Human Stem Cell-Derived Organoids Address Top Medical Leading Causes of Death in The US

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Received December 31, 2023
Accepted April 22, 2024
Electronic access May 15, 2024

Organoids are independent research tools synthesized from induced pluripotent stem cells (iPSCs) which can model various body systems and diseases in an *in vitro* setting, as well as resemble parent organ structures and functions. Organoids contain multiple cell types of the *in vivo* counterpart, they organize similarly to *in vivo* tissue, and they function the parent organ. Described as mini organs in a dish, organoids are kept in 3D cultures and have successfully modeled diseases without cures. Cancer, Alzheimer's Disease (AD), and Diabetes Mellitus are 3 of the top 7 leading medical causes of death2 and have been modeled using organoids. For cancers, tumor organoids can model stomach, colorectal, liver, pancreatic, prostate, and breast cancers. Cerebral organoids can be used to model Alzheimer's Disease pathologies, such as neurofibrillary tangles and beta-amyloid plaques, which have been a challenge to model in the past. Islet organoids used for modeling diabetes have grown to be the same size as their *in vivo* counterparts, making them easier to examine and study. While there are many benefits to using organoids to model these diseases, some limitations exist, including that organoids are still rough representations of what they are modeling and are hard to grow and maintain in a lab setting. Regardless, organoids have potential since they model *in vivo* systems more accurately than other 2D and animal models. The impact of organoid research is seen in drug screening, immunotherapy, and personalized medical treatments.

Introduction

Organoids, or mini organs in a dish, are 3D cultures of cells that mimic the cell types seen *in vivo* organs and have shown promise in disease modeling, drug screening, and regenerative medicine approaches for diseases affecting numerous organ systems. Cancer, Alzheimer's Disease (AD), and Diabetes Mellitus (DM) represent 3 of the top 7 leading causes of death in the United States and are representative pathologies in which organoids have been used[1]. The three diseases are interrelated in that if one is contracted, then the others might show up as symptoms or side effects. They are also shown to affect people of the same age group more than others. An example of a specific correlation between Alzheimer's and diabetes is that hyperinsulinemia from Type 2 diabetes is related to amyloid-beta production in the brain, a common cause of Alzheimer's and dementia because the body is competing for an insulin-degrading enzyme which causes disrupts the brain's ability to clear up the forming plaques[2]. Scientists currently have no standard or definitive cure for these diseases, highlighting the need for models that accurately replicate the complex physiology behind these correlated diseases. Cancer is a disease affecting over 2 million people in the US[3]. It is characterized by uncontrolled growth of cells that can spread (metastasize) throughout the body and impact other organ systems. Alzheimer's Disease, also known as senile dementia, is a form of dementia that slowly destroys memory, cognition, and the ability to carry out simple tasks. More than 6 million Americans are diagnosed with Alzheimer's today[3]. Diabetes Mellitus currently affects 37 million patients, mostly over the age of 45, and many younger Americans as well[4] by causing hyperglycemia (elevated blood glucose level) which in turn damages multiple tissues and organs.

The most common models for testing treatments to address these diseases are 2D cell cultures and animal models. Unfortunately, 2D cell cultures have been shown to lack maturity compared to 3D models and are typically limited to one cell type, lacking the complexity of multiple cell-type interactions. The problem with animal models is that they don't always accurately represent human physiology, and there are ethical concerns regarding their use. Scientists are researching regenerative medicine approaches using differentiated human stem cells to model different organ systems. This approach uses cell types found in the desired organ of study and starts to mimic the complexity of these organ systems.

Stem cells are undifferentiated, or "unspecialized," cells with two main properties: self-renewal by creating two daughter stem cells and the ability to differentiate into many different cell types. Extracellular signaling pathways control stem cell differentiation. Due to extensive evidence underpinning the molecular mechanisms that regulate stem cell fate, refined methods are widely used in a research setting *(in vitro)* to understand how these cells, which have unlimited potential, make cellular decisions. By doing this in a 3D culture, stem cells can generate organoids. Cells are put into the 3D cell matrix, and stem cells

grow very efficiently and easily self-organize into larger tissues and structures. This process is much more efficient than current models.

Organoids have enabled researchers to model different organ systems using human stem cells, which contain cell types found in the desired organ of study and begin to mimic the complexity of these organ systems. Organoids are flexible because they can be expanded, cryopreserved, genetically modified, and remain genetically and phenotypically stable. Additionally, these organoids can be used to model each disease with direct impacts on drug discovery and future implications for regenerative medicine approaches (See Figure 1 below). Organoids' ability to model disease mutations better allows them to represent the *in vivo* conditions (conditions in a living organism) of organs. This is because they contain many cell types of the *in vivo* counterpart (the many types of cells are kept in co-culture so that they interact the same way outside as they do inside the body). This causes cells to organize into tissues based on their cell type the same way they would *in vivo*, and the cells in the organoid act the same way their parent organ would. Organoids have many specific advantages in the industry today. First, organoids are human-derived and can recapitulate human physiological systems. The second is high efficiency, because organoids are quick and relatively easy to establish. Third, organoids show stability in all aspects during drug screening. Lastly, organoids can be generated from individual tissues or cells, contributing to the realization of precision diagnosis and treatment. These advantages provide that organoids are huge assets that possess a wide range of biomedical applications. These features further prove that organoids can mimic the development of organs *in vitro*, which can be used to model and study the mechanisms of organ-specific genetic diseases. For example, islet organoids are used to model insulin secretion-related genetic disorders like Wolfram Syndrome and congenital hyperinsulinism. They can also be used to model infectious agents and how pathogens spread, metabolic disease, cancers, drug research, direct medicines, and regenerative medicine, all of which are areas of research that aren't as successful using other means of study[5]. Some potential limitations of organoids also include the lack of accessibility to organoids because they are relatively new, it's harder to repeat procedures and get the exact same results and organoid cultures are often different from one batch to the next because identical cells in the human body rarely exist. This review will focus on what human induced pluripotent stem cells (iPSC)-derived organoids are, how they are synthesized and how they can be used to model and develop regenerative medicine approaches for cancer, Alzheimer's Disease, and Diabetes Mellitus.

Previous Models and Organoids

For decades, medical research has relied on 2D ell lines and animal models to study cellular and molecular mechanisms of pathogenesis. These approaches have contributed to a better understanding of biology and pathophysiology of human diseases and have uncovered aspects of development and differentiation and cellular behaviors of diseases. Animal models have also led to more complex dimensions of understanding and tremendous breakthroughs allowing for many therapeutic interventions in human disease.

However, most of the drugs that were developed with animal models had limited success in clinical trials because of the lack of models that can fully model human biology. These 2D cultures suffer disadvantages associated with the loss of tissue-specific architecture, mechanical and biochemical cues, and cell-to-cell and cell-to-matrix interactions making them relatively poor models to predict drug responses for certain diseases. Furthermore, animal models used in preclinical research are based on inbred strains where all test subjects are genetically homogenous liking the diversity of human genetics. This has led to the alternative of human 3D cell cultures, referred to as organoids, which can be maintained and followed for extended time periods due to their self-renewal and differentiation capabilities. They can model various organ systems in the body, and they have unforeseen modeling and studies recapitulating the development of human tissues and diseases. A major advantage is the ability to model patient specific disorders and use them to screen for drugs and treatments by expediating personalized medicine applications and drug screening[6].

For instance, compared with 2D culture, colon cancer HCT-116 cells in 3D culture have been found more resistant to certain anticancer drugs. The accelerating implementation of 3D cell cultures in early drug discovery is fueled by the need to continuously improve pharmaceutical research and the use of organoids would allow greater predictability of efficacy and toxicity in humans before drugs move into clinical trials. Besides organoids, other 3D cell cultures include spheroids, scaffolds and hydrogels, organs-on-chips, and three dimensional bioprinting.

In drug discovery, the complex process has been decreased using organoids by helping with target identification and screening. They have promised to bridge the gap between 2D culture and have been used to understand mechanisms of different diseases. 3D cultures have also been able to detect architecture transformation of multiple types of tissue, especially when it comes to diseases like cancer where cells change from normal to malignant. They also allow for CRISPR/Cas9 editing which is why many genetic disorders that were once difficult or impossible to model in animals and 2D cell cultures can now be modeled using organoid cultures through the introduction of mutations to the organoids.

