Mena, 1:00
Team R01

ADVANCEMENTS IN ALZHEIMER'S RESEARCH THROUGH THE APPLICATION OF PLURIPOTENT STEM CELLS AND CRYOGENIC LAYERING IN 3D PRINTING

Avery Klein ajk162@pitt.edu, Abigail Wuenschel aaw56@pitt.edu, Emily Gorges elg83@pitt.edu

Abstract *- Recent advancements in cognitive degenerative disease research is being made possible by developments in both soft-tissue cryogenic layering in 3D printing and in directed development of induced pluripotent stem cells (iPSCs). Cryogenic layering is a technique whereby a layer of printed tissue is frozen before the next layer is added. The freezing and subsequent thawing of the layers provides a degree of stability which is otherwise difficult to attain in alternative soft-tissue printing techniques; this is critical when printing models of such soft and fragile tissue as is found in the human brain, as it prevents the collapse of the intricate structure as it is being printed. Specific cells can be integrated into these models throughout the printing process, and with directed differentiation of iPSCs, these specific cells can be a patient's own. Through introduction of certain genes, adult cells can be reverted to an embryonic-like state. These, in turn, can be redeveloped into neural cells via addition of key developmental proteins. Thus, cells as accessible as a patient's skin can be transformed into neural tissue for models of their brain.*

Such improvements in technology allow degenerative brain disorders, specifically Alzheimer's disease, to be studied with a depth and capacity which was previously impossible to attain. Modeling an afflicted patient's brain enables better identification of quantifiable medical signs that the disease is present, and furthermore, such models facilitate research into the stages of the disease which occur before there are noticeable symptoms in a patient. This research is sustainable in its simultaneous focus on both the needs of the present and the future, in terms of both health and quality of life. Ultimately, being able to diagnose the disease earlier allows for treatment before permanent brain degeneration occurs. The ability to study a model of a patient's brain allows for research which could not otherwise be done on the patient's actual brain, and this can potentially lead to better treatment for the millions of people living with Alzheimer's and other cognitive degenerative diseases.

Key Words - Alzheimer's Disease, Amyloid Plaque, Brain Model, Composite Hydrogel, Cryogenic Layering, Induced Pluripotent Stem Cells, Quality of Life

CURRENT PROBLEMS WITH ALZHEIMER'S DISEASE RESEARCH

Research into cognitive degenerative diseases has conventionally been difficult. As a complex and delicate organ, the brain must be treated with care. Coupled with the fact that the brain is inside a live patient, this means that researchers are necessarily limited in the scope of research they can safely do. They cannot directly interact with the brain outside of very invasive surgeries, and all actions taken during such an operation must be deliberate and safe, leaving no room for experimentation. And indirect models of the brain, such as those produced by MRI and PET scans, offer only an image, to be viewed and not interacted with. The only other option that allows for controlled experiments is working with a patient's brain post-mortem, and this has the readily apparent drawback of the cells no longer being alive. This only gives insight into the state the disease was in when the patient passed away and does not offer much in regard to researching the disease's interaction with live cells.

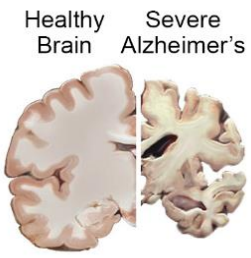

FIGURE 1 [1]
Damage of Alzheimer's Compared to Standard Brain

These problems have inhibited research into Alzheimer's, a cognitive degenerative disease. According to the National Institute on Aging's "Alzheimer's Disease Fact Sheet," Alzheimer's results in gradual irreversible destruction of a patient's memory and thinking skills, eventually leading them to be unable to perform simple cognitive tasks [1]. Yet the disease affects the brain long before these symptoms are readily observable, potentially over a decade prior. Abnormal protein buildup results in formation of amyloid plaques and tau tangles, and these buildups gradually cut off neural connection pathways and kill the cells. Figure 1 portrays the deterioration of a brain affected by Alzheimer's disease as compared to a brain in its typical, healthy state. As the disease progresses, the brain shrinks considerably. This is a direct result of the plaque buildup, which causes neurons to lose connection with one another and ultimately die. Only after the plaques and tangles have killed off a significant number of cells do the symptoms manifest, and without the symptoms, a diagnosis cannot be reached outside of a post-mortem assessment [1]. It is difficult to study how the disease begins and progresses due to this fact, since most cases of Alzheimer's are only identified years after its onset. And without the ability to perform controlled experiments on a patient's brain during the disease's progression, it can prove difficult to even understand, much less develop treatments for it.

