a decrease in myelinated axon levels can be caused by low TH levels, ultimately impairing proper myelination in the brain and decreasing optimal neurocognitive function due to poorly myelinated pathways. [43-44] Moreover, since myelination in the brain is most active during the first year of life, CH is especially detrimental due to its onset. [45] Improperly myelinated pathways contribute to symptoms such as "brain fog" because myelination is fundamental to optimal brain function as it allows for rapid transfer of information along neural fibers. [46] In early stages of neurodevelopment, myelination provides the building blocks of neural connectivity which subsequently aids in proper cognitive and behavioral functioning of an individual. [47] Consequently, hypothyroidism in earlier stages of development appears to be more negatively impactful than in later stages of life.

Along with causing disruptions in myelin cell activity, low TH levels are able to disrupt optimal myelination by disturbing oligodendrocyte maturation. [48] As a consequence, low TH levels can contribute to perturbations in ideal oligodendrocyte maturation which negatively impacts myelination since action potentials will not be able to transmit signals between neurons properly. [49] This cascade will cause problems in an individual's cognitive and behavioral functioning if the number of impacted oligodendrocytes is significant. Additionally, newer research by Williamson and Lyons (2018) also suggests that myelin cells have a regulating role in experience and learning, further exemplifying the importance of overall neuron health. [49] Moreover, properly myelinated neural circuits are able to transfer information significantly faster than unmyelinated cells. [49] For these reasons, it is likely that symptoms of hypothyroidism such as brain fog and difficulty remembering

information is partially caused by non-optimal myelination conditions caused by perturbations in oligodendrocyte maturation.

Conclusion

With over 5% of the general population diagnosed with hypothyroidism and another 5% estimated to be undiagnosed, hypothyroidism continues to be a highly pertinent endocrine disorder globally. [50] Most commonly, hypothyroid patients frequently report symptoms such as brain fog, memory deficits, and difficulty concentrating. [1] Current research demonstrates that the severity and type of neurological symptoms depends on a variety of factors, making hypothyroidism a multifaceted problem. The onset of hypothyroidism in early developmental stages and in the womb appears to be more detrimental to proper neurological development due to the increased neuroplasticity in the developing brain during this timeline [6, 45]. Hence, screening protocols for high-risk expectant mothers have been set in place, but there is still uncertainty on how low-risk mothers should be screened. [26] Though the precise cumulative effects of low TH levels are not fully understood, emerging research suggests that disturbances in parvalbumin, myelination, and cellular metabolic homeostasis have large roles in neurological function. [3, 32] Further research in the connection between low TH levels and these neurological aspects along with identifying the roles of T3 and T4 independent of each other will be beneficial in advancing hypothyroidism research and formulating treatments to mitigate and alleviate the neurocognitive symptoms associated with low TH levels.



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Review of the Link Between Pediatric Acute Lymphoid Leukemia (ALL), Acute Myeloid Leukemia (AML), and Down Syndrome (DS)

by Sohani Sandhu

Abstract

Pediatric acute lymphoblastic leukemia (ALL) constitutes 20% of all cancers that affect the general youth population, but when focusing solely on the down syndrome (DS) pediatric population, this percentage skyrockets by 10 to 30%.. This statistic suggests a strong correlation between DS and various AML and ALL symptoms, such as transient leukemia. Although there is little research at the moment that explains this correlation's cause, it is likely due to the different genetic makeup of DS and non-DS ALL/AML patients. These chromosomal differences also are the suggested reason behind the increased toxicity of methotrexate (MTX) and different medical outcomes from using it with DS patients vs non-DS patients. One study shows that the DS-ALL patient population has a greater occurrence of side effects related to the gastrointestinal system than non-DS patients and overall higher toxicity of relapse therapy as well due to higher overall treatment intensity. Therefore, the drug treatment plans used for non-DS-ALL patients need to be adapted for DS-ALL patients so that the intensity and type of drug matches the patient being treated. This is just one example demonstrating the need for increased research into the connection between ALL and DS in patients, and is the primary scope of this literature review. In this literature review, the aim is to synthesize observed relationships between DS and ALL in order to justify the investigation of better treatments for DS-ALL patients specifically.

Introduction

Pediatric acute lymphoblastic leukemia (ALL) is a type of cancer that causes the bone marrow to overproduce immature white blood cells, or lymphocytes, restricting the space and function of healthy white blood cells.[1] It is the most common cancer among children and adolescents less than 20 years old, with it representing 20% of all cancers that affect this population.[2] However, this already high percentage of cases increases drastically when focusing solely on the down syndrome (DS) population. Children with DS have a 10 to 30% increase in the likelihood of developing pediatric ALL or acute myeloid leukemia (AML). [3] Although scientists are still investigating the connection between DS and acute leukemias, there is an evident relationship between having DS and having different AML and ALL symptoms, specifically in having transient leukemia, likely due to the different base genetics of DS and non-DS ALL/AML patients.[4] As a result of the chromosomal abnormalities in DS-ALL patients, altered side effects and therapeutic outcomes of the drugs have been observed in them as well. Despite this trend, many of the therapies used to treat ALL today are the same for both DS and non-DS patients, even though studies show that each population reacts to the cancer and its treatments differently. Therefore, in this literature review, the aim is to outline and consolidate observed patterns connecting DS and ALL among pediatric patients in order to justify the investigation of new or adjusted treatments for DS-ALL patients specifically.

Discussion

AML Manifestation in Non-DS vs DS Patients

In general, pediatric ALL and AML manifest themselves in detrimental ways both in DS and non-DS patients. For both populations of patients, the following symptoms are most common: fever, easy bruising, petechiae (or flat, red spots under the skin caused by easy bleeding), lethargy, bone pain, shortness of breath, painless lumps, and loss of appetite[1]. However, a condition that usually only occurs in DS-AML patients is transient leukemia (TL). This occurs in 10% of infants with DS, and presents itself anywhere from no visible symptoms to bruising, respiratory distress, hydrops fetalis, hepatic failure and death. Although this condition spontaneously resolves itself in a majority of infants, 20 to 30% of infants with TL end up having AML 1 to 4 years after TL makes its appearance. [5]

The genetic cause behind TL has been attributed primarily to two factors: the presence of trisomy 21 and a mutation in the GATA binding protein 1. The trisomy 21 causes the increased production of megakaryocyte progenitors (MKPs) in the fetal liver, which can overwhelm regular bone marrow production and reduce the production of other vital cells, such as leukocytes (including neutrophils) and erythrocytes.[6] This, combined with the GATA1 mutation, initiates the onset of TL, although no explanation as to why GATA1 contributes to this has been discovered. This sheds a light as to why DS infants have a likelihood of getting TL: DS is caused by trisomy 21, which also facilitates the onset of TL. Therefore, TL is a major indicator of later onset of AML in DS patients that is rarely present in non-DS patients.

Although TL can cause death and can be an indicator of future AML, not all of its effects are dangerous, according to a study by Klusmann et al. in 2008. Their research shows that DS-ML patients who had an earlier onset of TL had a lower relapse rate, and therefore a better 5-year event-free survival (91% ± 5%) than those without TL (70% ± 4%).[7] Regardless, there are still a variety of risk factors associated with transient leukemia, making it one of the distinguishing factors between some DS-AML and non-DS-AML patients' conditions.

Survivability of Non-DS vs DS Patients with ALL

Various studies have been performed in regards to investigating the survivability of AML and ALL in non-DS and DS patients, each with slightly different results but a common theme. Firstly, in regards to AML patients, the results are vastly different, and much more positive, than expected. The first major study to identify the relationship

between AML and DS patient survivability was published in 1992, in which non-DS patients only had an event-free survival (EFS) of 28%, which was drastically lower than the DS group, especially since 12 of the 248 DS people had an EFS of 100%.[8] This high survival rate was attributed to a specific drug treatment plan using cytarabine, which is proven to be generally effective against AML, as will be further elaborated later.