Target identification and validation is often the rate-limiting step in preclinical drug discovery. 3D cultures can discover mechanisms and targets to accelerate target identification. In addition to this, organoids can do subcategory work with efficacy profiling, toxicity profiling, and pharmacokinetics profiling. In

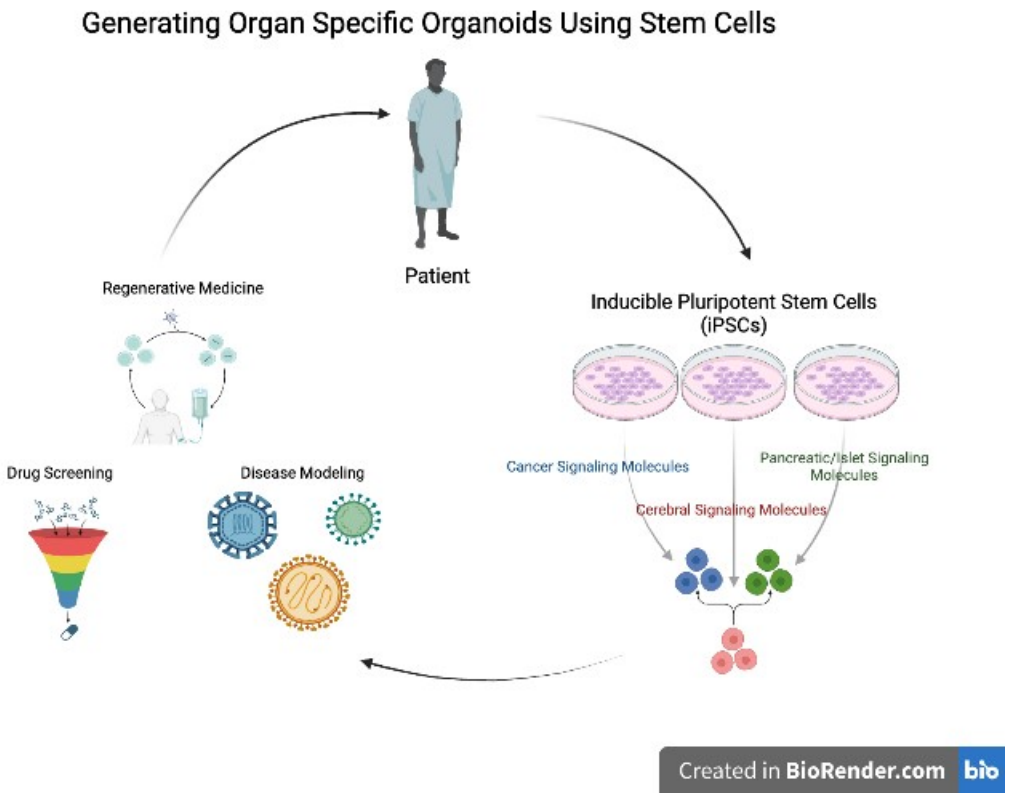

Fig. 1 Generating organ-specific organoids using stem cells. Induced pluripotent stem cells extracted from patients can be differentiated into different organoid types based on the activation of different cell signaling pathways. These organoids have the potential for applications in drug screening, disease modeling, and regenerative medicine.

addition, organoids can be used for transplantation, a process known as regenerative medicine, which is a process that 2D cell cultures can't go through. An example of this is making a 3D structured kidney with all the same properties as the *in vivo* counterpart making it easier for organ transplantation[7].

Diseases

Previous Cancer Models

Cancer research has advanced over the years, and diagnostic technologies have continued to improve. However, many obstacles are yet to be overcome to cure the growing number of patients affected by this disease. Many different types of cancer affect various organs throughout the body. The number of cancer deaths may be prevented through early detection or preventative measurements, but finding and testing viable treatments is difficult due to the complexity and differences between cancer types. Current cancer and drug testing models such as 2D cell line cultures and patient-derived tumor xenografts (PDTXs) don't do as good of a job at modeling the disease, causing the potential cures to either not be effective or have adverse side effects on the patient. While 2D cell cultures have led to many breakthroughs, they tend to lack maturity or the ability to model complex features compared to their *in vivo* counterparts. They are inefficient in modeling vital body systems such as the immune system, the tumor microenvironment, stromal compartments, and organ-specific functions. Many of these inabilities are due to limitations in PDTXs and their features.

Some disadvantages of PDTXs are that they lack the genetic heterogeneity in their tumor models. For many years, have been consuming money, time, and resources1. Drawbacks in current cancer models are often because generating the models themselves is a very inefficient process that involves an extensive selection of cells to use for the 2D *in vitro* conditions. Since the used cells are very selective, they have often already undergone enough genetic change and can no longer model the original disease. These poor cancer models lead to drug treatments working well on the cancer models but ultimately failing during a clinical trial. The current use of animal models as tumor models doesn't capture the pathogenic processes in the human body; for example, the histological complexity and genetic

heterogeneity of human cancers aren't reflected in mouse models for human cancer[8]. For these reasons, it's essential to switch to a new, refined method of modeling and testing.

Organoids for Cancer Models

Stem cell-derived tumor organoid models are 3D models generated from induced pluripotent stem cells (iPSCs). Tumor organoids have been created for stomach, colorectal, liver, pancreatic, prostate, and breast cancers. These tumor models express the different cell types and markers present in the tumors. Before using iPSCs, most organoid studies in the cancer field relied on adult stem cells (ASCs). However, iPSCs are a high throughput method for generating tumor organoids. CRISPR mutagenesis technology has also been applied to iPSC-based organoids to generate cancer-causing mutations and model brain tumors[9]. Cancer arises through a sequence of mutations which leave specific signatures, defined by a systematic analysis of mutation characteristics across many independent cancer sequencing data sets. A study shows that a set of mutational signatures in genome wide mutation collections of breast cancers predicts deficiency of BRCA1 and BRCA2 and a sensitivity to PARP. However, organoids have made the process to simultaneously depict tumors with unstable genomes a lot easier with the long-term expansion of epithelial tissue starting from a single adult stem cell. Organoids can also be modified using CRISPR-Cas9 Genome editing, and the study specifically uses it on human intestinal organoid cultures to decipher the mutational consequences of DNA repair deficiency. To dissect mutational signatures in human organoid cultures, there is a loss of function mutation in the MMR gene MutL homolog 1 (MLH1). Inactivating mutations in these genes predispose people to colorectal cancer and these tumors are characterized by immense mutational load such as base substitutions and small insertions and deletions at short repeat sequences in the genome. In human colonic organoids, MLH1 is inactivated by CRISPR-Cas9 by inserting a puromycin-resistance cassette. Then, organoids are expanded and genotyped to confirm correct biallelic targeting. Then, organoids were clonally expanded, and genes were inactivated revealing a substantial reduction in MLH1 mRNA expression organoids which became $MLH1^{KO}$ due to the degradation of nonsense mRNAs transcribed from mutant alleles. Then, the organoids were settled for 2 months to allow for the accumulation of sufficient mutations needed for downstream analysis. As expected, the $MLH1^{KO}$ organoids showed increased base substitution load and a change in type of base substitution, confirming that deletion of MLH1 is sufficient to generate the mutator phenotype observed in MMR-deficient-tumors. There was not an increase in structural variations either. To test somatic mutations, a genome-wide analysis was performed to replicate human intestinal organoids. Early, intermediate, and late replicating genomic regions were defined and researchers determined the relationship between somatic mutational load and replication timing. Accumulated mutations in normal organoids were more frequent in late replicating DNA. Because of the mutation modeling organoid system created in this study for colorectal cancer, mutational signatures have been shown, and genomic landscapes of genetically modified human organoid subclones were synthesized. The study concluded that the approach demonstrated that a high contribution of signature 30 mutations within a tumor can indicate cancer-predisposing germline mutations in the base excision repair gene NTHL1[10].

Generating organoids can vary in the time they develop depending on the type of cancer and the mutations involved in the desired cancer to model[8].

