Describe how organoids are being used to understand fundamental biology questions

Organoids are small, three dimensional clusters of cells that self organise and display characteristics of their organ counterparts. They are grown from stem cells, and under the right conditions, they successfully look and perform like the organs they are designed to resemble. To date, organoids produced have resembled various organs including the brain, kidney, stomach and lung. [1] This rapidly advancing area of biology has already demonstrated significant importance in drug discovery, personalised medicine and understanding complex processes in the human body. Organoids have huge potential to revolutionise the future of science - in 2013, *The Scientist* named the use of organoids as one of the greatest scientific advancements. [2] This essay will explore what organoids are, how they are made, their potential uses in modern medicine, and why they are so influential in understanding complex biological questions.

What are organoids?

Organoids are composed of cell clusters grown from tissues, induced pluripotent stem cells (iPSCs), or embryonic stem cells. They are able to self organise into three dimensional structures that resemble organs. [1] Although organoids self-assemble, scientists have recently been developing new ways to better control the development of organoids and more accurately simulate the environment inside the human body. One way in which this is occurring is using bio-printing. The bio printer works by positioning the cells into specific locations in a polymerised collagen gel. This technique can be used to simulate the environment of the body more accurately, integrating other tissues and body systems with the organoid. This has been shown to help understand the interaction between tumours and the microenvironment they are found in, especially with regards to how cancer progresses. [3]

iPSCs are the most common type of stem cell used in the production of organoids. To produce these iPSCs, somatic cells from the body are removed and are reprogrammed back into an embryonic stem cell state, or pluripotent state. This process commonly uses virus vectors to integrate the required DNA with reprogramming factors into the cell's original DNA. The iPSC is then able to differentiate into any type of human cell, and these cells are used in the production of organoids. [4] The tissues or organs made using iPSCs from a specific patient will be a close match and so if transplanted into the same patient it is unlikely there will be an immune rejection. [5] This could be a significant advancement in the future of organ or tissue transplants.

General Importance of Organoids

Although organoids have been a relatively recent biological advancement, they are already significant in research. The dominant alternative to 3D cell culture is flat cell cultures where a monolayer of cells is grown in a growth medium. The flat surface is not entirely representative of the original cell environment in the human body, whereas when using 3D organoids there is added structural complexity and cells are able to interact with other cells in all directions. Through 3D printing, it is possible to integrate many different flows of fluids including blood and urine. Consequently, the cells grow more realistically and therefore provide a more accurate representation which can be used in scientific research. [6]

Another pivotal use of organoids is in drug development. Despite the success of many drugs which have been tested on animals, there are limitations to this technique. As well as ethical complications, animals often do not respond to certain drugs as humans would. It has been suggested that 90% of all tests on animals as models for human diseases fail due to the intrinsic physiological differences between animals and humans. [7] Currently, drug development is a costly process - an average of $2 billion is required for each new drug to reach consumers. [1] The use of organoids could reduce this by increasing the success rate and reducing the time needed for research and clinical trials. Organoids are a suitable alternative; as the cells are derived from humans, the

organoids respond to drugs in a similar way to how they would in the human body. Additionally, many diseases that require new drugs and treatments are uniquely human, such as HIV. The use of organoids would help scientists both understand these complex illnesses and develop effective treatments, where it is not possible to do so on animal models. It is worth noting however, that organoids are not designed to completely replace animal models for research and drug development, rather complement and work alongside them. Testing drugs on organoids not only shows whether a drug will work against a specific condition, but also gives scientists an insight into how the cells interact with the drug. Furthermore, the recent addition of fluid flows and other cells and tissues will enhance our understanding of drugs and treatments for specific illnesses. [6]

Cancer

According to the World Health Organisation, cancer is responsible for around 1 in 6 deaths globally and is the second leading cause of death worldwide. [8] Although there has been rapid progress in potential treatments, this disease is incredibly difficult to treat due to the heterogeneity between different patients. Variations include the growth rate of tumours, drug sensitivity and the prognosis. Organoids have the potential to revolutionise cancer research as they retain the unique physiological characteristics of the original tumour. Therefore, they can be used in many areas of groundbreaking research from drug screening to studying the growth of tumours. There are two main ways to produce a cancer organoid. One method produces patient derived tumour organoids (PDTOs). They are produced from either a surgically removed biopsied tissue or the cellular components from blood. Either of these are taken from the patient and grown in the correct conditions to create a tumour organoid. (see Fig 1 [9]) The second method is where iPSCs are grown to form a 'normal' organoid, and then gene editing is used to create a tumour organoid. [9] (see Fig 1 [9]).

