Organoids:
Uses, Procedures and Advancements

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1. Abstract

Organoids are three dimensional self-organizing masses of cells produced in vitro, which resemble organs in terms of biological, functional and structural complexity of said organs. They show great promise for cultivating our understanding of human biology. The capability to replicate diseases and research human development with the same depth as with animal testing has been considerably advanced by these 3-D cultures. Due to their improvement in modelling organs or tissues in humans, the study of them could propel medical research forward, making creation of new regenerative medicine, tissue engineering and drug screening possible. They are also of interest to developmental biologists, who use these ideal models to investigate a number of relevant aspects of human biology, such as genetic variability or tissue dynamics. However, this topic also has a controversial side to it regarding its ethics. This is for reasons such as the concern of commercialization. Overall, organoids are still a relatively new branch of biology for humanity and while they have weaknesses as well as strengths, they are something worth being studied and could have a major impact on us in the future.

2. Preface

Organoids is one of the most fascinating research topics that exist. The field is only taking its first steps but already shows tremendous potential. Already in the 80s doctors were able to create skin cell cultures that could replace the skin of patients with severe burns giving them a chance of survival. Cells from cancer tumours can be taken to create personalized medicine that ensures that the treatment is effective at killing the cancer. Although the industry is still reliant on animal testing organoid models are improving quickly and give better and more humane ways of testing medicine. And in the far future we might even be able to transplant lab-created organs into patients.

Such a vast topic has the potential for many questions: How are organoids created? What advancements allowed organoids to be created? What are the capabilities of the applications of organoids in the present day? How do organoids help researchers? What is the environment that organoids are grown in? What are the challenges that researchers face today?

3. Introduction

The field of organoid research and organoid application is advancing at a great pace.[i] Organoids have become an essential tool for an assortment of fields in biological research, from genome editing and drug

research to the studying of tumours. Organoids even hold promise in aiding the diagnosis of dangerous conditions in human fetuses. Though the number of research papers including organoids has been plentiful over the past ten years, the technology's origins stem from before that.[ii]

The past 50 years of research have shown that cells grown in vitro (in the lab) in 2D do not accurately mimic those in vivo (in life, in practice).[ii] 1987 is one of the earliest papers mentioning the use of type I collagen gels and compare them with an EHS matrix (which is a compound including type IV collagen). EHS today is also referred to by its trade names Matrigel® or Geltrex® and are used to produce gels. These gels allow the formation of environments of cells which we call 3 dimensional structures. Certain cells, such as those in the aforementioned paper, thrive far better in these environments than on plastic. This discovery (amongst others) is a milestone in the use of 3 dimensional structures in research and is the ground upon which the field of organoids is built.[iii] [iv] [v]

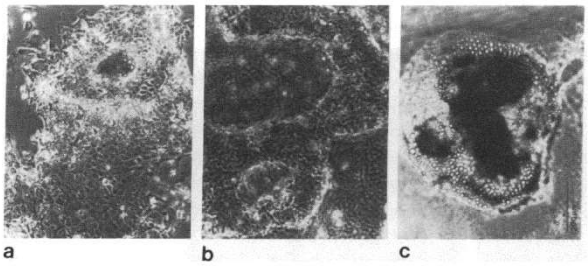

FIG. 1. Phase-contrast micrographs of PMME cultured on plastic and EHS matrix. (*a*) Cells on tissue culture plastic. (*b*) Cells cultured on EHS matrix. (*c*) Cells cultured on a different batch of EHS matrix.

Image 1, Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells [v]

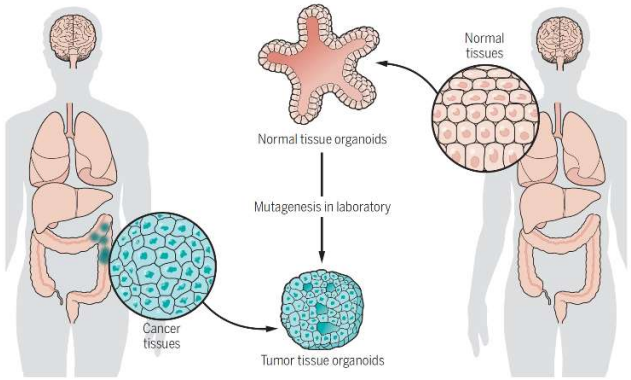

Fig. 1. Methods to generate a human cancer organoid biobank. A biobank of human cancer organoids can be generated directly from neoplastic tissues (left) or by genetic modification of organoids developed from normal tissues (right).