Current advancements, however, offer potential solutions to many of these problems hindering Alzheimer's research. New 3D-printing technology allows for soft brain-like tissue to be stably printed into the form of a model, and this model can be populated with the patient's own cells through directed stem cell development. With a physical brain model, researchers can simulate Alzheimer's by inducing protein buildup, and can observe its progression in real time. The more significant benefit, though, lies in the fact that they can conduct experiments that they could not otherwise do on a live patient's brain. This, in turn, will not only help researchers understand the disease better, but it can also facilitate finding better treatment.

Such treatment could be directly applicable to patients already diagnosed with Alzheimer's, ameliorating symptoms or inhibiting the disease progression. This addresses present patients, but of equal importance is its prospective use in treating future patients as well: earlier diagnosis, subsequent earlier treatment, or even outright prevention of the disease are all possible results of more comprehensive research into Alzheimer's. Embodied in this is a principle of increasing significance in fields of scientific research, that being sustainability. Such research reflects sustainability in its focus on attending to present generations' needs while not detracting from the ability of future generations to address their needs. Beyond simple noninterference with future needs, though, such research proactively searches for solutions to these needs before they have even arisen. In order to sustainably advance this research, proper means of doing so require an appropriate apparatus. A soft-tissue brain model can act as the intermediate in conducting research on Alzheimer's disease progression using pluripotent-stem-cell-cultured neural tissue.

APPLYING CRYOGENIC LAYERING TO PRODUCE 3D-PRINTED MODELS

Printing soft tissue is a relatively recent advancement in the field of 3D printing. In an article discussing the applications of 3D printing in the medical field, C. Lee Ventola explains that 3D printing technology has been conventionally used to create products like prosthetics, orthopedic implants, pre-surgical models, and human tissue. By using a computer-designed file and by providing ink, biomaterials, plastic, powder, and other product-specific materials, 3D printing allows these devices and structures to be printed layer by layer to form a 3D formation [2]. This technology has advanced so that a particularly intricate design can be printed with great precision. Specifically, such detailed printing can be used to form a complex organ like the brain.

Conventional Printing vs. Cryogenic Layering

A structurally sound and compositionally-similar model as that to an actual brain has, until recently, been difficult to replicate. According to Zhengchu Tan et al., past methods for creating 3D brain models have included cast molding, but due to hollowed formations within a brain, it proved difficult to successfully achieve such detail using the cast molding technique [3]. Given the narrow hollowed inner portions of tissue within a brain, accurate molding of these parts on such a precise scale is challenging. Additionally, in an analysis of 3D-printed brain models, Caitlin C. Ploch et al. underline that the use of such molding and forms of gelatin allows for mechanical properties, including rigidness, tactility, and flexibility, of the model to better match that of the brain [4]. However, using a 3D-printed mold does not hold the same precision as that of a 3D-printed model. A fully printed brain structure allows for increasing precision as each layer is applied, using varying ink speeds and biomaterials. Typically, these fully 3D-printed models that do not rely on the molding process have properties too dissimilar to the brain. Such models, even though they are anatomically accurate, are much more structurally rigid than an actual brain. Furthermore, these models face deformation as the weight increases with each layer added, decreasing structural precision [4]. While completely printed brain models offer greater precision than molded models, molded models allow the composition of the model to be much more favorable in comparison. An ideal model incorporates both similar mechanical properties to an actual brain and similar physical appearance of the intricate structure.