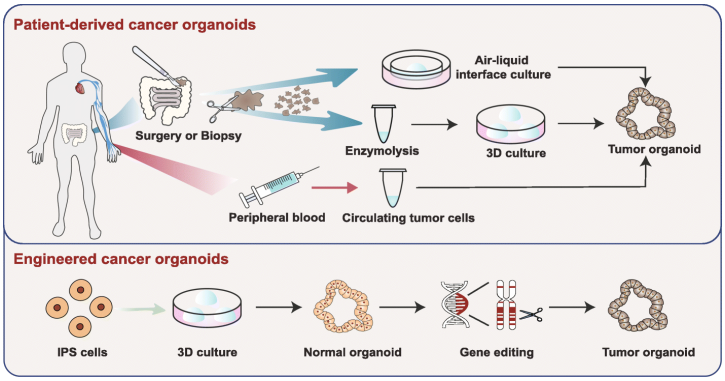

Figure 1

One of the key uses of cancer organoids is in personalised medicine. This can be used to predict which drug or combination of drugs will work on a specific tumour, avoiding any complications or side effects that are unfortunately often seen in cancer treatments. Drug testing on organoids is often very accurate at predicting what will happen in the patient's body. This is because the organoids capture the exact gene expression and genetic alternations specific to that cancer. [9] Additionally, they self-assemble to preserve the original structure and function of the tumours they are derived from.[10] In an experiment by Emile Voest and his team at the Netherlands Cancer Institute, organoids were grown from cancer patients and tested with a drug, Irinotecan, for six days. These tests correctly predicted how 80% of the people responded to this treatment. [11] This illustrates that organoids can be very accurate models when used for drug screening, where the drug often acts differently in different patients. Furthermore, organoids can be used to predict the sensitivity to other treatments including immunotherapy, chemotherapy and the effect of multiple drugs together. Organoids are also proving instrumental in cancer research, for example, in the understanding of cancer metastasis. This is a complex yet fundamental process where cancer cells spread to other organs from the primary site. Cancer metastasis is the major cause of death in cancer patients. Therefore, improved understanding of this process, using organoids, would be invaluable in cancer research. [9]

Recently, cancer organoids have been collected in biobanks - these are collections of organoids along with medical information about their origin. These are stored for lengths of time and are accessible for scientific research. Biobanks provide immediate access to organoids and improve the cost effectiveness of scientific research which will hopefully lead to more advancements in cancer research. [12] The Wellcome Sanger Institute in Cambridge has established a cancer organoid production line, where they have already produced 100 organoids. [15] The researchers have included profiles of each organoid's genome, medical information and susceptibilities to hundreds of drugs. These data are stored on the Cell Model Passports website. This website allows scientists to select the best cancer organoid models for their research and provides lots of information about each type of tumour, allowing all scientists to benefit from organoids. [13] Dr Mathew Garnett at the Sanger Institute states "Organoids are a great model – enabling us to study how tumours develop and how they respond to treatments." [15]

The Brain

The brain is a complex organ which is very hard to study *in vitro*, meaning that neuroscience is a highly elusive area. Organoids can be made into small brains which can show neuronal activity and some complex brain features, such as photosensitive cells. Indeed, small brain organoids have been successfully implanted into mice, proving these organoids are almost identical to normal brain cells. [14] Brain organoids are being used to better understand complex neurological diseases such as Zika virus disease, Autism Spectrum Disorder (ASD) and Alzheimer's disease. The organoids are excellent models, showing how different brain cells interact and any differences in gene regulation. Research into the Zika virus using brain organoids has shown that the virus causes neuron producing cells to differentiate prematurely. Another significant advancement in neuroscience using organoids has been research into ASD - organoids have helped provide knowledge regarding cell proliferation in autistic patients. [1]