Image 2, Cancer modeling meets human organoid technology [vi]

Today organoids are used in studies and papers all across medical biology:

Tumour organoids have changed the idea of an organoid being only a microscopic 3 dimensional structure to one that can be indefinitely expanded. These models can be created using tumour tissue of various carcinomas. Non-cancerous organoids may also be used to create tumour organoids via use of CRISPR-gene editing. These tumour organoids are used in drug testing to more accurately gauge the response of the cancer to the drug in vivo.[vi]

Patients suffering from harmful conditions may be able to receive personalised treatments thanks to organoids. Normal and malignant tissue from a patient may be extracted and with organoids be used to help diagnose conditions and their progression.[vii] 2D models do not accurately reflect their in vivo counterparts, which is why the move to 3-D models has been crucial in improving the accuracy of drug tests. In the pharmaceutical industry it is vital for the research of new drug candidates to ensure as best it can safe and effective treatments before moving on to development clinical trials. 3-D models greatly improve the reliability of this step. Organoids can also serve as a more complex model due to their self-organizing nature and providing a tissue-autonomous mechanism more accurately reflecting in vivo situations.[ii] [vii] [i]

Research is being done into the possibility of using organoids for diagnosing fetuses with dangerous and harmful conditions. From fetal cells extracted from amniotic fluid organoids can be cultured and analysed to diagnose conditions. Some condition such as congenital diaphragmatic hernia (CDH) can be treated before the fetus is born and prevent the condition from causing greater harm to the patient when born. [viii]

4. Description of engineering technique

The definition of an organoid isn't very precise as it has been used to describe different culture techniques. A 2014 review article provides some examples of the definitions used in various papers: (I) The extraction of epithelial ducts into 3-D gels, (II) the growing of clones of epithelial stem cells without mesenchyme (the cells responsible for producing epithelial stem cells in an embryo) or (III) cultures derived from ES(Embryonic stem) cells or iPS (induced Pluripotent stem) cells.[ix]
Most uses of the word organoid fall into one of these techniques. For that reason, an understanding of stem cells is important.

Stem cells can create copies of themselves and they can evolve into different types of cells in a process called differentiation[x]. They build and maintain our bodies throughout our whole life and can be found in nearly every tissue. There are three types of stem cells used for organoid cultures. ES cells can be found in the early human or mouse embryo and they are capable of building every tissue in our body (pluripotency). They can multiply indefinitely but they can be hard to maintain in a lab. Furthermore, ES cell generation is associated with the ethical controversy of destroying human embryos[xi]. Skin cells can be converted into cells with ES properties. These iPS cells don't face the same types of challenges. For that reason, iPS cells are now mainly used in research. The third type of stem cells are Tissue derived cells (TDC). These stem cells can be found in every major organ of the body and they are specialised in repairing and building the organ that they are located in. [xii]

The starting number of cells and their heterogeneity has a big impact on the ability to conduct precise experiments down the line. Thus, precise processes need to be established to sample seedlings[xiii]. For the generation of organoids from TDCs tissue samples are collected. They are then cut into smaller pieces usually around 2-4mm to increase the surface area. To break the sample down into even smaller samples enzymatic digestion[xiv] is used. The type of enzymatic mixture used depends on the type of tissue. Breaking tissues into cells can also be done using mechanical methods. This is a lot faster and much cheaper than the enzymatic method but the useability and amount of cells left behind is low. Instead, mechanical and enzymatic breakdown methods can be combined for better results. After several rounds of washing the cells, they can be used for seeding and the creation of organoid cultures. To create organoid cultures from iPSCs you maintain them through adding substrates to the extracellular matrix (ECM). Single iPSCs do not survive well in lab environments. Usually multiple iPSCs cells are used to start of an organoid culture as they produce populations with higher viability. The process to get the tissue of tumours is similar to getting normal tissue. One difference is that tumour tissue is attached to normal tissue and to get clean research results these two need to be separated. As of right now, there is no reliable method to separate normal and