The application of cryogenic layering makes this challenging task of creating such a model possible. This innovation, in Tan et al.'s research, allows a composite hydrogel, similar in stiffness to that of the brain, to be used to print a layer of tissue-like structure. After being formed, this layer is frozen upon contact with the base using liquid nitrogen in an isopropanol bath, which maintains the cold temperatures necessary to keep the printed figure frozen. Another layer is then stacked upon the prior layer, which is once again frozen upon first contact. As the number of layers increases, the time it takes for each additional layer to freeze also increases. To account for this and to prevent premature thawing, the composite hydrogel ink release speed is adjusted accordingly. The structural design allows for deposits of water in the formed model to maintain the level of hydration necessary and uphold its structural integrity when frozen. Hence, by freezing each layer, the delicate structure containing inner hollow portions is able to maintain its shape as more layers are added. Without this process, the weight of each subsequent layer would cause the model to collapse, destroying the fragile layers below it [3]. The application of cryogenic layering achieves what other brain models have failed to do. The process prevents collapse and upholds structural integrity while it also allows for the model to simulate the brain's delicate compositional make-up with the implementation of the correct biomaterial. In Figure 2, one such 3D printed brain using cryogenic layering can be seen in both its fully frozen and thawed states. The leftmost image illustrates that the freezing application maintains the model's complex formation after the structure is completely constructed. Once thawed, the rightmost image depicts the structure in its unique state of elasticity.

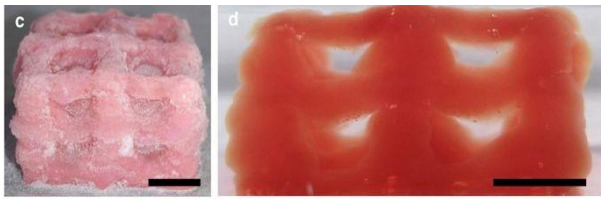

FIGURE 2 [3]
Cryogenically printed brain form using biomaterials

Material Compatibility

A model of such structure can be assessed to confirm its similarities to the brain's properties. In Antonio Forte's dissertation on brain tissue biomechanics, he discusses several procedures to test this material comparability. These tests include compression-relaxation, which shows stress over time with constant strain; cyclic indentation-relaxation, which shows stress over time with strain applied at constant intervals; rheometric tests, which show viscoelastic behavior; and oedometric tests, which show permeability of the tissue [5]. Performing each of these procedures on a material and comparing the results to the results of the brain is useful in order to assess which materials are most appropriate to simulate brain tissue behavior.

In research conducted by A. Forte et al., the use of composite hydrogels in the 3D printing process of a brain model is investigated on the basis of Forte's previous research. One such composite hydrogel used consists of polyvinyl alcohol (PVA) and phytagel (PHY). By using a concentration ratio of 6 percent PVA to 0.85 percent PHY, the polyvinyl alcohol and phytagel "could be combined to form a stable coupled network with porosity, elastic, and viscoelastic properties representative of the brain" [6]. Consequently, this composite hydrogel can be used in the cryogenic printing process to form a model with properties, such as stiffness and porousness, similar to that of a human brain. This allows the 3D printed structure to accurately model its soft tissue-like composition. Typically, this type of composite hydrogel would have to go through a cycle of freezing and thawing. This would be necessary because bonding must form between the materials so that the composite hydrogels properly reach and maintain the brain-like consistency. However, by using the cryogenic layering process, composite hydrogel bonding can occur in one step as the process continues rather than a freezing and thawing cycle [3]. The utilization of composite hydrogels to copy the human brain's tissue in the 3D cryogenic layering printing process successfully forms an apt brain model that can be used for research.

Application of the Model in Housing Living Cells

To provide a suitable environment for living cells, a brain model must have a composition like an actual brain where the cells can develop and tissue can be formed. Using the aforementioned composite hydrogel consisting of polyvinyl alcohol and phytagel, the cryogenically layered model can properly accommodate living cells. Tan et al. detail that by applying a collagen coating, the survival of cells was near 97 percent. This shows that the composite hydrogel, with additional proteins, establishes a sufficient environment that can be populated with cells. Additionally, the collagen-coated composite hydrogel allowed for the cells to be applied consistently, meaning the application did not cause any abnormal clustering of cells [3]. Cell viability and compatibility to the biomaterial are critical when creating an appropriate 3D brain model for research purposes.