The narrative for ALL patients, however, is quite different. One study that particularly focused on this connection compared 30 DS-ALL children with 60 non-DS-ALL children who matched with each other for age, diagnosis, sex, etc.[9] Adverse events and days of hospitalization due to toxicity were recorded for each group. The results showed that the number of adverse events and hospitalizations was drastically greater in the DS-ALL population compared to the non-DS-ALL population (Table 1, Table 2). Similarly, when looking at the survival and relapse rates of the DS-ALL vs non-DS-ALL populations, non-DS-ALL patients had a significantly higher survival rate than DS-ALL patients before 1999 (Graph 1). This is due to the high cancer relapse rates, as well as an increased risk of contracting life-threatening infections due to a weaker immune system. Although the survival rate improved after 1999 with a new drug, the toxicity of the drug to the DS-ALL patients was higher than that experienced by the non-DS-ALL patients.[9] Therefore, the DS patients in general seem to have a worse experience with ALL compared to non-DS patients. This goes to prove that the current drugs used to treat DS-ALL are not maximally effective, which will be further discussed in the following section.

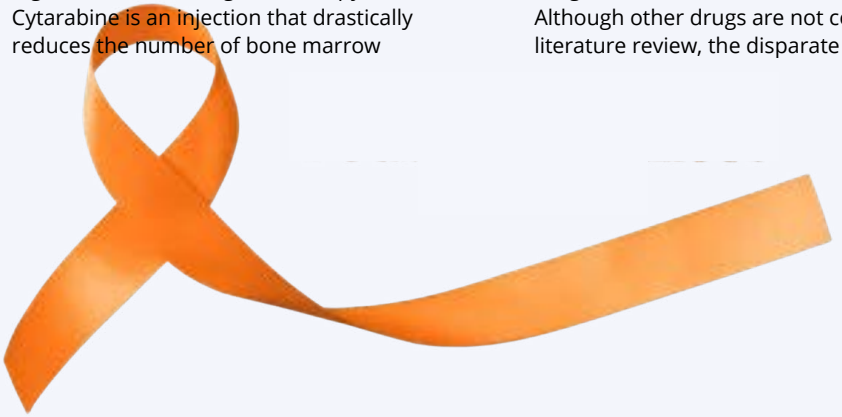
Effectiveness of AML and ALL Drugs in Non-DS vs DS Patients

In regards to current AML drugs, a study by Caldwell 2014 shows that cytarabine is already fairly effective, as seen in the survival rates cited in the earlier section. One of the proposed explanations for this phenomenon was the early introduction of high doses of the drug into therapy.[8] Cytarabine is an injection that drastically reduces the number of bone marrow cancer cells by stopping them from making and repairing DNA that they need to multiply. It is currently used alongside chemotherapy as treatment for non-DS-AML patients. In this study, a high dosage of the same drug proved to have equal benefits for DS-AML patients as non-DS-AML patients. Therefore, the AML medication plan with cytarabine that is currently set in place is relatively satisfactory in making up the difference between DS-AML and non-DS-AML patients.

However, a greater need for updating lies in the medication used for DS-ALL patients. The drug used in the Caldwell et al. study referenced above was intermediate and high-dose methotrexate (MTX). The results showed that DS-ALL patients had more side effects related to the gastrointestinal area compared to non-DS patients because it inhibits the function of folic acid and corresponds to an overall higher toxicity of relapse therapy. This increase in incidence was attributed to the higher treatment intensity and longer infusion time that was delivered to the patients.[9] The reason why a higher dose was delivered in the first place was because the treatment was deemed effective for DS-ALL patients only when the dose was increased compared to the normal amount. However, this dosage, despite being needed to be effective against the AML cells, also heavily negatively affected the patient's health, more so than with normal treatment.

Therefore, it is crucial that an improved treatment plan using methotrexate be discovered for DS-ALL patients. The study specifies dose reduction or escalation trials should be performed to find a balance in dosage so that it is high enough to be effective against DS-AML without causing too many negative side effects (Shah et al., 2008). Without achieving this balance that the study suggests, the current methotrexate dose used to treat DS-ALL either under or over-treat the disease, making them either ineffective or dangerous to the patient.

However, these are just two of the many drugs that are used to treat ALL and AML. Although other drugs are not covered in this literature review, the disparate results between DS and non-DS patients receiving these common drugs exemplify the conclusion: more time and funding must be put into discovering the link between Down syndrome and leukemia so that DS leukemia patients can be better treated.



Conclusion

In this literature review, we found that Down syndrome can cause certain conditions to develop in children that can then make them more prone to developing leukemia. Down syndrome also, as a result, affects the way leukemia takes a biochemical hold on patients' bodies, which can lead to altered effects of drugs on them that have not been completely studied or accommodated for in research yet (such as in the case of MTX for ALL). Therefore, this shines a light on the need for better DS-ALL treatment, and urges scientists to pursue this area of research. Research in this area will be significant, as it will encourage the discovery of newer and more effective drugs for DS-ALL patients, as well as demand a deeper understanding of the reason why there is a biological connection between DS and ALL. This will hopefully lead to greater success rates of treatments of such patients, and possibly even lower rates of DS-ALL development if the connection between the two conditions can be found and severed.

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DESIGN BY Jessica Huang
EDITED BY Karen Ryu

TABLE I: Proportion of Cases With a Grade 3 and 4 Adverse Events Among Patients With (DS) and Without (NDS) Down Syndrome During the Duration of Treatment for ALL [9]

Toxicity	DS (n = 30) (%)	NDS (n = 60) (%)	*P*-value adjusted
Skin	3.33	1.67	0.999
Hematology	96.66	83.33	0.131
Infection	100	83.33	0.041
Positive culture[a]	70	46.67	0.566
CVL infection	20	11.67	0.999
ICU admission	16.67	11.67	0.999
Respiratory	63.33	36.67	0.027
Cardiac	0	0	na
Renal	3.33	0	0.911
Endocrine[c]	13.33	1.67	0.278
Gastrointestinal	53.34	18.34	0.001
CNS[b]	6.66	5.00	0.999
Musculoskeletal	20.0	5.00	0.278
Other[d]	13.33	15.06	1

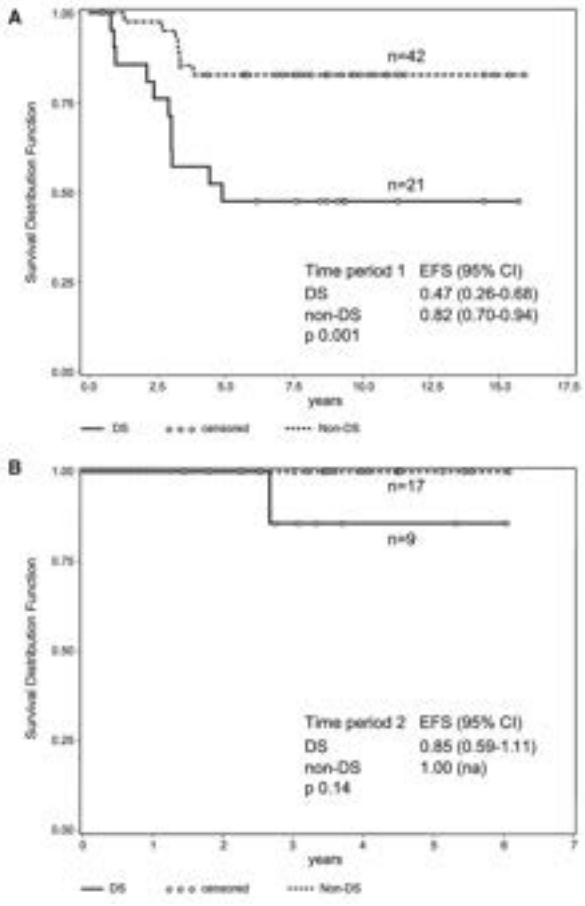
[a]Bacterial blood culture; [b]*Candida albicans* meningitis; [c]Hyperglycemia; [d]Veno-occlusive disease, vulvovaginitis, jugular vein thrombosis, orbital cellulites, na not applicable.