Most importantly, the tumor organoids have been vital for drug testing since they have been shown to respond to known anti-tumor medications[9]. The process for drug testing for these anti-tumor medications is extensive but rewarding. To exploit the full potential of immunotherapy, it's necessary to find ways to predict which patient will respond to the treatment and who will eventually relapse, and how to overcome the rise of resistance mechanisms. To transfer away from the use of 2D models, a lab by Tsai et al. Describes how to co-culture human pancreatic cancer organoids together with autologous CAFs and T-cells. This is suitable for the precision of testing drug responses and for testing the lymphocytes infiltrating capacity into the tumor tissues of these models. A coculture of pancreatic organoids and CAFs has also been used for the identification of tumor secreted ligands capable of promoting inflammatory CAF subtypes. Another interesting method to investigate the toxicity of certain cells like CAR-NK has been to select suitable target antigens for CAR therapy by comparing the cytotoxic effect of CAR-NK cells against matching tumoral and normal colon organoids. These methods focus on the interaction between tumor organoids and a single immune population of interest. A path taken by Dijkstra et al. Is where patient-derived tumor organoids are cocultured in the presence of autologous circulating peripheral blood lymphocytes without any selection of a particular subset of immune population. This study provided a new strategy for the generation of tumor reactive cytotoxic T cells for patients with colorectal cancer or non-small cell lung cancer. Other ways to form drug therapies are through genetically engineered organoid models using CRISPR, dynamic organoid cultures with vascularization and fluidic technologies, and personalized models and biobanking. This method has been deemed effective enough to show 90% of somatic mutations as the original tumor from which they were derived.

Altogether, the ease in organoid culturing and expansion makes organoid culture models attractive in studying personalized anticancer treatment. The success rate of establishing this treatment spans from 70-90% for colon tumors. On the more clinical side, organoids are a helpful pre-clinical model. After multiple oncological studies, the pooled sensitivity was

0.81 and the specificity was 0.74 for using organoids to discriminate patients with a clinical response. Most screening efforts using tumor organoids use small collections of FDA-approved drugs for multi-dose evaluation. Chemically diverse compound collections are less likely to provide a high hit rate and the binding affinity of these compounds to certain receptors, kinases or other druggable targets is unlikely to be high. To screen larger compound libraries, assay miniaturization and automation needs to occur. For assay miniaturization and automation, screens with organoids follow the same optimization process as for any other cell type. First is assay optimization with a plate format of 384-well plates, then further optimization and extensive testing occurs for comparison of the performance of automated screening setup. To enhance the possible throughput of screening pipeline, it's important to identify drug dilution or the readout. Specialized scheduling software provided in lab automation generally helps identify these issues and allows for optimal scheduling of the experiment. In addition, this software helps track samples in the system. Screens are successful if the z-factor is 0.5 or higher and would indicate sufficient separation between positive and negative control conditions. Organoids vary from batch to batch, but most of them do not hit 0.5 or higher. However, to provide data screening of good quality, it's important to minimize variation using selected readouts, which have large effects on the z-factor. For example, image-based screens typically have a lower z-factor than whole-well measurements like viability assays[8].

Organoids can also model many of the tumors' mutations inside the body, which helps scientists gain insight into mutational processes that occur during tissue homeostasis and tumorigenesis (See Figure 2 below). They use single stem cell-derived organoid cultures to determine genome-wide mutation patterns in the small intestine, colon, and liver[11]. Tumor modeling will be a pivotal branch of organoid technology because organoids can model cancer progression mutation-tumorigenesis processes and genetic carcinoma[12].

When developing these tumor organoids, it must be considered to introduce the proper cell signaling molecules to direct differentiation towards the desired lineage, tailoring the extracellular matrix (ECM) to match that of the target organ, and optimizing media conditions. They must also focus on adjusting growth factors, including epidermal growth factor (EGF)[12]. Tumor organoids may develop slower than most other organoids, leading to higher rates of mitotic failure and cell death. The overgrowth of tumor organoids by healthier epithelial cells must be controlled by growing them in more controlled environments, like their *in vivo* conditions, or using pure tumor organoid cultures. It's essential to find the optimal culture for organoids to live in (depending on factors unique to each type of tumor, such as mutations, cancer type, or markers) so that researchers can derive potentially curative drugs from studying the tumor organoids. Many scientists and researchers have developed a co-culture system to develop this organoid micro-environment. Often, using pure tumor cells to generate the organoids is a prerequisite before considering any other factors that may affect the organoids' microenvironment in which they live[11].

Organoid modeling systems have already improved in many ways, which is why organoids can explore the link between pathogens (infectious diseases) and cancer development. One out of 5 cancer cases are correlated to infectious agents, but many don't know how a particular pathogen can turn cancerous. Many studies have established co-culture organoid systems from different types of pathogens, and now organoid technologies can be used to explore how this transformation works. For example, liver organoids could be used to study how hepatitis infections lead to the development of liver cancer, or stomach organoids can be used to evaluate the connection between the Epstein-Barr virus and the development of gastric cancer[13].

Benefits

Organoids make drug screening for many types of cancers very accurate. 3D breast cancer cultures can be easily observed to study breast cancer biology. Many factors that affected the collection of cells to make the organoids, or the growth factors involved with the organoids to make them easier to study, were successfully tailored and adjusted so that tumor organoids for breast cancer are now consistent with parent tumors in morphology, histopathology, hormone receptor status, human epidermal growth factor receptor 2 (Her2) status, mutational landscape, and DNA Circulating Nucleic Acids (CNAs) which are segments of DNA or RNA found in the bloodstream. This has contributed to the organoid's ability to model structural and functional changes when breast ductal cancer begins to affect other areas of the breast. They have also become an excellent tool for evaluating tumor invasions in the breast and interactions between tumor, ECM, and stromal cells.

Another example is organoids helping with stomach (gastric) cancer and some of its implications. The organoids did a significantly better job showing the essential characteristics of their parent tumors, including their markers, like carcinoembryonic antigen, Cadherin 17, cytokeratin 7 (KRT7), and periodic acid Schiff reaction. The organoids also showed some crucial mutations prevalent in stomach cancer, such as mutL homolog 1, mutS homolog 6, phosphatidylinositol 3-kinase catalytic subunit, ERBB2, and TP53[12].

Limitations

There are a few drawbacks related to organoids modelling various forms of cancer. Cancer organoids grow slower than other organoids, contributing to too much of the tumor spreading too fast within the organoid, yielding inaccurate results[5]. Because of their time to mature fully, these organoid cultures consume

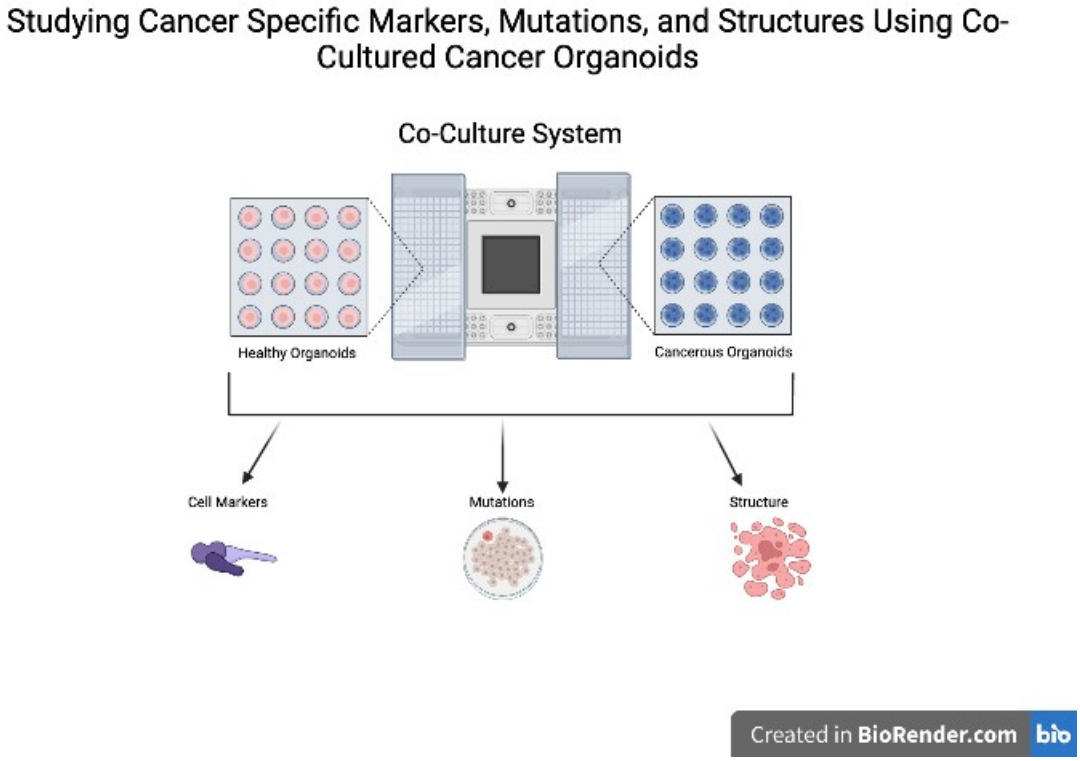

Fig. 2 Applications of co-cultured organoids in cancer research. Cancer organoids can be cultured with healthy organoids in a co-culture system, enabling the discovery of specific cell markers, identification of mutations, and phenotypic structural changes.