When utilizing a brain model for research and experimental purposes, the model must be characteristic of an actual brain so that effective research can occur. A structure that is too rigid or dissimilar would not provide a model that can simulate the brain's environment, which is necessary to conducting research where the model must house human cells and allow a neural tissue complex to be formed. The cryogenic layering process allows a model that is necessarily

similar in both structure and stiffness to the brain. The process prevents structural collapse and allows for the complex brain matrix to be accurately formed while the composite hydrogels mimic the brain.

INDUCED PLURIPOTENT STEM CELLS TO SIMULATE BRAIN TISSUE

A Brief Overview of Pluripotent Stem Cells

Once cryogenically printed, the fully formed brain matrix can be populated with the patient's own cells, without extracting brain tissue, using induced pluripotent stem cells (iPSCs). iPSCs are a type of stem cell which can be produced from any adult cell, even from something as mundane as skin. In an article discussing the potential applications of iPSCs, C. Goldthwaite explains that through introduction to genes pivotal to initiating pluripotency, they can be reverted into an embryonic-cell-like state, and from there, they can be induced into developing into any other type of adult cell via addition of genes characteristic to that specific development [7]. Figure 3 outlines the cellular process of transforming a developed somatic cell into its undeveloped form. Here, a virus acts as the vector of reprogramming information necessary for the cell to revert to this state. Once in this new form, these now pluripotent stem cells can become any type of cell when exposed to specific differentiation factors.

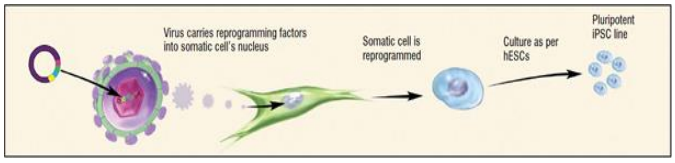

FIGURE 3 [7]
Simplified Induced Pluripotent Stem Cell Process

Notably, this allows for production of neural tissue. This tissue can then be used in models to analyze functionality and progression of neurological diseases such as Alzheimer's. The significance of this becomes apparent when contrasted with other methods currently used for conducting research into such diseases. As Cantley et al. state, "commonly used brain imaging techniques such as fMRI and EEG are unable to attain single-cell level of resolution even when used in combination… More involved techniques, such as the implantation of neurotrophic electrodes, are able to provide information at the single-cell level but require invasive procedures" [8]. Aside from those techniques, much of what humans know about the brain is from animal models, which while useful in obtaining a general understanding, are imperfect because human brains are far more complex and respond to disease differently than animal brains.

Brain models seeded with iPSC-derived neural tissue circumvent many of these problems: one can obtain a single-cell level of resolution, without invasive procedures on a patient, and can get an understanding of how cognitive diseases affect actual human brains, rather than how they may possibly affect them, based on observations of animal brains. Such models are not without their drawbacks, as the neural networks they house may not be fully consistent with those of the brain, but they nonetheless remain a promising advancement in the field of neurological study.

Techniques Assessing iPSC Viability

Techniques to produce neural cells are still in development, but some methods have shown success thus far. One study by Frega et al. rapidly generates mature neurons in 3 weeks, with upwards of 95 percent conversion efficiency of transduced cells [9]. The protocol uses a process known as lentiviral transduction, which utilizes a virus's ability to transfer genetic information to a desired cell in order to modify and reprogram its genetic composition. This procedure is applied to human iPSCs obtained from skin fibroblasts, which are reverted to an embryonic-cell-like state using the lentivirally-transduced reprogramming factors cMYC, SOX2, OCT2, and KLF4, all genes necessary in achieving cell pluripotency. Then, lentiviral transduction is again used to inject and expose the cell with rtTA (reverse tetracycline-controlled transactivator) and Ngn2 (neurogenin-2), beginning the transformation into functional neuronal cells. Thus, this process converts adult human skin cells to pluripotent stem cells, and then directs the stem cells into neural tissue development. Rat astrocytes also played a role in the protocol, as they "actively contribute to the refinement of developing neural circuits by controlling synapse formation, maintenance, and elimination" [9]. As a result, these astrocytes provide the links necessary to allow for proper neural functioning. The study goes on further to evaluate the electrical functionality of the neurons, with results showing that, "[A] few weeks after the induction of differentiation, the neurons derived from healthy-control [human] iPSCs formed functionally active neural networks, showing spontaneous events" [9]. This demonstration of spontaneous electrophysiological activity suggests that these iPSC-derived neurons can, to a certain extent, mimic the electrical behavior of human neural networks.