TABLE II: Days of Hospitalization During Different Phases of Treatment for ALL in Children With (DS) and Without (NDS) Down Syndrome [9]

Phase of treatment	DS (n = 30) mean days (95% CI)	NDS (n = 60) mean days (95% CI)	*P*-value
Induction	18.53 (14.2-22.7)	10.78 (7.7-13.7)	0.004
Consolidation	9.10 (3.2-14.9)	7.96 (3.8-12.1)	0.753
Re-induction	5.93 (1.7-10.1)	3.93 (0.9-6.9)	0.440
Maintenance	13.73 (9.0-18.4)	8.40 (5.0-11.7)	0.068

CI, confidence interval.

GRAPH 1: Event-free survival of children with ALL and Down syndrome during different treatment eras. A: During time period one (prior to 1999) the probability of EFS of children with ALL and Down syndrome estimated according to the Kaplan–Meier method was significantly lower than in the non-Down syndrome control group B: In the recent time period (since 1999) such a difference is not been detectable. CI, confidence interval; na, not applicable. [9]



Using Anti-FLAG Immunoaffinity

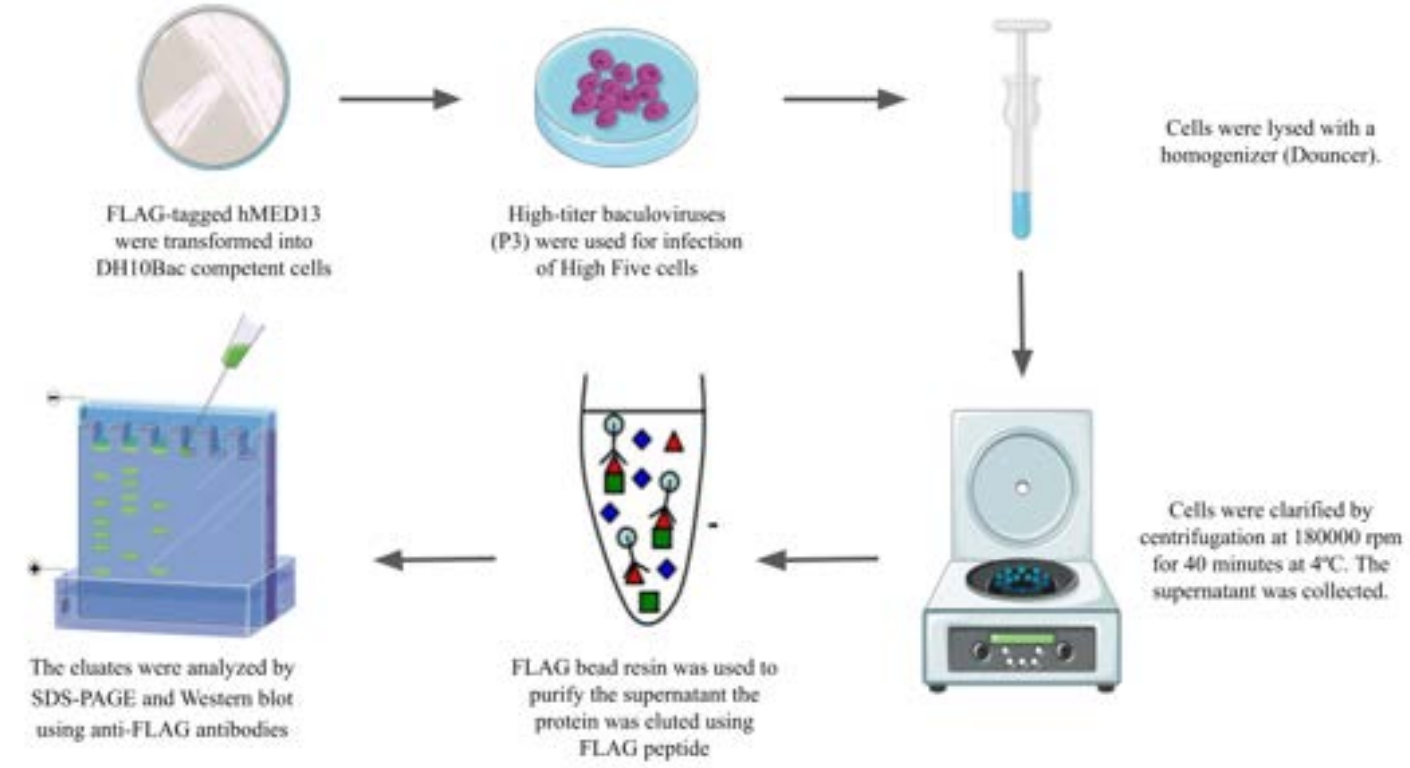


Figure 1. The procedure conducted to purify the MED13 protein followed this pathway, which consisted of expressing FLAG-tagged hMED13 into DH10Bac competent cells, then transfecting the vector into High Five insect cells. 48 hours after infection, cells were harvested with a lysis buffer and centrifuged. FLAG Immunoprecipitation assay was conducted and protein characterization was conducted with SDS-PAGE and a Western Blot.

ABSTRACT

The Mediator complex is an essential transcription regulator that bridges transcription factors with RNA polymerase II. This interaction is controlled by interactions between Mediator and the CDK8 module, but the mechanisms of the Mediator—CDK8 association remain poorly understood. Mediator 13 is a component of the Mediator multi-protein complex that facilitates the initial steps of gene transcription. In this study, FLAG-tagged human MED13 (hMED13) were transformed into DH10Bac competent cells, which were then used to transfect Sf9 insect cells. The Sf9 cells containing the MED13 protein were lysed with a Douncer and purified by using an anti-FLAG Immunoprecipitation assay. The results of a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed a single band of protein of the molecular mass at 240 kDa. Thus, immunoaffinity chromatography using anti-FLAG antibodies would be an economical and safe method for the purification of MED13.

INTRODUCTION

The transcription of genes in eukaryotic cells is highly regulated and extremely intricate. This regulation occurs mostly via a protein complex, called the Mediator, which plays an important role in the activation of RNA polymerase II-dependent transcription by conveying regulatory signals from enhancers to promoters. However, the detailed molecular mechanisms of the mediator in gene regulation remain poorly understood. The Mediator is composed of a large Core and a dissociable Cdk8 kinase module (CKM) which regulates gene transcription by RNA polymerase II (1). The CKM, first identified in yeast, consists of four subunits: Cdk8, CycC, MED12, and MED13 (2). MED13 is the largest subunit in the Mediator and helps associate the CKM with the Core of the Mediator (3). Recently, a structural study also discovered that MED13 possesses Argonaute (Ago) architecture (4). Based on its protein sequence, Li et al. predicted MED13 to be a member of the PIWI protein family; this was confirmed when Li et al. discovered an apparent PIWI module that is characteristic of the Ago/PIWI superfamily of proteins(5).

Ago proteins play a critical role in transcriptional and posttranscriptional gene silencing (6). Ago proteins are characterized by the presence of PAZ domains, which contribute to the binding of microRNAs to control gene expression. Ago proteins cause mRNA degradation via the RNA-induced silencing complex, cleaving the target mRNA strand complementary to their bound siRNA (7, 8). Therefore, these proteins are essential for gene regulatory mechanisms. The regulatory ability of Ago proteins can play an important role in essential biological processes, such as in germline stem-cell division (9). The dysfunction of these proteins can cause several human disorders, such as cancer. Characterizing quantities of MED13 would help understand its functional role in Mediator-dependent transcription regulation.

The finding of MED13 Argonaute within the Mediator suggests that MED13 has the ability to bind the nucleic acids involved in gene regulation.

Chromatography to Purify the MED13 Protein

By Sachi Kishinchandani, Tsai Laboratory (1)
1. University of Texas McGovern Medical School

However, the function and nucleic acid binding ability of MED13 remains unknown. Therefore, we want to structurally and biochemically analyze the MED13 Argonaute protein to understand its role in gene regulation, specifically in mRNA regulation (10). In my project, I will be investigating the nucleic acid binding ability of Human MED13 Argonaute.