more time and money than standard 2D models. When looking into prostate cancer, cancer cell lines derived from primary patient material are inefficient to generate, only rare clones are able to expand and no longer recapitulate the heterogeneity of original tumors, culture conditions for prostate tumor organoids are not strictly defined which is why the researchers of Palacky University were unable to confirm the growth of tumor cells because of growth factors AMACR and ERG overexpression. The length of cultivating these organoids is long and inefficient, and only yields data and results for 3 weeks before becoming ineffective[9].

This varies with breast cancer organoids and some of its limitations. Breast cancer organoids take between 4 to 6 weeks to cultivate and for patients who are in urgent need of treatment. However, even though breast cancer organoids can reflect the patient's disease situation, when culture systems are being built, complete technology is still lacked to simultaneously connect both organoids and tumor environments. At present, the breast cancer-like organs that are cultivated from patients cover different numbers of breast cancer cells and some tissues making it difficult to ensure that all are tumor tissues and the number of cells obtained after enzyme digestion is different which affects the survival rate of organoid culture. Also, Matrigel and cytokines (tools to create breast cancer organoids) have different concentrations in different laboratories which is not accurate for uniform evaluation of the formation rate of breast cancer organ cultures[13].

Not only does this mean that cancer organoids themselves still have a long way to go before being successful and accurate for all cell types and patients, but the problems and inefficiencies of organoids also vary between different cancer types.

Alzheimer's Disease

Alzheimer's Disease is the most common form of dementia. It is a leading cause of death in the US, as 50 million Americans are diagnosed with it annually[14]. A healthy adult brain without Alzheimer's has approximately 100 billion neurons, where each neuron contains the cell body, dendrites, and an axon. Each part of every neuron in the brain needs to do its job without any hindrances. The neurons of patients with Alzheimer's are injured, and connections between neuronal networks can break down. Often, Alzheimer's patients suffer from memory loss and confusion. The two fundamental causes of Alzheimer's

are beta-amyloid plaques and neurofibrillary tangles (Tau tangles). Beta-amyloid plaques are insoluble plaques that block neuron communication between the dendrites in the brain. Beta-amyloid plaques are generally found on the neuron's surface, where they release their beta-amyloid precursor protein into the brain. The protein is cut free by enzymes such as beta-secretase and gamma-secretase. The beta-amyloid fragments join to create plaques that attach to the neurons. They can also be formed by the aggregation of monomeric amyloid beta peptides which are turned into amyloid beta oligomers which generate insoluble fibrils. These plaques can cause many adverse effects on the brain. For one, they cause vascular issues in the brain, such as no blood or oxygen flow, causing tissues in the brain to die, becoming insoluble in the brain, and blocking signaling between neurons. They also trigger inflammatory responses and Reactive Oxygen Species (ROS) production, resulting in neuronal death. Neurofibrillary (Tau) tangles are another major cause of Alzheimer's Disease. Tau proteins stabilize microtubules (important molecules that transport nutrients from the cell body to the axon and dendrites). When misfolded (generally into C-shaped structures when the brain is affected by Alzheimer's), it will accumulate inside the neurons and affect the number of nutrients available to the brain and communication within the brain. Tau tangles can cause neurons in the brain to be injured and die. Connections between networks no longer work, and brain regions begin to shrink. While researchers know a lot about the pathophysiology of Alzheimer's, they have only developed medications for patients that ease symptoms, but there are still no treatments to cure the disease [14,15].

Current Models for Alzheimer's

Current models that are used for Alzheimer's Disease, such as various cellular and animal models, aren't able to adequately capture the pathophysiology of the disease. For example, the everyday use of transgenic mice cannot capture Tau pathology and the development of Alzheimer's features early in life. Additionally, monolayer neuronal cultures from AD patients don't have the plaques or tangles, and they express toxic proteins, limiting their potential to model AD. Now, researchers are finding different methods to model diseases, and a more efficient way to model Alzheimer's is using organoids [14,15].

Early 2D AD models were derived from somatic cells and were induced to present AD characteristics. Although this method showed certain pathophysiological features of the disease, such as neuronal cytotoxicity, the neuronal cells typically lacked other features, such as beta-amyloid plaque formation. Different techniques were tested, such as using somatic cells that carried AD mutations or using CRISPR-Cas9 to edit the disease's genome and induce the mutant. However, in all these versions, the 2D cell cultures and monolayer cultures could not show all aspects of the disease. iPSC-derived neurons successfully showed essential features of AD pathogenesis at early stages. Still, they failed to present several morphological and functional properties of the human brain, which limits their use as model systems for this disease. Monolayer cultures don't accurately represent functions observed in the human brain's oligodendrocytes, astrocytes, and microglia [14].

When talking about animal models for Alzheimer's, some prove to model certain phenotypes of Alzheimer's over others. Aging is an important AD pathogenic factor, and aging models can be divided into those of natural aging and those of rapid aging. Although the naturally aging mice may show some changes of the nervous system in their brain tissues, they have poor feeding ability and a high mortality. The rapid-aging model shows AD pathological characteristics, such as astrocytic response to oxidative stress and early-phase energy metabolic abnormalities, while other phenotypes such as NFTs and tau hyperphosphorylation are not present. In addition, metabolic disorders and learning and memory deficits in mice have been seen present as well.

While there have been significant improvements from the mice that were first used, there are still shortcomings. The APP transgenic (modified) mice do not have NFTs, tau hyperphosphorylation, or neuronal apoptosis and they show early Aβ deposition and gene expression instability, represented by various appearance times and severities of Aβ deposition among the animals from different sources. Unlike human patients with AD in whom neuronal apoptosis occurs with increased age, in transgenic mouse models, brain atrophy is known to occur at early development and precedes the formation of Aβ accumulation.

Although the above-mentioned models recapitulate some pathological and behavioral features in AD patients, the levels of Aβ deposition and NFTs are still inconsistent. Overall, AD animal models have limitations including high mortality and poor representation of the structural, cellular and protein abnormalities in the human brain. In addition, murine brain cortices are small and smooth, while human cortices are larger and have many sulci and gyri, which are closely related to the learning and memory abilities of humans. Therefore, it is very difficult to mimic the human nervous system disease using animal models [11].

Organoids For Alzheimer's

On the other hand, 3D cells cultures (where organoids are synthesized) allow the iPSCs to self-organize and become organs outside the body. They begin displaying morphological and functional similarities to their *in vivo* counterparts and can even represent complex structures in the brain. Studies also showed that cells derived from AD patients displayed some features associated with AD pathogenesis, including higher production of more amyloidogenic variants of the amyloid-beta protein, increased Tau phosphorylation, higher susceptibility to

amyloid-beta-mediated neurotoxicity, and presence of cellular stress markers. Since the brain is such a complex organ in the human body, scientists attempt to create different parts of the brain (forebrain, midbrain, hippocampus, etc.) separately before combining them all into the brain. This process may be time-consuming since the separate organoids take months to grow and develop. Still, it ultimately yields a more efficient and accurate way of modeling conditions in the brain, such as Alzheimer's. Other studies [16] talk about how scientists repeated the procedure of many other researchers to develop cerebral organoids. They developed these directed organoid cultures to contain different cell lineages at stable proportions, limiting variations across different batches and cell lines. In trying to create this level of homogeneity between cell lines, CRISPR-Cas9 technology has helped create mutations for each batch to test the effects of different factors on the brain. However, the success of this approach is largely because of the use of isogenic cell lines. Studies deploying isogenic cell lines(citation) allow multiple lineages of stem cells to be studied and compared at the same time and use CRISPR to fix these mutations in each of the different scenarios. It allows for side-by-side experiments with the different disease-causing variants and the human sample to test if the patient's symptoms correlate to a previously discovered disease-causing variant. This approach is a lot more efficient instead of culturing multiple lineages at the same time, each with different viral mutations [17].