Other research further substantiates this claim. A similar study done by Cantley et al. constructed a 3D brain model utilizing polyornithine and laminin coated scaffolds, which were then seeded with iPSC-derived neuronal cells. In regard to electrical activity, they state that "The presence of both spontaneous and inducible activity was observed by 10 weeks, indicating the presence of healthy, functioning neurons… The 3D tissue models continue to be functional out to 9 months (ongoing)" [8]. They also make note of the fact that the electrical responses were partially blocked using

receptor antagonists, which they say "[indicates] the presence of functional glutamatergic neurons within the model" [8]. The findings in this study are consistent with those of Frega et al. in that the iPSC-derived neuronal cell networks exhibit the electrical functionality and activity akin to that of the human brain.

The similarities between the generated neural tissue and patients' brain matter open up many applications. Cantley et al. speculate that the cells "could be used to uncover early stage biomarkers of the disease state, in turn supporting earlier diagnosis and improving understanding of disease progression." They also mention its potential in determining drug targets for neurodegenerative diseases [8]. Investigating into these proteins inherently involved with Alzheimer's is one method of finding treatment, since it identifies what potential treatments may need to affect in order to produce their therapeutic effects.

Both studies make note of potential drawbacks, however. Frega et al. highlight the procedure's reliance on rat astrocytes, which detract from the neuronal cell network's capacity to accurately represent a strictly human network [9]. It is a distinct possibility that rat cells being used to guide the development of the neural circuits may do so in a manner contrasting human astrocytes. Cantley et al. similarly mention potential variation factors, specifically that "when working with stem cells even under perfect conditions, the neurogenic nature… generates a variety of neural subtypes as well as astrocytes… this results in a complex network organization" [8]. The complexity of the networks, while in a sense representative of the complexity of the brain, nonetheless can introduce a level of factors which cannot be controlled for in some experiments.

Personalized Treatment for Alzheimer's

Using pluripotent stem cell generation techniques, new cells are produced which are genetically identical to the original cells. This is especially important when considering Alzheimer's disease research, given that certain individuals display a genetic predisposition to the disease [1]. The relative sameness of the cells should enable a more consistent modeling of Alzheimer's progression, as inducing it into cells known to be susceptible eliminates a degree of variability. This extends beyond simply ensuring that the model's cells are susceptible to Alzheimer's, though; using the patient's own cells can enable personalized treatment, something which has already seen use when dealing with other illnesses and diseases. According to Mukesh Verma's analysis of cancer treatments, "information about a patient's proteinaceous, genetic, and metabolic profile could be used to tailor medical care to that individual's needs." Furthermore, these can also be used to assess risk factors for other conditions and develop preventative treatments [10]. While propagation of plaque masses in Alzheimer's are not entirely analogous to propagation of cell masses in cancer, the two types of diseases both have genetic links, both progress in stages, and both progress at different rates based on the patient. As such, considering the success of personalized treatment for cancer patients, it could likewise be applied for the purpose of treating Alzheimer's patients.

CREATING WORKING MODELS OF THE BRAIN

Cryogenic layering, in conjunction with pluripotent stem cell application, can enable the production of a working model of a human brain. First, the soft-tissue structure itself is printed layer-by-layer, with stability being offered from the freezing at each step. At the end of the printing process, it is thawed, and retains its structural integrity. At this point, neural cells produced from pluripotent stem cells can be seeded in the structure to populate its brain-like matrixes with neural tissue. Once the model is fully populated, Alzheimer's disease can be induced by overexposure to certain proteins.