METHODS

Expression of Recombinant MED13 Protein

Full-length, N- and C-terminal regions of FLAG-tagged hMED13 were transformed into DH10Bac competent cells (Invitrogen). Colonies containing recombinant bacmids were identified by disruption of the lacZ gene inside the bacmid DNA. The isolated recombinant bacmid DNAs from white LacZ colonies, which were confirmed by PCR, were used for transfection of Sf9 (High Five) insect cells. After three rounds of viral amplification, high-titer baculoviruses (P3) were used for infection of High Five cells (Invitrogen) for expressing MED13 proteins.

Harvesting and Lysis of Recombinant MED13 Protein

48 hours after infection, cells were harvested with a lysis buffer. The cell culture was centrifuged at 1000 rpm for 6 minutes at 6ºC. The cell pellet was washed with 1X PBS buffer, and centrifuged for 8 min at 1000 rpm, then resuspended using lysis buffer (150mM NaCl, 0.1mM EDTA pH 8.0, 20mM HEPES pH 7.9, 0.01 % NP40 detergent, 10% glycerol) and 1X protease inhibitor. Cells were then lysed with a homogenizer (Douncer). The lysed cells were clarified by centrifugation at 180000 rpm for 40 minutes at 4ºC, after which the supernatant with the protein was collected.

FLAG Immunoprecipitation Assay

The FLAG bead resin (Sigma ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich, F1804)) was equilibrated three times with lysis buffer (150mM NaCl, 0.1mM EDTA pH 8.0, 20mM HEPES pH 7.9, 0.01 % NP40 detergent, 10% glycerol), each time for 5 minutes. The resin was then centrifuged for 1 minute at 1000 rpm at 4ºC. The lysed cells were added and incubated on the resin for 1 hour at 4°C on a rotation platform. Then this was centrifuged at 1000 rpm for 1 min, and the resin was collected. The resin was washed five times using the lysis buffer, and the protein was eluted using FLAG peptide (Sigma-Aldrich F3290). The eluates were analyzed by SDS-PAGE and Western blot (BIO-RAD Trans-Blot Turbo) using anti-FLAG antibodies.

Protein Characterization

To estimate the total protein concentration of the MED13 protein, a Bradford assay was used with BSA as a standard. A secondary SDS-PAGE was conducted, and a Western Blot with anti-FLAG antibodies was performed to determine the extent of purification of the protein.

RESULTS & DISCUSSION

In this study, nickel affinity chromatography and FLAG immunoprecipitation were applied for polyclonal antibody purification against MED13. Full-length MED13 was found in a strong and clear band in SDS-PAGE and Western blot analyses after anti-FLAG immunoaffinity chromatography, and the purity of prepared MED13 was up to 98% via enzyme activity assay, whereas data for nickel affinity chromatography was not significant.

Thus, immunoaffinity chromatography using purified anti-FLAG antibodies is an economical and safe method for purifying MED13.

Half-Length hMED13 Molecular Weight Analysis shows that the C-terminal of MED13 can be used to Purify the Protein with an Anti-FLAG Immunoaffinity Chromatography

Initially, cells were transfected with only the first or second half of the MED13 protein. This was because the region responsible for nucleic binding was of interest, and it would be easier to identify this region with two fragments rather than the whole peptide in nucleic base binding characterization studies. The structures of Ago proteins reveal a common architecture composed of four globular domains (N, PAZ, MID, and PIWI) and two linker domains, which form two lobes (N-PAZ and MID-PIWI) with a central nucleic acid–binding cleft between them (11).

Based on the MED13 structure determined and the alpha-fold structure prediction, MED13 consists of four domains (N, PAZ, PIWI, and MID domain) along with several loop regions (4). The N-terminal half of MED13 was designed to contain the N and PAZ domains (the first half) and the C-terminal half of MED13 contains MID and PIWI domains (the second half). It was hypothesized that only the C-terminal of MED13 (MID and PIWI domains) would bind nucleic acid, as its structure suggests a nucleic acid binding module (11). Splitting the protein into two halves and adding a FLAG-tag on the C-terminus of each half of the protein sequence allowed for detection of the MED13 protein via SDS-PAGE (Figure 3) and an anti-FLAG tag Western blot (Figure 4).

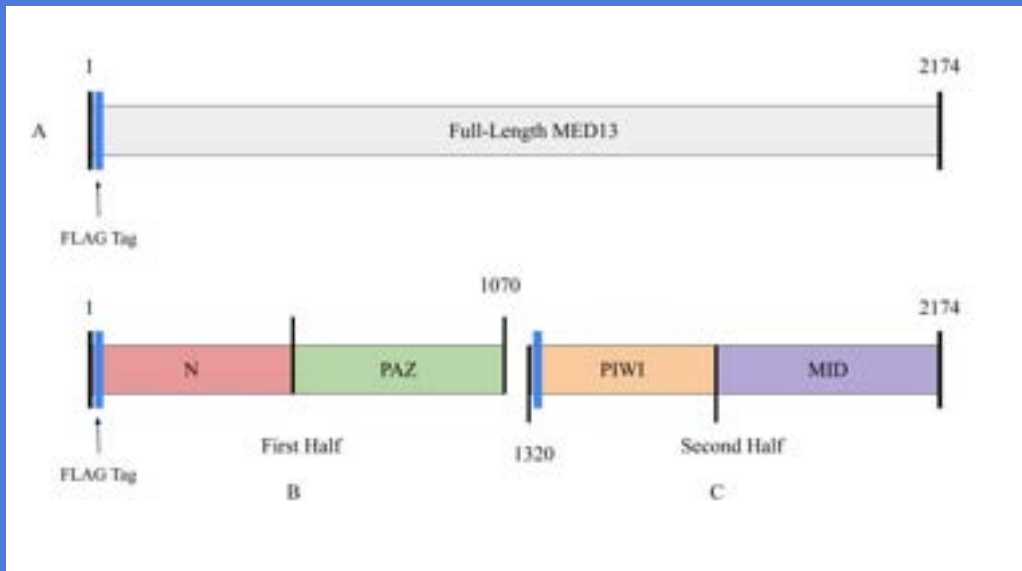


Figure 2. A diagram showing the MED13 protein and its domains. A: depicts the full-length MED13 construct of length 2174 peptides and the FLAG tag located on the C-terminus. B: depicts the first half of the MED13 protein from 1 to 1070 peptides, consisting of the N and PAZ domains. C: depicts the second half of the MED13 protein from 1320 to 2174 peptides, consisting of the PIWI and MID domains.

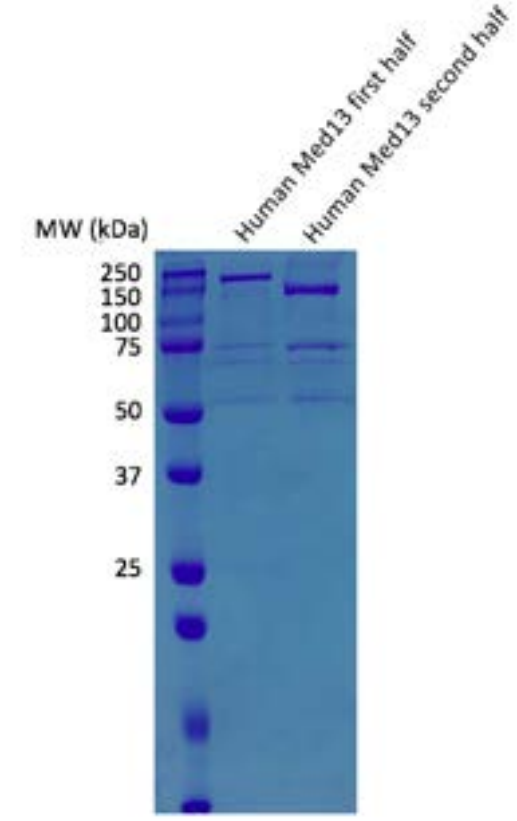


Figure 3. Significant protein bands from elution of two halves of MED13-transfected Sf9 insect-cell pellets: the first half was found around 175 kDa, and the second half around 140 kDa. SDS-PAGE was conducted under reducing conditions. Samples were run through Tris-HCl SDS acrylamide gel 10% in running buffer (25 mM Tris, pH 8.3, 192 mM glycine, 0.1% (w/v) SDS), stained with Coomassie blue solution, then visualized with white light. Lane 1: Bio-Rad Precision Plus Protein ladder. Lane 2: the purification proteins of the first-half MED13-transfected insect cells via anti-FLAG-tag. Lane 3: purified proteins from the second-half MED13-transfected insect cells via anti-FLAG-tag.