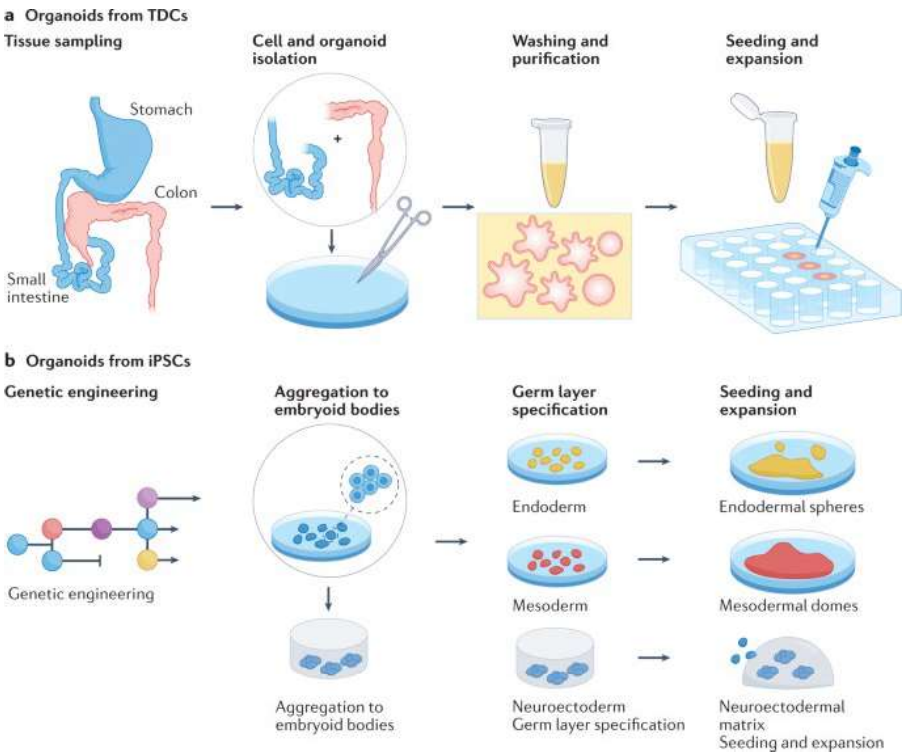

Image 3: Overview of getting single cells from TDCs or iPSCs to start organoid cultures [xii]

tumour cells. One approach to this problem is to limit growth factors that normal cells need in the extracellular matrix they are grown in.

After cells have been isolated from each other they are placed into a 3D matrix. These are matrices like Matrigel or a natural Extracellular matrix (ECM) such as collagen. Collagen accounts for 30% of our bodies protein and it provides structure, support and strength so it is a useful tool in all matrices.[xv] The ideal organoid support structure should be stress-relaxing and be very dynamic in physical and chemical properties to make it possible to control organoid features and especially to control organoid structure. Matrigel can support organoid cultures, but it doesn't allow a lot of control over the chemical or physical influences that decide how organoids grow. That's why matrices with defined mixtures have been tested as alternatives. Examples of these are human collagen, fibrin or synthetic hydrogels. Out of the mentioned substances synthetic hydrogels have proven especially useful.

To control the growth and differentiation of the cells soluble growth factors need to be used. These cues use signal pathways to control which parts of the DNA are transcribed or not. One of the pathways that is used the most by stem cells is the Wnt pathway. Interestingly this pathway is very often mutated or altered if a cancer tumour is present[xvi]. This approach to controlling organoids is often very imprecise making it difficult to replicate experiments. Apart from chemical cues physical cues are also very important. Nutrient supply and waste removal of the cells needs to be closely monitored. Bioreactors exist that can precisely monitor pH, temperature, oxygen and glucose levels.

To culture organoids on a larger scale, so-called organoid farms exist. They can be owned by institutes to serve the purposes of the institute or even by companies to provide organoid culturing as a service.[xvii] [xviii] To satisfy the demand for a higher number of organoids automation is used. Automation is achieved with the use of robotic arms equipped with pipettes.

5. Documentation and pictures of research institutions visited

Researching organoids, we quickly came across the work of Hans Clevers. We promptly contacted him asking for the possibility for an interview. At the time we didn't understand the significance of Hans Clever's position at Roche: Head of Pharma Research and Early Development (pRED).[xix] We assume his position is of great significance considering that Roche invested 14.2 billion Swiss Francs into research in 2023 he must be somewhat important.