Inducing Alzheimer's

The use of one such protein has already successfully induced the disease in mice. In an overview of transgenic mouse models for disease research, G. Elder et al. discuss information and techniques surrounding such research for Alzheimer's. Notably, they mention that the amyloid plaques and tau tangles responsible for the degradation of brain matter and the loss of neural communication network connectivity in Alzheimer's have well-defined constituents. ß-amyloid peptides and hyperphosphorylated forms of neurofibrillary (tau) tangles make up the plaques and tangles respectively, and their buildup can be induced by overexposure to the amyloid precursor protein (APP) [11]. When this technique was used on transgenic mice, it was observed that the use of APP induced protein buildup pathologically similar to, and induced symptoms in the mice clinically similar to, buildups and symptoms of Alzheimer's patients [11]. The introduction of APP to the mice's brains successfully causes the disease to both begin development and progress through its stages.

The success of this experimental procedure in inducing Alzheimer's in mice suggests that the process can be likewise implemented in an external brain model. If the transgenic mice, who have genetic material from humans, can have the disease induced in them, then it follows that similar exposure to APP could induce the disease in a brain model constructed with human cells. With a model that mimics the structure and composition of the brain, which is populated with living neural tissue, and which can be afflicted with Alzheimer's in a similar manner to humans, researchers can potentially overcome the problems which plagued research on the disease in the past. They would no longer be constrained by the limitations imposed on them by working with a live patient. Not only would researchers be able to have a visual

representation of the disease, which they already have to a certain extent from MRIs and PET scans, but they can also observe how the cells physically respond during the disease progression. Controlled experiments can be performed at any stage, and this offers a flexibility of research that is simply not feasible with a live patient.

Specifically, the preliminary preclinical stage of the disease can be studied before symptoms are even observable, which is arguably the most important stage to be able to identify and work with. During the preclinical stage, lasting damage has not been fully inflicted on the brain [1], and if any treatment is to be found, this is the most important stage to do so, such that the patient suffers the fewest long-term adverse effects of the disease. Study of this stage can allow researchers to potentially identify pathological links that could enable earlier diagnosis, which would improve upon the current symptom-based diagnosis. Study of other stages after the onset of symptoms is also significant, because it facilitates the search for treatment for the patients whose Alzheimer's has already caused cognitive damage. In this way, though the disease cannot be prevented outright, its detrimental effects can be limited.

The Potential of Experimental Brain Models and Their Drawbacks

Although the presence of amyloid plaques and tau tangles in the brain begins in the early stages of Alzheimer's, it is difficult to discover these internal plaques. However, recent studies have shown that the immune response to this foreign buildup can be potentially significant in early diagnosis of the disease. In an analysis of neuroinflammation in response to Alzheimer's disease, Frank Heppner et al. elucidate that "immune activation in AD [Alzheimer's disease] has the capacity to facilitate and trigger the pathophysiology of AD" [12]. This means that the plaque associated with Alzheimer's can initiate an immune response, which influences disease progression. The fault of the immune system action is that myeloid cells, key components of said response, have been connected to increasing progression of the disease by being the driving force in inflammation [12]. More research would be necessary to determine how to control the potential negative effects of this response. Consequently, "interfering with neuroinflammatory pathways and molecules requires precise knowledge about the underlying immune events - which may change during the disease course" [12]. However, this can be made possible with a brain model using cryogenic layering and induced pluripotent stem cells. The significant link between a brain model populated with live cells and researching immune response is that this model allows the full progression of the disease to be studied. In turn, this provides the potential for early indicators of the disease to be well-defined. These indicators are essential in concern to treatment of Alzheimer's in its earliest stages, where it has the opportunity to be most successful in halting disease progression.