There are strong bands present between 150 and 250 kDa for the first half, and 100 to 150 kDa for the second half, which aligns with the predictions of the MED13 protein components by Li et al. 2021. Additional analysis of the samples with SDS-PAGE qualitatively show that the protein samples for each half have a similar purity of MED13, as the bands are similar in size, width, and darkness. Further experimentation characterizing the nucleic binding ability of MED13 is necessary to determine whether the C-terminus is involved in RNA binding as the Li et al. 2021 paper predicts.

Full-Length hMED13 Molecular Weight Analysis determines that FLAG Immunoprecipitation is the Best Method of Purification for MED13 and that it is Possible to Purify a Protein of a Large Size (240 kDa)

Once the baculovirus was reinfected, the full-length Flag-tagged hMED13 plasmid with a FLAG tag was transformed into DH10Bac cells then transformed into Sf9 insect cells for heterologous protein expression. The enzyme was lysed and purified by anti-FLAG-tag affinity chromatography. SDS-PAGE analysis under denaturing conditions showed three clear bands of protein in the fractions eluted: MED13, and the heavy and light chains of the anti-FLAG-tag antibody. The molecular mass of the purified MED13 was approximately 240 kDa which is shown in a clear zone around a single band at 240 kDa (Figure 5), demonstrating that the protein had been effectively purified.

OUR PROTOCOL ALLOWS MED13 PURIFICATION WITH STANDARD PURIFICATION EQUIPMENT AND BUFFERS.

In addition to the anti-FLAG immunoaffinity chromatography, we created a His-tagged hMED13 plasmid which was transformed into DH10Bac cells, then transfected in to Sf9 insect cells. Purification with a Ni-NTA resin yielded a band around 240 kDa, as expected, but the column shows impure results (Figure 5). We hypothesize that this may be due to some of the proteins of the insect cells having chelating capacity, that the protein sample had too high of a concentration, or that the MED13 protein had not been completely denatured and required a stronger denaturing component. Additional studies can reduce the concentration of the protein before conducting an SDS-PAGE, but as mentioned earlier, this experiment has found a way to best purify MED13 with immunoaffinity chromatography.

Further analysis was done with a Western Blot for functional assessment of anti-FLAG purified MED13. Figure 6 represents the Western blot analysis of the anti-FLAG purified protein from Sf9 cells, showing the presence of a MED13 protein band with a molecular mass of 240 kDa in the “bound protein” columns. In eukaryotes, Ago proteins are around 100 to 200 kDa, which helps them play a central role in gene silencing processes guided by small RNAs (12). The result of this experiment was consistent with the molecular mass of a full-length Ago protein in the Meister paper, as well as the size that we hypothesized based on the genetic sequence from Li et. al 2021 (MM ≥ 200 kDa).

In this report, we describe the purification of MED13 from transfected Sf9 insect cells. Our protocol allows MED13 purification with standard purification equipment and buffers. MED13 can both bind to a Nickel and a FLAG immunoaffinity column, the latter used to effectively separate the tag from the purified protein. Based on preliminary results, it can be reasonably concluded that the purification of MED13 via affinity chromatography using anti-FLAG antibodies is a reasonable method for purification. This result is of great significance, as we were able to purify a protein with a molecular mass of 240 kDa. Proteins of a large size, i.e, larger than 200 kDa, are difficult to purify (13), so a successful purification of the full-length MED13 with an anti-FLAG immunoprecipitation assay is important for purification procedures and for future experimentation of MED13.

The purification of MED13 is important due to the possible transcriptional and posttranscriptional gene silencing it may conduct (5). Since it is a member of the Ago family, MED13 is likely to cause mRNA degradation via the RNA-induced silencing complex. Thus, MED13 protein may be essential for gene regulatory mechanisms (6, 7). We present a protocol yielding pure MED13 suitable for a wide array of studies.

CONCLUSION

In summary, an approximately 240 kDa protein from Flag-tagged hMED13 was heterologously expressed in insect cells and purified by anti-FLAG-tag affinity chromatography. In the future, we would like to optimize protein purity, as the purity of the protein is essential in conducting further analysis of MED13 with any sort of functional assay; for example, using an additional purification strategy after an anti-FLAG immunoaffinity column has been conducted, such as size exclusion chromatography or ion-exchange columns, or modifying buffer conditions, could increase the purity of the protein to conduct any further experiments, such as characterizing MED13 enzymatic capabilities to determine its effects on DNA or RNA regulation.

A next step can employ the use of FLAG purification and FLAG peptide to elute peptide, as the eluate has FLAG peptide, which may interfere in further experiments. To solve this, the protein can be purified further to remove the FLAG peptide, with purification methods such as ion-exchange chromatography or dialysis. Additionally, conducting a structural analysis of the anti-FLAG-tag-treated protein would be a way to further understand the extent of purification of the protein.

ACKNOWLEDGEMENTS

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Designed by: Taylor Schultz

Figure 4. Western Blot of the first and second halves of Human MED13 protein where the molecular mass of the first half was around 175 kDa, and the second half around 140 kDa. The half-length MED13 proteins were purified using anti-FLAG antibodies. SDS-PAGE was subjected onto Human MED13 and electroblotted onto PVDF membrane. Lane 1: prestained low molecular mass marker. Lane 2: nothing. Lane 3: the purification proteins of the first-half MED13-transfected Sf9 insect cells via anti-FLAG-tag Immunoprecipitation assay. Lane 4: the detected band shows the second-half MED13-transfected insect cells via anti-FLAG-tag Immunoprecipitation assay.

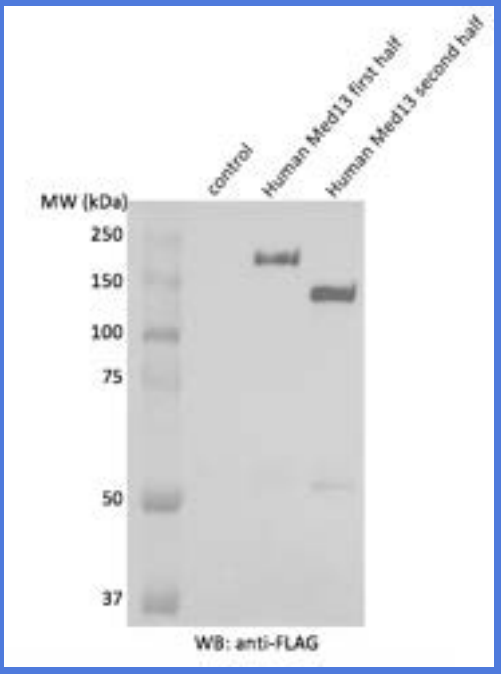


Figure 5. Significant protein band from elution of full-length MED13-transfected Sf9 insect-cell pellet found around 240 kDa. SDS-PAGE was conducted under reducing conditions. Samples were run through Tris-HCl SDS acrylamide gel 10% in running buffer (25 mM Tris, pH 8.3, 192 mM glycine, 0.1% (w/v) SDS), stained with Coomassie blue solution, then visualized with white light. Lane 1: low molecular mass marker. Lane 2: the purification proteins of the His-tag Ni column. Lane 3: is purified proteins from the FLAG-immunoprecipitation column. The heavy chain and light chain bands present in the FLAG-immunoprecipitation column come from the anti-FLAG-tag antibody.