The neurodegenerative disease Alzheimer's currently has no cure and is ultimately fatal. Alzheimer's causes dementia, with symptoms including memory loss, personality changes and hallucinations. This disease has distressing emotional and financial impacts on families, doctors and health care systems. Many potential drugs have been tested on mice but have failed to work on human patients. Organoids could be instrumental in understanding the complex causes of this disease, as well as potential treatments and ways to prevent it. All the main brain cell types are now able to be made into organoids, including microglia and astrocytes. Somatic cells from patients are reprogrammed into iPSCs and then differentiated into different types of brain cells. Gene editing techniques such as CRISPR-Cas9 can then be used to introduce or change mutations linked to Alzheimer's. Scientists often study the differences between healthy and diseased cells, both genetic differences and differences in their phenotypes. Brain organoids often involve co-culture models, which incorporate many different types of brain cell - these models more accurately display the interactions of these cells as they would appear *in vivo*. Microglial cells have been made into organoids - these cells are known as the immune cells of the brain and are involved in synaptic pruning and neuro-inflammation. Previously thought to have only responded to Alzheimer's disease, organoids have helped scientists understand that microglial cells are also partly responsible for causing the disease. Organoids have shown that there are differences in the microglia of healthy patients compared to Alzheimer's patients, for example regarding the process of phagocytosis and the production of cytokines. [16] Organoids could also be of use in personalised medicine through the identification and treatment of the disease and also in drug screening, to see whether a particular drug will work in a particular patient.

Transplants

Currently there is an alarming shortage of organ donors and often organs or tissues are rejected by the immune system once transplanted - around 40% of heart transplants result in an episode of rejection within a year of the transplant. [17] One alternative is growing human organs in animals.

However, there are risks of transmitting infectious diseases and there are many ethical issues connected with this option. The use of specific organoids from the patient could be a better alternative. As the original cells are removed from the patient there is little risk of tissue rejection as the antigens present on the cells will be self-antigens. These organoids can be made by first removing the original organs or tissues from the patient and destroying the cells with a chemical. Left behind is a 3D platform for pluripotent cells to differentiate into the organ cells *in vivo*. [18]

One specific example of organoid transplantations are artificial ovaries. After cancer treatment such as chemotherapy or radiation, many women are infertile; this process will allow these women to have children after cancer. Before cancer treatment, follicles are extracted from the patient and frozen. Once the patient is free from cancer, the follicles would be injected into an artificial ovary grown from stem cells, and then this would all be transplanted back into the woman. The artificial ovary provides the follicles with the right conditions to survive, allowing the patient to menstruate and/or have a child. The artificial ovary is an example of a 3D printed organoid mainly made from collagen, growth factors and elastins. Artificial ovaries have been successfully transplanted into mice, which reproduced after transplantation. As well as helping women have children after cancer, artificial ovaries could have various other uses including testing drugs *in vitro*, providing transgender individuals with a more natural hormone delivery system and helping older women produce hormones to combat diseases such as osteoporosis and heart disease. [19]

Ethics and Limitations

Although organoids have the potential to revolutionise biological research, they have a few limitations. Firstly, there are various ethical arguments in this field. The rapid advancement of brain organoids has led to the discussion that, in the future, there is a possibility that these organoids could feel pain or pleasure - this is a highly controversial step. Furthermore, organoids from human cells can be transplanted into animals which raises the question as to whether these animals should have different moral rights compared to a 'normal' animal. Some researchers argue that organisms which are 'substantially human' should have human rights. However, this is very subjective - how do you define being human? [20]

Another ethical dilemma is when people donate their cells or tissue for research and organoids are developed. There are many questions surrounding the donor's influence concerning how the cells are used and disposed of. [21] Another limitation is that the organoid is not an entirely accurate representation of the body. It often doesn't take into account other organs, cells or systems that would be present in the body. However, compared to other alternatives such as flat cell cultures and animal models, in many circumstances they are more complex and accurate models. [22]

Conclusion

From the first attempts to grow organs *in vitro* in 1907, the development of physiologically analogous three-dimensional human models has been a vast scientific challenge. Organoids have revolutionised how we understand fundamental human biology and despite some limitations, they have lead to many significant advancements. New drugs have been tested which have produced successful results in several studies. They have also been used in transplants, for example artificial ovaries, as well as in important research into diseases such as Alzheimer's and cancer. As organoids so accurately resemble the structure and physiology of *in vivo* organs, there is great hope that they will continue to accelerate medical research and improve in the understanding of fundamental biology questions.

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