AD is modeled by cerebral organoids with the ability to take multiple transcription factors and receptors and model different cells that make up the brain and how pathophysiological factors like Beta-Amyloid plaques and Tau tangles affect chemical and physical compositions of the brain. The majority of AD-iPSCs focus on neurons, but they also address other phenotypes like fAD-causing mutations and long-term self-renewing NSCs. The study shows the expression of APP and amyloid-beta secretion. They detect very low levels of APP and amyloid beta while the expression of both proteins increased to detectable levels only after three weeks of neuronal differentiation. In general, initial studies using iPSC-derived neurons demonstrated the presence of typical AD pathological features like amyloid-beta accumulation, altered secretion of Amyloid-beta40, the presence of protein inclusions, activation of glycogen synthase kinase 3 beta, and tau hyperphosphorylation. They also demonstrate loss of synapses and decreased synaptic activity, altered electrophysiological activity, increased oxidative stress, and reactive oxygen species. These pathologies were all found in the neurons from the cerebral organoid [18].

More specifically, cerebral organoids can model astrocytes in AD, and they show reduced cholesterol levels and elevated expression of the regulatory element binding protein (SREBP). They show significantly less complex morphological approaches, atrophic profiles, and abnormal localization of key functional astroglia markers. Oligodendrocytes are another phenotype modeled in cerebral organoids that generate myelin sheaths around axons which play a role in neural repair. AD studies showed that the morphology of oligodendrocytes have been altered in AD and how Amyloid beta oligomers caused a decrease in myelin proteins. They showed the morphology of microglia. Neuroinflammation is a defining feature of neurodegenerative diseases and microglia begin to react with changes in structure and function. Reactive glia cells cluster around amyloid beta plaques showing their inability to clear the plaques. Microglia are also implicated in the neuroinflammatory aspect of AD. IPSC derived microglia manifest the ability to phagocyte AD related substances including amyloid beta and Tau oligomers. They were able to express different gene expression profiles with increases among microglial AD risk genes [18].

Cerebral organoids have been able to model different neurological disorders, including autism, Parkinson's, and Zika virus infections. Indeed, organoids carrying familial AD mutations have been able to model Alzheimer's and allowed the simultaneous presence of beta-amyloid plaques as well as Tau tangles. The researchers continued to perform tests on the cerebral organoids to make sure that the organoids were able to model the neurodegenerative features (neurofibrillary tangles and amyloid-beta plaques) of AD and compare them to their *in vivo* counterparts in the brain (see Figure 3 below). Recently, a new 3D human tri-culture model was developed to model AD, including neurons, astrocytes, and microglia. The model displayed critical features of AD pathology, such as beta-amyloid aggregation, Tau hyperphosphorylation, neuroinflammatory activity, microglial recruitment, and axonal cleavage resulting from neurotoxic activities [16].

Since cerebral organoids could express the complicated pathology of AD, they were then used to test drugs and treatments to counter AD. Studies showed that the medications tested on the organoids reduced amyloid-beta peptide levels and alterations in Tau pathology. In other studies, researchers found that their treatments partially reversed the brain's beta plaques and Tau pathology. Thus, numerous studies were able to prove that cerebral organoids can serve to model AD, as well as serve as a drug development platform for AD [16].

Limitations

The current AD Cerebral Organoid lacks microglial cells which are important to the immune system during AD development. The activation of microglial cells induced by amyloid-beta plaques occurs early in AD pathological process. Microglial cells stimulated by amyloid beta can have a variety of effects with inflammation causing neural injury or death, synaptic damage, and neurotransmitter changes, all parts of the AD pathological process. To solve this problem, some researchers have made cerebral organoids containing microglial cells by improving the cerebral organoid culture. They have attempted to combine

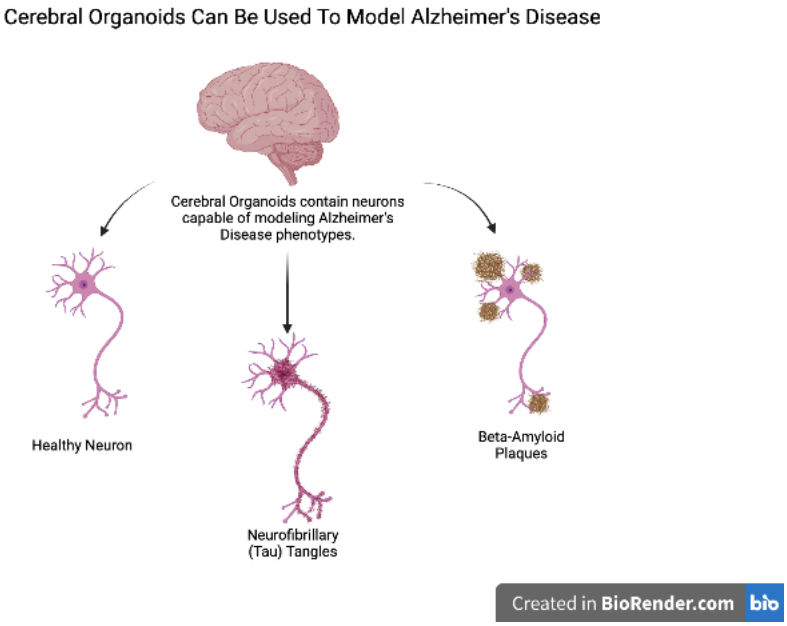

Fig. 3 Cerebral Organoids Can Be Used To Model Alzheimer's Disease. Cerebral organoids contain neurons with different phenotypes representing Alzheimer's disease. This includes healthy neurons, neurons with Neurofibrillary (Tau) Tangles, and neurons with Beta-Amyloid plaques.

human microglial and microvascular endothelial cells, peripheral nerve cells, astrocytes, and oligodendrocytes to form an organoid with a brain barrier that includes microglia. These newly developed cerebral organoids have now enhanced the inflammatory response of the cerebral organoids [11].

Non-Vascular AD cerebral organoids have many disadvantages including the inability to model age-related diseases because cerebral organoids may demonstrate volume shrinkage and cellular apoptosis since nutrients and oxygen can't enter the innermost organoid regions without a vascular system. Therefore, AD cerebral organoids can't stimulate real AD brain well because they aren't old enough. Also, vasculature is important in the accumulation and clearance of neurotoxins during AD pathogenesis. Vasculature is fundamental to the blood-brain barrier because it supplies essential energy and nutrients to sustain neurons and their functions, as well as limiting the entry of blood components. These components of the neurovascular unit work together to sustain the normal permeability of the blood-brain barrier. When the blood-brain barrier is blocked, various toxic substances accumulate in the brain, resulting in pathological changes. Third, brain hemodynamics is related to amyloid beta production and tau phosphorylation. The lack of a blood-brain barrier in organoids makes the development of anti-AD drugs challenging, as the permeability to anti-AD drugs in cerebral organoids cannot truly simulate their diffusion and absorption in the real AD brain. Several attempts have been made to create cerebral organoids with a vascular system by using differentiated human pluripotent stem cells to form vascular endothelial growth factors and using CRISPR/Cas9 gene editing to make human embryonic stem cells express vascular genes ETS variant 2 (ETV2) and combining normal human embryonic stem cells and ETV2-embryonic stem cells to create human cerebral organoids with a vascular system [11].

Lastly, another big problem is the lack of connection with other organs. The brain is dependent on the rest of the body, and AD pathogenesis has close relationships with other organs like the gut, kidneys, and heart. Gut microbiotas communicate with the brain through the gut-brain-axis. Evidence supports that intestinal dysbacteriosis is closely associated with the development of AD and disruptions of intestinal microbial diversity could induce neuroinflammation and amyloidosis. Similarly, the brain and kidney communicate through the brain-intestine-kidney axis. Immune cells in the bone marrow are activated by the gut microbiome and enter the bloodstream causing inflammatory response to the kidneys. Problems in the kidney, gut, and heart all lead to an increase in neural death and inflammation or cause an increase in amyloid beta plaques and tau tangles, making it essential to develop and model that relates to other organs and the body. Organoid transplantation experiments have demonstrated that cerebral organoids have formed connections

and circuits with the host, grow blood vessels in the implant, and have neural synapses and glial cells. This implanted human cerebral organoid demonstrates the same developmental processes and characteristics of neurogenic dynamics as the host grows. Microfluidic technologies have been used to develop artificial vascular systems, incorporating signaling molecules, nutrients, and oxygen that can reach inner regions. This can extend the survival of many types of organoids to obtain older organoids. Microfluidic technologies can establish connections between cerebral organoids and other kinds of organoids in the future as well[11].