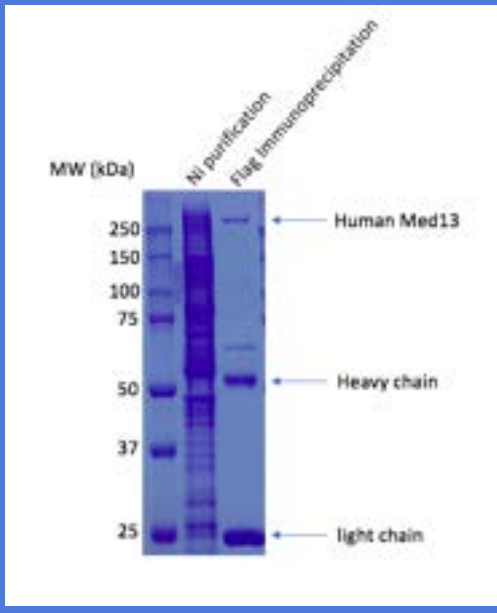
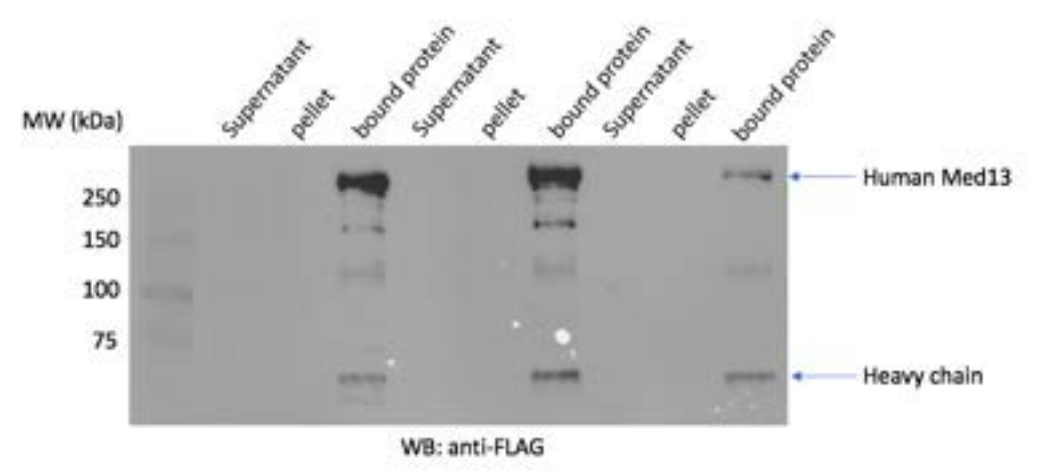


Figure 6. Western Blot of the Full Human MED13 protein that shows the Molecular Weight of full-length MED13 is 240 kDa. Full-length MED13 protein was purified using anti-FLAG antibodies. SDS-PAGE was subjected onto Human MED13 and electroblotted onto PVDF membrane. Lane 1: prestained low molecular mass marker. Lane 2: the detected band shows purified full-length MED13 via anti-FLAG Immunoprecipitation assay.



USES OF HYDROXYAPATITE IN DENTAL ENAMEL BIOFABRICATION

BY WILEY LIOU

ABSTRACT

Hydroxyapatite (HAp) is the primary component of tooth enamel and is important in the prevention of bacterial and acidic decay. Over a lifetime, however, the wearing of this outer tooth layer may lead to recurring pain and disease, making both prevention and restoration of the teeth crucial for daily routines like chewing and physical appearance. Although HAp is a molecule already found in bone tissue, scientists have explored methods of artificially embedding HAp to complement already existing enamel due to its biocompatibility and rigidity. In this review, multiple uses of HAp are explored, as well as ways to artificially synthesize the molecule. First, using HAp as an alternative to fluoride in toothpastes may decrease the toxicity of unintended ingestion. In addition, immersing impacted teeth in a HAp powder solution has demonstrated evidence of rehardening, while a hydrogel mat similarly facilitated the formation of HAp crystals on the surface of sampled teeth. Alternatively, creating a HAp sheet to cover the surface of the tooth is shown to be a less invasive procedure while accomplishing the same task. Finally, three 3D printing techniques of HAp are explored. While vat polymerization has to be conducted at high heat, leading to possible destructuring of HAp crystals, both inkjet printing and extrusion printing can create highly specific scaffolds at the expense of high cost. Overall, mimicking tooth enamel through the fabrication of HAp, while tedious, can be a minimally invasive alternative to crowns and bridges for dental patients.

INTRODUCTION

The thin outer layer of a tooth, or the dental enamel, is formed in a process known as amelogenesis, in which cells called ameloblasts are generated from oral epithelium tissue. [1] As these cells secrete amelogenin and enamelin, calcium and phosphate ions crystallize on top of the dentin surface, the structural layer of teeth underneath the enamel. The result is a hardened matrix composed of long, parallel, and cylindrical rods around 3 μm wide. [2] Dental enamel maintains this rigid structure owing to the fact that it is 95% by weight HAp, which stabilizes the enamel structure through hexagonal phosphate and calcium ion repetitions. (Figure 1). [1, 3]

Evolutionarily, HAp minerals in our enamel have been used to protect against bacterial decay of sensitive dentin, allowing the safe consumption of sugars and acidic substances. [4] However since enamel is acellular, it does not naturally regenerate, leaving it vulnerable to demineralization. [4] When bacteria combines with ingested food, it forms plaque, an acidic substance that further decays the tooth. [5] Thus, enamel restoration is necessary for individuals who have deteriorated their outer teeth layer and intend to prevent tooth pain and gum disease.

As scientists consider candidates for the synthetic fabrication of enamel, artificially synthesized HAp is a particularly attractive material due to its biomimetic capabilities. [6] Because of its natural presence in human teeth, biofabricated HAp is more

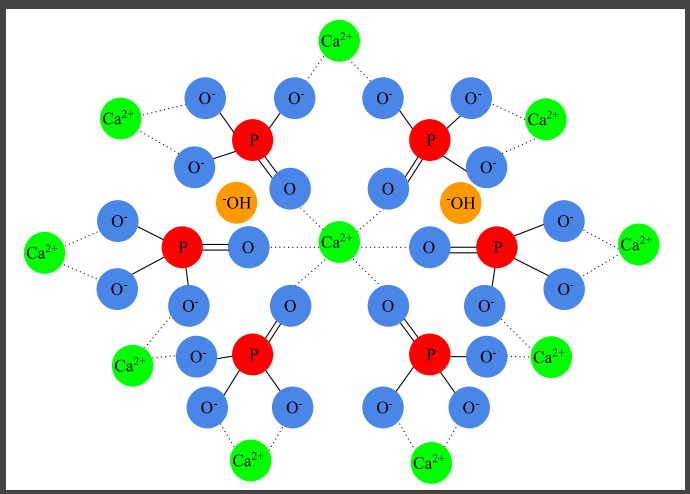


Figure 1: Molecular structure of hydroxyapatite Ca10(PO4)6·2(OH). The crystal structure of hydroxyapatite allows for the formation of hydrogen bonds between the hydroxyl ions and the phosphate groups, which stabilizes the compound. The presence of calcium ions in the crystal lattice also gives hydroxyapatite a high degree of thermal and chemical stability. [3]

likely to integrate into the bone compared to alternatives not produced during bone development. In this scientific review, various methods of artificial HAp synthesis will be explored, as well as their possible uses and potential follow-up research.