Although these brain models have significant potential benefits to Alzheimer's research, there are a few notable drawbacks that the models present. Since the 3D printed brain is an isolated entity, researchers would only be able to study the impact of Alzheimer's disease on neural cells and would lack information on how the disease affects the rest of the body: information that could likely assist in the development of a treatment of the disease. In addition to this isolation, the models would lack the environmental factors that may potentially contribute to the progression of the disease, such as diet and physical activity [1]. Therefore, only genetic factors that influence the development of the disease could be studied. This would be disadvantageous because the environment of the patient is believed to have a considerable influence on the advancement of the disease, in addition to genetics. Furthermore, the 3D-printed brain models are simply models. They mimic the composition and behavior of a brain however, they are not themselves an actual brain. Conducting research on a model rather than an actual brain could lead to variations of the effects and progression of the disease compared to the actual brain of a patient. It is finally important to note that research on neural diseases using brain models is largely in its infancy, and as such, its commercial and economic viability is difficult to accurately assess. It will take further investment and development for this technology to reach a point at which such an estimate can be determined, and possibly even more development to be affordable for patients. Despite their indisputable flaws, the brain models are potentially the best possible way to obtain research on cognitive degenerative diseases next to conducting research on a live patient's brain which is controversial and invasive.

As a result of its unintrusiveness, the outlined research procedure has the capability to not only positively impact patients directly, but also account for Alzheimer's treatment in the future. These methods are sustainable, which, as defined by the United Nations Brundtland Commission, means that they "[meet] the needs of the present without compromising the ability of future generations to meet their own needs" [13]. Once developed, the treatment itself will be sustainable, in the fact that it is treating current patients while also laying the groundwork for treating future patients. Earlier diagnosis, personalized treatment, and/or preventative treatment will only be attainable in the future based on the groundwork research established in the present.

THE POTENTIAL OF FUNCTIONAL BRAIN MODELS FOR RESEARCH

The intersection of printing advancements and stem cell applications is significant in laying the groundwork for furthering the development of future research. These preliminary experimental models allow for more controlled,

comprehensive research on Alzheimer's in each specific patient's case, since the models use the patient's own cells. Furthermore, by using the 3D printed brain models, researchers are able to gain a better understanding of the brain's structure and of how cognitive degenerative diseases affect that structure; this can help to determine how to treat patients with Alzheimer's disease. In this way, the models allow for research results to be obtained in a safer way and pose less risk to the patient because research can be conducted on a copy of the brain without the patient being involved. Not only is it safer, it is also more convenient for researchers and less intrusive for the patient, as they are not required to frequently meet each other in order to conduct such research.

Since the brain models are able to be observed more closely, researchers can potentially find more concrete perceptible physical and behavioral alterations to a brain afflicted with Alzheimer's, which gives insight into the current state of the disease as opposed to only being able to study the diseased brain after the death of the patient. These observed alterations of the brain could then be studied and potentially be used for diagnosis in future patients, rather than relying on subjectively observed symptoms, such as memory problems and impaired judgment. When these symptoms appear, it is an indication that the disease has already progressed and inflicted significant damage to the brain [1]. At this point, even if better treatment is found down the line, little can be done, as the damage is permanent. This irreversible damage means that once neural connection is lost in specific parts of the brain, as a result of the plaque buildup, the neurons in the affected areas fail to have the ability to send and receive signals.

Future patients with Alzheimer's and other cognitive diseases, because of this developing technology of 3D printed brain models, may receive earlier, better treatment, and if the disease is diagnosed in its earliest stages, then the majority of cognitive damage can potentially be averted. Being able to better research and potentially attain and provide treatment for this distressing disease can significantly improve the quality of life of Alzheimer's patients; not only will slowing the progression of the disease lengthen lifespan, due to its eventually terminal nature, but it will allow the patient to maintain fuller cognitive function for longer as well. As a result, they can maintain their independence for longer, not needing to completely depend on others for everyday activities.

The improvements to quality of life extend far beyond just the patient with Alzheimer's. Their family, too, has to cope with watching their loved one slowly mentally deteriorate, to the point of not even knowing family members' names. By slowing the progression of the disease, they will have more time to enjoy life with the patient in the future. Family members also often function as caregivers for patients, which can place heavy mental, emotional, financial, and time-related burdens on them, so the patient maintaining their independence for longer improves both their own quality of life and that of those who will eventually take care of them [1]. In these ways, the effects of the treatment are sustainable in that the quality of life of those diagnosed is improved in the present, and the quality of life of those close to them is improved both in the present and in the future. Moreover, this technology has the potential to mitigate or even halt the progression of an insofar-irreversible disease, and possibly other cognitive degenerative diseases, that affect millions of people worldwide.