Diabetes Mellitus

The 3rd most widespread disease in the United States is Diabetes Mellitus (DM). Diabetes is the most common metabolic and chronic disorder, affecting more than 400 million people worldwide[19]. DM is a multifactorial disease triggered by a combination of genetic, epigenetic, and environmental factors. The increase in life expectancy and unhealthy lifestyle habits are risk factors for the development of obesity and other side effects like insulin resistance syndrome[20].

Type 1 (T1D) and Type 2 (T2D) are two significant variations of this disease. Both are characterized by an elevated blood glucose level, called hyperglycemia. T1D is where the pancreas doesn't make insulin because the person's immune system has attacked the body's islet cells, whereas T2D is where the body still makes insulin but less and in addition is resistant. This condition is generally found in patients older than the age of 45. Diabetes is caused by defective insulin secretion by pancreatic beta-cells and the inability of insulin-sensitive tissues to respond to insulin. Often, diabetes is inherited from a parent because of family history and genetic factors. Other factors include obesity, low physical activity, and an unhealthy diet. Elevated blood glucose levels damage the heart, vasculature, eyes, kidneys, and nerves. In addition to the body's high glucose levels, if the condition stays in the body for too long, it begins promoting insulin resistance (IR) in the body[21].

Preclinical biomedical models of DM currently include rodents. However, there is no animal model that presents all the phenotypic or genotypic alterations of DM in humans. Most studies on rodent models are focused on the development of strategies for the prevention and early treatment of DM. However, because of this, there's a gap in the knowledge of the long-term complications of DM. One of the main reasons is the difficulties of maintain animal experiments long enough to compare them to what happens in advanced stages of DM in humans. Therefore, new models must be synthesized to carry out long term studies to investigate the effects of hyperglycemia and its impact on organs of the disease. Also, the scientific aspects of using experimental animals are subject to strict ethical guidelines which also limit the extent of research and testing scientists can perform on their animal test subjects[20].

The pancreatic islet is an endocrine organ that secretes hormones for blood glucose homeostasis. It comprises insulin-producing beta-cells, glucagon-producing alpha-cells, somatostatin-producing delta-cells, polypeptide-producing /PP cells, and ghrelin-producing epsilon-cells. During development, islets come from pancreatic buds and start to expand through cell proliferation. Unfortunately, a lot is still unknown about islet cells, such as the morphogenesis, signaling pathways, and critical factors involved in islet development[21].

Organoids in Diabetes

Using organoids to find a cure for diabetes would once again prove the ability to provide new models for developmental biology and disease research. Since organoids come from human-derived cells and aren't animal models, they are more accurate and can better model processes inside the human body[21]. Islet organoids could grow the same size they would if they were in the body with the help of many additional chemicals and genes inserted into the organoids to help with *in vitro* maturation. The islet organoids can mimic pancreatic fibroblasts and pancreatic endothelial cells. They can also form organ-like and vascular structures and have many similar functions as beta-cells in the body. This means that organoids can indirectly provide glycemic control through a combination of genetic and environmental factors that destroy beta-cells[22]. More recent studies have shown that iPSC-derived islet organoids are great for regenerative medicine due to their high proliferative capacity, multilineage differentiation, ease of access, and safety. To generate the islet organoids, researchers combined different versions of islet cells found in the human body to make the organoid. The functionality of the *in vitro* organoids was better than their *in vivo* counterparts. This is because they produced more insulin as glucose increased in the testing microenvironment, which was much better than the function of islets and beta-cells in a diabetic patient[23] (see Figure 4 below).

In addition to the elaborate versions of chemical, genetic, and environmental factors on the developing organoids, the *in vitro* 3D microenvironment has been investigated. Islets are densely packed 3D structures with cell distribution patterns, where cell interactions have significant roles in how the islet organoid will act and react and all its functions. This is why 3D organoids, which consistently deliver induction signals or nutrients while removing metabolic wastes, are put into microfluidic devices. Organoids also do a much better job of modeling the overall physiology of the organoid and disease than 2D cell line cultures can. For example, these cell lines respond poorly to glucose stimulation. They can't model interactions between beta cells and other cell types, which are crucial processes for islet function and the onset of diabetes. Islet organoids grown and matured in a 3D setting, on the other hand, provide a novel system in

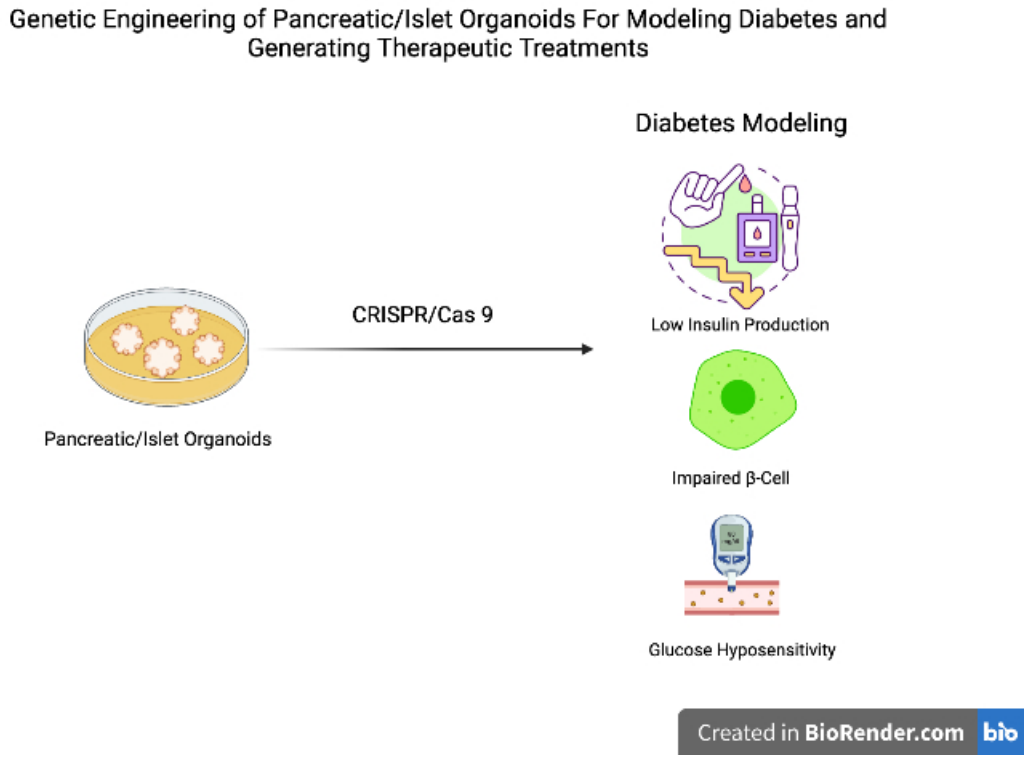

Fig. 4 Genetic Engineering of Pancreatic/Islet Organoids For Modeling Diabetes and Generating Therapeutic Treatments. Pancreatic/Islet organoids can be kept in dishes and genetically modified using CRISPR/Cas 9 technologies. This can lead to the organoids modeling different aspects of diabetes, including low-insulin production, impaired β-Cells, and glucose hyposensitivity.

which scientists can study diabetes. For example, the combination of gene editing technologies such as CRISPR-Cas 9 and refined iPSC technology allows for the manipulation of islet organoids to investigate developmental, functional, and pathological mechanisms of normal and diabetic human islets, which can potentially be used to develop personalized and informative drug screening platforms[21].