TOOTHPASTES

Fluoride is currently the standard hardening material in toothpaste due to its remineralizing abilities on damaged teeth[7] For children under the age of eight especially, however, accidental ingestion of thess toothpastes may lead to permanent discoloration from fluorosis if consumed over the 0.07 mg/kg body weight level of tolerance. [8] Further studies have shown that fluoride can have neurotoxic properties, impacting neurons in the hippocampus. [9] A meta-analysis study on children in China found a decrease in IQ for those with fluoride exposure, with an average standardized weighted mean difference of -0.45 and a p-value < 0.001. [10] In contrast, HAp remineralizes enamel by introducing structural elements that are naturally occurring in the human bone like calcium and phosphate ions. The first time HAp has been used in toothpaste was in 1970, when the U.S. National Aeronautics and Space Authority provided it to their astronauts in space due to enamel's demineralization in the absence of gravity. [6] Its clinical and commercial use, however, did not begin until later, when Sangi Co. Ltd bought the right to use HAp in toothpaste to repair enamel. [6]

To determine whether HAp possesses the same hardening potential as fluoride, a 2019 study conducted by the UTHSA School of Dentistry asked 32 consenting individuals to brush their teeth either with fluoride-containing toothpaste or HAp-containing toothpaste. [11] After two two-week periods of treatment, both groups had similar increases in rates of mineralization on their teeth compared to brushing with no toothpaste (55.8% and 56.9% respectively, p=.81). After intentionally creating an indentation, measurements of its depth reduction with both HAp and fluoride increased similarly in both groups (27.1%, 28.5% respectively, p=.68) [11]. Taken together, these results demonstrate that neither toothpaste has a significant advantage over the other in hardening enamel. Another study using bovine teeth found that nano-hydroxyapatite toothpaste actually demonstrated higher mineralizing capability compared to its fluoride counterpart, with the former having significantly less mineral gain on enamel and dentine compared to HAp toothpastes ($p < .05$). [12] However, a limitation of this study was that it was conducted in vitro, meaning that it was not able to replicate the environment of an organismal mouth; specifically, the large variation in pH under different conditions.

Tooth Sample	Hardness (HV)
Unindented, Unimmersed	311.96
1 Month	283.36
2 Months	283.6
3 Months	311.96

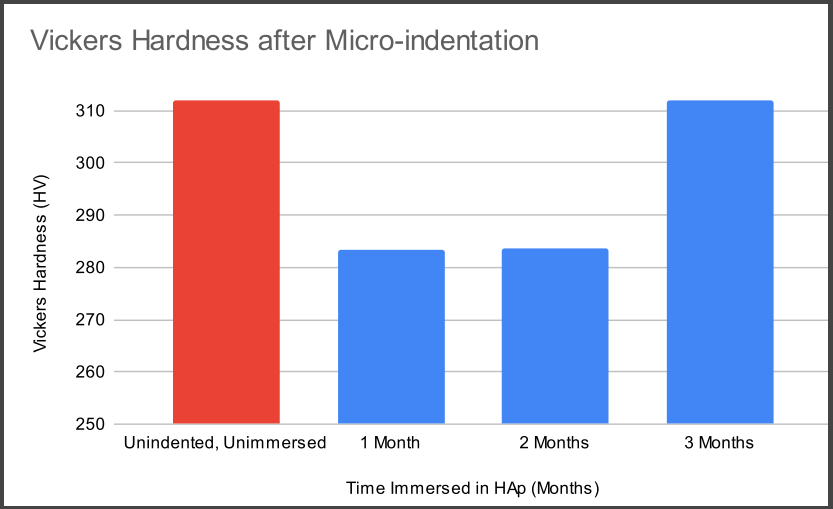


Figure 2: Vickers hardness of the three treated tooth samples. After the samples were micro-indented and immersed in the HAp, the tooth rehardened back to its original hardness after 3 months. [14]

In order to determine the efficacy of HAp toothpaste relative to fluoride toothpaste in the presence of pH variation, Huang et al. attempted to find how mineralization varies when situated in a pH-alternating solution of acetic acid. [13] They found that fluoride-containing toothpaste had a greater increase in surface microhardness recovery compared to the nano-HAp toothpastes. Data indicates that the fluoride toothpaste experienced a surface microhardness recovery rate greater than 50% after 12 days of pH cycling; on the other hand, for toothpastes made of 10-15% HAp, the recovery rate was only around 40%. [13]. Nonetheless, HAp still showed evidence of hardening relative to the negative control ($p < .05$), with crystals forming aggregates in scanning electron microscope (SEM) images. [13] Overall, though HAp toothpastes are promising, more research is needed to improve its reliability compared to fluoride under pH varying conditions.

IMMERSION AND COATING

While HAp toothpaste resolves minor enamel erosions, more severe decay can be resolved through immersion and coating of teeth in HAp in the forms of HAp pastes, gels, and sheets. A 2008 study conducted at Pusan National University used a HAp powder solution to repair a tooth that was scratched with sandpaper and a micro-polisher. [14] To mimic the properties of the bone, a powder containing 635 threads of HAp/in2 was dissolved at 70 wt% in distilled water. Four samples of teeth were immersed in the solution, allowing the ionization of calcium and phosphorus in the following equation:

$$Ca_x(PO_4)_y(OH)_2 + nH_2O \leftrightarrow xCa^{2+} + y(PO_4)^{3-} + z(HPO_4)^{2-} + mH_2O$$

Following this immersion, the positively ionized calcium integrated with the tooth surface, which itself is also charged from the accumulation of acidic saliva. [15] The results of SEM images provide evidence of recrystallized HAp on the tooth through the presence of white dots. [14] To check the embedding capabilities of this powder, teeth were tested after diamond-shaped dents were created. Their results showed that after three months, the hardness of the microindented area was similar to that

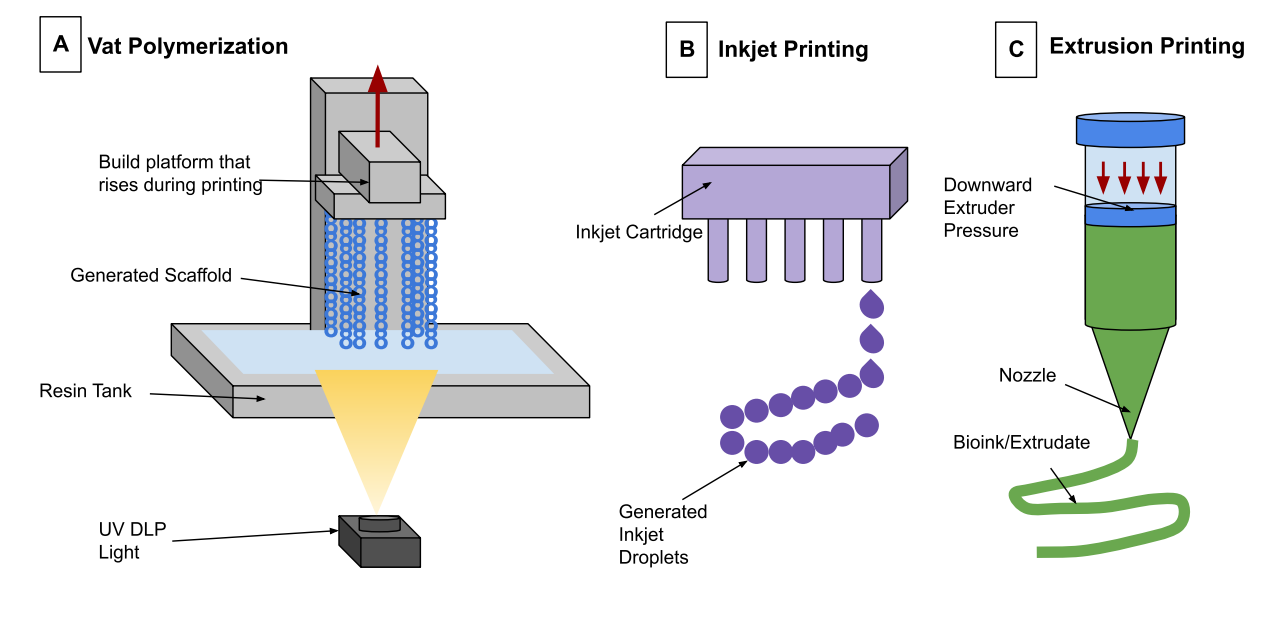


Figure 3: A comparison of the three 3D printing techniques discussed. A. Vat Polymerization generates a scaffold by using a DLP projector to harden a scaffold layer by layer, with a platform rising as the resin hardens. [20] B, C. Inkjet printing and extrusion both involve pushing out a scaffold through a nozzle that is able to be specific in size and shape. [26]

of the non-HAp-immersed tooth (Figure 2). These results together display evidence that the HAp powder had rehardening properties, with calcium ions adsorbing onto teeth, and phosphate ions acting as an agglomeration tool. [14]