SOURCES

[1] "Alzheimer's Disease Fact Sheet." National Institute on Aging. 8.17.2016. Accessed 1/24/2019. https://www.nia.nih.gov/health/alzheimers-disease-fact-sheet.

[2] C. L. Ventola. "Medical Applications for 3D Printing: Current and Projected Uses." Pharmacy and Therapeutics. 2014. Accessed 3/2/2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4189697/.

[3] Z. Tan, et al. "Cryogenic 3D Printing of Super Soft Hydrogels." Scientific Reports. 11.24.2017. Accessed 1/13/2019. https://www.nature.com/articles/s41598-017-16668-9.

[4] C. Ploch, et al. "Using 3D Printing to Create Personalized Brain Models for Neurosurgical Training and Preoperative Planning." World Neurosurgery. 2016. Accessed 3/2/2019. https://www.sciencedirect.com/science/article/pii/S1878875016003260.

[5] A. Forte. "Brain Tissue Biomechanics: New Tissue Phantoms, Mechanical Characterisation, and Modelling Strategies for Enhanced Surgical Procedure." 2015. https://spiral.imperial.ac.uk/handle/10044/1/48062.

[6] A. E. Forte, et al. "A Composite Hydrogel for Brain Tissue Phantoms." Materials & Design. 12.15.2016. Accessed 1/17/2019. https://www.sciencedirect.com/science/article/pii/S0264127516312370.

[7] C. Goldthwaite. "The Promise of Induced Pluripotent Stem Cells (iPSCs)." National Institutes of Health. 2016. Accessed 1/24/2019. https://stemcells.nih.gov/info/Regenerative_Medicine/2006Chapter10.htm.

[8] W. L. Canley, et al. "Functional and Sustainable 3D Human Neural Network Models from Pluripotent Stem Cells." ACS Biomaterials Science and Engineering. 10.1.2018. Accessed 1/15/2019. https://pubs.acs.org/doi/10.1021/acsbiomaterials.8b00622.

[9] M. Frega, et al. "Rapid Neuronal Differentiation of Induced Pluripotent Stem Cells for Measuring Network Activity on Micro-electrode Arrays." Journal of Visualized Experiments. 1.8.2017. Accessed 1/24/2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5407693/.

[10] M. Verma. "Personalized Medicine and Cancer." Journal of Personalized Medicine. 2012. Accessed 3/6/2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4251363/.
[11] G. Elder, et al. "Transgenic Mouse Models of Alzheimer's Disease." The Mount Sinai Journal of Medicine. 1.25.2010. Accessed 3/3/2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2925685/.
[12] F. Heppner, et al. "Immune Attack: The Role of Inflammation in Alzheimer's Disease." Nature Reviews Neuroscience. 5.20.2015. Accessed 3/5/2019. https://www.nature.com/articles/nrn3880.
[13] G. H. Bruntland. Chapter 2. Our Common Future: Report of the World Commission on Environment and Development. 3.20.1987. Accessed 3/26/2019. http://www.un-documents.net/ocf-02.htm.

ADDITIONAL SOURCES

R. Parimi. "A Beginner's Guide to Lentiviral Transduction." Bitesize Bio. Accessed 3/6/2019. https://bitesizebio.com/41748/a-beginners-guide-to-lentiviral-transduction/.
T. Pultarova. "Scientists 3D-Printed Squishy, Brain-Like Tissue for the First Time." Live Science. 1.13.2018. Accessed 1/13/2019. https://www.livescience.com/61416-3d-printed-brain.html.

ACKNOWLEDGEMENTS

We would like to thank Beth Bateman Newborg for guiding us through the writing process by giving us a directed path to follow and for helping us to narrow the subject of our topic. We would also like to thank Judy Brink for helping us solidify our approach for integrating sustainability into our paper. Finally, we would like to acknowledge the Benedum library for giving us an encouraging space to research and work on our paper.