Currently, iPSCs derived from patients with T1D are used to generate new models demonstrating that cells respond to different forms of beta-cell stress *in vitro*. The study of T2D-related genes showed standard endocrine specifications but impaired insulin secretion *in vitro* and *in vivo*. These phenotypes showed symptoms in patients carrying mutations in specific genes, which may have led to the rise or development of the disease. In contrast, animal models were only able to show irrelevant phenotypes[24].

Because of advancements in human genetics, geneticists have found that one of the most representative forms of diabetes to model is the monogenic form and it is the most straightforward scenario for personalized modelling. The two main types of monogenic diabetes are maturity-onset diabetes of the young (MODY) and neonatal diabetes mellitus (NDM). MODY develops later in life, although sometime before 30 years of age. NDM develops in the first 6 months of life and results from mutations causing abnormal insulin production and secretion, islet cell destruction or abnormal pancreas development. Mutations in different genes can cause either type of diabetes and iPSC derived organoids were able to uncover the mutations in the specific insulin locus that caused NDM or MODY. By using a combination of patient mutations and knockout strategy, many different genes were shown to be involved with endocrine dysfunctions. Other mutations were shown to cause functional impairment of the coupling between glucose sensing and insulin secretion. Another approach involves hPSCs to study developmental consequences of the mutations that cause different forms of diabetes and whether they are naturally occurring or loss-of-function mutations. Several other studies have also generated human 2D *in vitro* models of monogenic mutations over the years which rely on generating iPSC lines from patients and their differentiation into pancreatic lineages to study genes and mutations. 3D culture methods have also been applied to syndromic forms of monogenic diabetes mellitus. Besides

studying genes of diabetes mellitus, the culture environment of organoids can also be manipulated which will allow for the study of fetal programming of diabetes mellitus by nutrients and environmental toxins[25].

Indeed, previous models of organoids still had flaws that are still being addressed in the present state. Some recent developments include the pancreatic differentiation of PSCs which display similar functions to an adult beta-cell that's important to displaying dynamic insulin secretion and was found a success through calcium signaling. Modeling adult-beta cells also promoted mitochondrial metabolic maturation and improved insulin secretion in response to glucose. Improvements in culture resulted in more functionality like human islets and it was assessed by dynamic insulin secretion assays. In addition to current iPSC-derived islets, a report from 2020 suggests that adult stem cells could be isolated from adult mouse islets and could be used for the formation of islet organoids composed of multiple endocrine cell types. IPSC derived beta-cells were also initially designed for transplantation but were often synthesized in small batches making it inefficient for large scale screening. Suspension cultures have enabled the assembly of larger islets that offered reproducibility and consistency in size so that they can format multiple conditions. Lastly, pancreas-on-chip models have enabled the cultures of 3D clusters of islet cells in microwells where samples can be coupled on microfluidic chips. These systems allow multiparametric assessments for continuous measurements of oxygen consumption rate, insulin secretion, calcium imaging and precise glucose stimulation. Pancreas-on-a-chip systems also enable the cell clusters to be lysed inside a device to analyze gene expression, DNA methylation, and micro-RNA secretion. These systems can model oxidative stress induced diabetes mellitus, study intra-islet communication based on rodent islets, improve functionality and glucose sensitivity of iPSC derived human islets, study insulin kinetics, and maintain islet physiology[25].

Because diabetes is related to so many organs and tissue, models should be able to incorporate multiple pancreatic cell types and organs to recapitulate certain aspects of diseases. Some models have integrated non-endocrine cell types for organoid cultures that can create more complex and mature systems. There are ongoing efforts to produce endothelial, mesenchymal, neuronal, and exocrine culture systems with pancreatic organoids. The aim of some 3D co-cultures is to improve vascularization in organoids and human islets prior to transplantations associated with regenerative medicine and connecting the individually synthesized organoids to blood vessels. In addition, to model T1DM and T2DM, models combined beta-cells and immune cells from healthy individuals and patients to compare phenotypes and results of how different human processes relate to the contraction and infection of diabetes. Following this work, other organoids have also been synthesized like liver organoids, intestinal organoids, muscle organoids, and adipose organoids because they are all related to diabetes and have some relation to the development of diabetes and its functions[25].

Why Islet Organoids are Better

It has been proven that organoids grown in 3D cultures yield more accurate results than animal models or 2D cell line cultures, which has many positive implications for the diabetes field. First, islet organoids are efficient platforms for drug screening in diabetes, and they may have some implications for advances in iPSC technology and personalized medicine. Patient-derived beta cells and islet organoids can serve as informative disease models that recapitulate the pathogenesis and phenotypes of specific patients so that scientists can assess specific drug reactions upon screening. Second, islet organoids can be beneficial in regenerative therapy, where if there's a shortage of islet donors, there will be an unlimited number of functional islet cells for treatment. Combining iPSC and CRISPR technologies can modify the genes to make the islet cells more personalized for each patient[19]. Islet organoids also have more impact on treating other diseases that might develop because of diabetes. Diabetes affects various regions of the patient's body, so, for example, patients are more likely to have heart disease[26]. Scientists are now studying how to use islet organoids to enhance revascularization[27].

Limitations of Islet Organoids

In the past few years, organoid technology has continued to advance, but not yet to the point where it can model all forms and phenotypes of diabetes. Generally, islet organoids don't fully recapitulate the behavior of their *in vivo* counterparts because they have poor insulin secretion ability and lack dynamic insulin release. To improve islet performance, a fundamental effort is characterizing discrepancies between islet organoids and native islets which have been beneficial in revealing molecular defects hindering the maturation and application of islet organoids. Second, islet organoids have lost parts of their heterogeneity compared to islets in the body. Islet organoids contain many cell types, but islet organoids have different concentrations of each type of cell than actual islets in the body. Also, nonendocrine supporting cells like endothelial cells and neurons are barely seen in islet organoids which are essential parts of vascular and innervation networks which facilitate islet development and integration into the circulatory and nervous systems. There's also a batch variation in the efficiency of differentiation, organoid quality, and organoid viability or functionality. These are all significant for signaling of islets and complete a detailed understanding of the islet development process[14].

There are also some limitations as sources of treatment for diabetes, such as issues with clinical safety and the incorporation of stem cells derivatives in the body raises concerns with respect to

tumors. Other factors including iPSC tissue origin and strategies for reprogramming, differentiation, or transplantation are thus affected because of the improper safety precautions that need to be resolved. Also, transplantations of islets have low survival and limited duration of euglycemia after transplantation. Low vascularization and immune rejection are also significant for islet cell loss. To overcome this issue, scientists have proposed elaborate encapsulation devices to shelter organoids from the immune system. Lastly, there are a lot of undefined ingredients, like Matrigel, which is not an approved material for clinical applications because of the presence of undefined ingredients. This can lead to the contamination of cellular microenvironments and organoid morphology or functions. To be able to be transplanted, organoids need to be purified to accelerate the clinical applications of islet organoids [14].

There are many underlying side effects of contracting diabetes such as problems related to the gut, kidneys, and brain which often makes islet organoids not enough to study all aspects and causes of diabetes. However, progress is being made to solve these problems. Diabetes patients suffer with vascular complications and previous organoid models were unable to model vascular systems as discussed in the Alzheimer's section. For islet organoids specifically, 3D vascular networks differentiated from iPSCs have been produced by generating organoids composed of endothelial cells and pericytes which were they tested and exposed to hyperglycemic conditions or inflammatory cytokines. These conditions were like the effect observed in patients with diabetes. Other genetic factors were further identified as drivers of diabetic vasculopathy using this system and helped with the realization that the vasculature is different molecularly and morphologically in different organs, and protocols were emerging to mimic the vasculature within specific organs. Bioprinting is also being used to create blood vessels and to design flow that can present growth factors or therapeutic drugs on endothelial cells to promote their maturation. Next, diabetic nephropathy affects 1/3 of patients with diabetes which is why kidney organoids can be used for glucose induced metabolic programming to recapitulate the genetic susceptibility of diabetes. Certain organoid models are still being tested and are in the process of modeling other phenotypes [15].

Limitations

Although organoids have shown great promise in drug discovery and regenerative medicine, and there is a broad spectrum of possibilities for organoids, a lot of work still needs to be done to make them more accurate and provide more reliable results.