Yet another approach to covering enamel is to mimic the gel-like environment that surrounds the teeth during amelogenesis, which involves the transport of calcium and phosphate ions from ameloblasts into the enamel. [16] Unlike past experiments that were conducted in aqueous solutions, Cao et. al created an agarose-based calcium chloride hydrogel mat to provide a one-way transport of ions into damaged teeth. [17] The researchers treated 2 mm-thick teeth samples with phosphoric acid for deterioration, then created a four-layer model by covering the damaged teeth slices with the calcium chloride hydrogel, an ion-free agarose gel, and a phosphate solution. Looking at SEM photographs, HAp crystals were visible after just two days of immersion for the treated teeth. Over the next few days, these rods organized themselves into one axis, and after six days, the gel that had previously been located between the crystals had been effectively eliminated. Furthermore, a nanohardness test demonstrated similar hardness values for the control and repaired teeth, with a hardness increase of 3.04 ± 0.75 gigapascals (GPa)(p=.05) after 6 days. [17] Although this gel can be cheaply produced, a limitation of the matrix formed is that it is inorganic, meaning that it is dissimilar to real enamel; further research can be done to explore how to embed enamel proteins like amelogenin and enamelin into the matrix without sacrificing low-cost.

Although gel immersion may enhance dental enamel, its clinical roles are limited due to the difficulty of isolating individual teeth from patients for the sole purpose of enamel repair. Rather than immersing the entire tooth, Hontsu and his team at Osaka Dental University created a film of HAp that demonstrated evidence of tooth hardening. [18] To create this sheet, they deposited a film of HAp on a soluble substrate via pulsed laser deposition, after which the substrate is dissolved and the remaining flexible HAp sheet is collected. [18] To observe its ability to bind to the dentin and enamel layers of teeth, photographs provided evidence of sealing owing to the moisture content of the created layer. [18] However, research has yet to be done to demonstrate whether or not this protective coating demonstrates hardening capabilities. Overall, though, the techniques of powders, hydrogels, and mats have all provided possibilities for enamel repair and solutions to ailments like dentine hypersensitivity.

3D PRINTING

A more advanced method to produce synthetic grafts is through 3D printing biomimetic material, also known as biomanufacturing. The advantages of these 3D-printed scaffolds are that they are replicable, have great biocompatibility, and can be customized to individual needs. Though there are numerous ways to develop artificial HAp through 3D printing, the ones that are successful must have certain traits. Notably, the HAp must be porous enough to allow for mass transport, strong enough to demonstrate hardening capabilities in enamel and prevent degradation in the mouth microenvironment, and nontoxic to ensure an absence of side effects. [19] Three methods of 3D printing are described in this discussion.

Vat polymerization is a method of using UV light in Digital Light Processing (DLP) to manufacture a HAp scaffold synthetically. [20, 21] In this process, liquid resin inside of a large tank hardens due to UV light causing molecules to bond. By lifting a platform and exposing resin to light, the 3D print is generated layer by layer [20] However, after the fabrication stage, the resin must be sintered, or burned, at a high temperature. To prevent cracks from forming, this must be done in a gradual process to an optimal temperature of 1250 °C, with a high flexural and compressional strength compared to fabrication at 1200° C and 1300° C. [21] Although this method can create a scaffold that is accurate and specific for an individual tooth, the sintering process at extremely high temperatures leads to concerns about

its bioactivity, or its ability to maintain integrity after the print is completed. [22]

In contrast, direct ink writing, also known as inkjet printing, is a method that can create scaffolds at room temperature. Using a paste consisting of α-tricalcium phosphate, it can harden into calcium-deficient HAp, which has previously been proven to foam into a pattern that is osteoconductive. [23] Konka et. al were able to find that by including gelatin micropores in the bioink, the porosity of the matrix increased by more than 60% [24]. More specifically, because these pores were concave rather than flat or convex, HAp deposition and adhesion were more likely with a more optimal geometric orientation. [24] Because gelatin makes the paste more flexible, the paste can be administered through a needle, making the process more effective than its brittle counterparts. A significant advantage of this process, moreover, is that it is scalable by altering the sphere sizes. [24] It also solves the problem of not needing to conduct 3D printing at a high temperature, which preserves bioactivity. A limitation of this process, however, is that its compressive strength is decreased, making it susceptible to degradation over time. [24] Also, current methods of extrusion, when applied to this ink, only produce pores that are convex, limiting bone growth. Thus, an extrusion method that is able to overcome this limitation and form concave pores is necessary before inkjet printing can be viable.

Similar to direct ink writing, a study of extrusion 3D printing conducted in 2021 found a novel printing technique to produce an HAp scaffold that was at low temperature, osteoconductive, and uniform. When creating their bioink slurry, the research team used a calcium phosphate cement (CPC) that was then dissolved with either a Polyvinyl butyral (PVB)/Ethanol (PVB/EtOH) solution or a PVB/Tetrahydrofuran (PVB/THF) solution. [25] During the ejection of the ink, the cement would harden into HAp when reacted with a nozzle containing sodium phosphate dibasic (Na_2HPO_4). Because of the small size of the nozzle (210 μm), the scaffolds produced were precise, higher resolution, and present promising avenues for creating specialized 3D scaffolds for individuals. When comparing the scaffolds between the EtOH- and THF-exposed cements, they found that the thickness and porosity of the former were greater, demonstrating that EtOH is a more promising dissolvent to maintain flexibility, as well as allowing water to penetrate the scaffold better, which makes more uniform HAp. Not only will this technique be applicable to tooth enamel, but it could also be applied to the skull and limbs by creating an artificial biosynthetic graft. Even though this study discovered a way to biomanufacture highly precise 3D scaffolds, research has yet to be conducted to identify the ideal scaffold properties to optimize HAp biocompatibility. Nonetheless, this extrusion process is a promising avenue for specific and biomimetic tissue.

CONCLUSION

Because of dental enamel's inability to regenerate after erosion through both extrinsic and intrinsic factors, the field of dentistry has looked for ways to re-harden the outer layer of teeth in a way that will retain hardness. Hydroxyapatite, the most prevalent compound found in enamel, has been artificially used to biomimic the crystalline structure of enamel, allowing integration into the tooth. In this review, various methods of manufacturing HAp were discussed. HAp-embedded toothpastes were presented as possible alternatives to fluoride toothpastes due to their nontoxic and hardening properties. For more extreme lesions and caries, however, more complex procedures may be conducted. Both submersions in a HAp powder and a hydrogel to imitate the amelogenesis environment are ways to immerse entire teeth to restore the loss of calcium and phosphate ions. On top of those methods, using a HAp sheet could be a way to form a flexible layer of fabricated enamel with less intervention on patients in clinical dentistry. Furthermore, three 3D biomanufacturing techniques are discussed: vat polymerization, inkjet printing, and extrusion printing. While these advanced techniques are the most specific and produce precise scaffolds, oftentimes they are more expensive and tedious. Further research should be conducted to create a scaffold that is flexible without sacrificing stability. More specifically, if high-precision scaffolds that are also easy to apply onto tooth enamel are manufactured, their likelihood of wider adoption in dental clinics is greater. With greater acceptance of HAp in restorative dentistry, enamel mineralization no longer has to rely on incompatible materials and may even present an avenue toward natural enamel regeneration.

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