From a broad perspective, many challenges remain for the widespread adoption of 3D cell culture technologies in the drug discovery process. There are very limited 3D screens done with large compound libraries. Many organoids exhibit more complex morphology and function leading the challenges in systematic assessment. They are also more diverse in terms of complexity, size, morphology, 3D architecture, and assaying protocols. This leads to challenges in standardizing analysis. Second, there's a lack in understanding the relevance of a 3D phenotype measured to drug effects and for 3D screening. With 3D screening, high content imaging could measure many different phenotypes, but it's critical to find a clinically relevant phenotype that's measurable to expediate the screening process. Third, assays using 3D cell models are less developed in imaging, analysis, quantification, and automation compared with established 2D methods. Fourth, the predictive values of 3D cell cultures for drug efficacy and toxicity need to be determined using existing human data since only a small amount of data from organoids matched clinical data. Lastly, authorities haven't accepted data obtained from organoids for preclinical animal testing [16].

Right now, organoids are still rough representations for many types of diseases and cancers, and some drawbacks remain. For one, organoids are imperfect reproductions. The organoids only include the primary tissues that need to be studied but don't have the native microenvironment they live in in the body, including the surrounding mesenchyme, immune cells, nervous system, or muscular layer. Scientists must continue to refine their organoids' environment by adding additional cellular elements, such as immune or neural cells, and further enhancing their co-cultures. Organoid research is by its nature reductionist. Researchers are testing cells/tissues/body parts in isolation from the whole. Thus, while organoid research has the advantage of studying how different cell types are made, organized into tissues, and connected, they are limited in their ability to test/address more complex physiological and behavioral questions. Organoids cannot be used to test for behavioral defects such as learning/memory/dementia for Alzheimer's, organoids are not ideal for studying how the lack/gain of beta cells impact blood sugar in response to different diets, and while drug screening for treating cancer using organoids can happen(for example to treat breast cancer cells), it is hard to use organoids to test if that treatment is toxic to other organs/cell types such as the kidney. However, fixing this problem may be harder than it seems because organoids' *in vivo* microenvironments are challenging to model because they are very diverse and vary from patient to patient. Second, it's hard to have the organoid fully mature in a lab environment, which can affect how accurate organoids are when used for modeling and drug testing. Third, some organoids can't stay expanded or physically larger sizes for long periods, mainly because their microenvironments aren't similar enough to those in the body. Fourth, current organoids come from epithelium, meaning that non-epithelial organoids still need to be studied. Lastly, growth factors used on the organoid may affect their gene expression and signaling pathways, which affects how drugs may react with the tumors in the human body [11]. To overcome these limitations, work is being done to develop

multi-organ-on-chip systems to increase micro vascularization, and different approaches have been developed that can further enhance the maturation of other organoid systems.

Looking at the three conditions more precisely, organoids have drawbacks in each type. Cancer organoids grow slower than other organoids, contributing to too much of the tumor spreading too fast within the organoid, yielding inaccurate results[5]. Because of their time to mature fully, these organoid cultures consume more time and money than standard 2D models.

In the Alzheimer's Disease models, typically older adults are affected by this condition, and generally, cerebral organoids are developed using cells from a relatively younger brain. Cerebral organoids also develop through epithelial-based tissues, even though neurodegenerative disorders occur in non-epithelial tissue conditions. Additionally, organoids can't model the process of vascularization, which is the transportation of nutrients and oxygen around the brain. Because of this, the organoid doesn't become an accurate representation of the disease since it's not as dynamic as the brain *in vivo*, and it doesn't demonstrate the formation of active synapses or the blood-brain barrier. Often, when neurons are self-organizing into organoids, the cells in organoids group together but develop into different shapes and sizes compared to the brain structures, making them inaccurate in modeling diseases like AD. Lastly, reprogramming iPSCs to become cerebral organoids can change the cell's genome, making the organoid inaccurate for modeling diseases[22,26].

Lastly, one limitation for diabetes patients is that islet organoids don't fully recapitulate the behavior of *in vivo* islets at the molecular and functional levels. They have poor insulin secretory capacity and lack dynamic insulin release. Islet organoids also don't contain all islet cell types. They generally have a higher concentration of beta-cells compared to native human islets. Instead, human islets have more non-beta endocrine cells with different activities and properties than the types of islet cells used in organoids. In addition, many supporting cells that interact with islet cells in the body aren't seen in the organoid. These essential components form vascular and innervation networks, facilitating islet development by integrating islets into the circulatory and nervous systems. Islet organoids have a specific undesired heterogeneity, where most derived islet organoids make up large numbers of proliferating progenitors or precursors and irrelevant or uncharacterized cell types, which hinder their *in vitro* application and *in vivo* transplantation. Lastly, in addition to the previous limitations, islet organoids are made in separate groups or batches. Each group or batch made will have a slight variation, making the organoids ineffective in drug screening since each organoid will have different properties and yield different results[27].

Future Directions

Despite some current limitations in organoid technology, there have been many breakthroughs. Organoids can model many organ systems in the body, including the small intestine, colon, stomach, prostate, liver, pancreas, breast, lung, and skin. Organoids have also been able to recapitulate more of a disease's physiology and pathology compared to previously used models (animal models).

In drug discovery, organoids have been shown to respond accurately to their engineered microenvironment, including eliciting injury responses when tested with known toxins. This is because they have the closest pathophysiology to the actual diseases in the body, *in vitro*. They can also help explore the epigenetic and genetic alterations causing drug resistance through CRISPR gene editing techniques with the help of isogenic cell lines. Organoid technology can also be pivotal in pharmacokinetic testing, which is essential for drug testing. This works because organoids can be generated for healthy and infected cells, allowing for drug screening, only affecting the infected cells[7]. This is seen in cancer, Alzheimer's, and diabetes research, where organoids have been shown to respond to drugs, including cisplatin, Aducanumab, and dapagliflozin, respectively[28–30]. Remarkably, the responses to these drugs showed significant sensitivity compared to 2D cell cultures, more closely mimicking how these drugs would behave in the body. The advantages of organoids in drug discovery and pharmacokinetic testing have begun to lead biotech companies to invest in and incorporate organoid drug screening methods into their drug development pipelines.

Organoids are also used for applications in immunotherapy. In the body, pathogens develop resistance against many types of antibiotics, making them stronger and more challenging to kick out of the body. The patient's immune system is observed to get rid of the affected cells. When infected cells have properties and characteristics of their host's immune system, they can generate proper immune responses. Whether or not the infected cells have features of the immune system is determined by whether there are specific mutations in the disease to develop antigens for the body. The exact antigen that is created depends on the condition that the patient has[4]. Here, organoids are used to expand the intensity of immune responses *in vitro* so that the body's immune system can fight against the infected cells within the body[19]. Another example of regenerative medicine is already being tested with diabetes, where islet organoids were transplanted into mice with hyperglycemia, and researchers found that the organoids lead to more balanced levels of glucose[28].

Lastly, organoids have the potential to have a significant impact on personalized treatments. Organoids can identify better treatment patterns for each disease affecting each person since organoids can accurately model the disease itself. An example of customized treatment, in cancer specifically, is photody-

namic therapy, which supplements conventional chemotherapies through a combination of oxaliplatin and neoadjuvant photodynamic therapy, and it serves as a treatment for pancreatic cancer[19]. In addition to seeing how specific treatments affect certain diseases, organoids can help predict the individual patient's treatment response. They can be used for personalized medicine treatment programs[30]. Similarly, cerebral organoids are being studied for similar impacts in regenerative medicine and personalized treatment.

Considering that the organoid field is continuing to grow due to its increased accuracy and efficiency over alternative models, researchers are working to achieve breakthroughs in the field.

Conclusion

Organoids are stem cell-derived 3D models that more closely recapitulate the physiology of organs and disease behavior in the human body, giving organoids a relatively high potential to be the future of drug testing, with many other positive implications. They show great potential to help with many diseases, including cancer, Alzheimer's, and diabetes. Previous studies demonstrate that organoids do a great job of replicating human pathology and can be used to model various body systems in the human body accurately. Although organoids are currently limited by their lack of maturity relative to their *in vivo* counterparts, vascularization, and multi-organ complexity, work is being done in the field to overcome each of these obstacles, which will, in turn, lead to more accurate organoid models for drug screening, disease modeling, and eventually regenerative medicine approaches.

Acknowledgements

I would like to thank Polygence and all of their support throughout writing this paper. I would also like to thank my mentor, Jeffrey Aceves, who guided me throughout this journey.

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