His personal assistant responded and recommended another scientist at Roche: Joep Beumer. We contacted him next and he kindly agreed to the interview.

1. Introduce yourself: Who are you and what is your title?

"I work at the IHB (Institute of Human Biology). It is a part of Roche, but it does not really work like a company itself. Its more of a university environment. The goal of IHB is to improve drug developments and more specifically drug testing models. I am a junior group leader and my group focuses on research on the intestine. Especially on how it senses and knows how to respond to what we eat."

2. How did you come to your position at the IHB and Roche? What led your career in the direction?

"I was interested in molecular biology. I like asking questions and using interesting tools. Then I wrote my PhD and post-doc with Hans Clever, who's referred to as the father of the organoid field."

3. How did the field of organoids come about? How was it invented/found?

"Hans Clevers made some important advancements in the research of natal and prenatal development. Especially his research into stem cells was very important. The research made it possible to grow long-term organoids and have long-term cultures. The field was originally dominated by developmental

biologists that know how cells grow and expand. But now that the models exist for organoids any scientist can use them to do with that what they want to."

4. How is an organoid created?

"You can grow organoids from an adult's tissue. The tissue needs to be broken down with enzymes (e.g. Polymerase). After this has been accomplished the 3-D structure can be formed. Stem cells are used to grow organoids and a better understanding of them makes it possible to grow organoids. You can reprogram cells to their fetal development stage and these cells can then develop into any cell in the body. These cells can then be put into a 3-D extracellular matrix. This structure gives the cells support and provides the materials for the cells to stay alive. This matrix gel also adds growth factors to the cells. In a specific order you can give the cells signals which tell them to develop in a way that you want them to."

5. What are the advantages/uses of an organoid?

"Organoids has been a revolution in what questions you can ask. You can do very simple experiments with them because they are not part of an animal or human. It makes asking questions a lot easier. You can grow organoids from specific patients. You can for example grow their cancer tumours and then test drugs on it to see which would work the best against the cancer that the patient has. Which is very good because in the past sometimes the drugs wouldn't work. It's also great that we can expand cancers so easily for the first time because it allows us to see more easily what mutations allow for cancers to be formed. And you can do the same thing with normal tissue. As you can multiply it indefinitely you can play around with specific nucleotides with CRISPR/Cas9 and see what genes influence which function of a cell."

6. How close to a real organ is a lab-created organoid?

"When you grow an organoid in vitro you get small balls of structure, not a real organ. These small parts are not like actual organs because they don't have the real 3-D structure of real organs. For example, the liver consists of like 17 different tissues connected in complicated ways. If we had a real organoid that has the same structure of an organ it would still be really difficult to transplant into a human, as we don't know how the immune system would respond to the organoid. But now you can already take skin cells from a patient use it to grow skin in a lab and transplant it to the patient. Some cells are also a lot easier to grow than others. The heart consists mainly of muscles which makes it easier to replicate its 3-D structure."

7. What fields and competences are involved in growing organoids? What fields of biology and/or other fields?

"As I already mentioned before: The stem cell field is immensely important for organoids. The natal and prenatal development of animals is also very interesting. For example, it is very difficult to create blood vessels in organoids. So that is something that people are researching a lot right now."

8. How did organoids help you in your recent work (Hallmarks of stem cells)?

"Hallmarks of stem cells wasn't a research paper in the traditional sense but rather a review paper. I can instead tell you about the snake venom gland paper[xx] that I wrote. Some time ago I was drinking a few beers with some friends and we thought about which organs would be cool to create in a lab. We came up with snake venom glands. And actually, snakes and humans aren't that different in their development and the signals you need to send to the cells for them to develop the way you want them to. And we actually managed to have organoid snake venom glands that can create snake venom. Which is very useful because you can then produce antivenom. We won't be so reliant on the by hand harvesting that still exists in the snake venom industry."

9. Could you grow the organ that spiders use to grow spider web silk?

"The way in which invertebrates grow is very different from vertebrates. The same signals that you can use to control cell growth and differentiation don't work with for example spiders. It's a very interesting idea but a lot more research would have to be done to make it happen."

10. What machines does the team you are working on use for stem cell- and organoid research?

"We mainly just use pipettes. Of course there are other machines that for example control the environment that organoids grow in. 50 percent of the time you do experiments the rest is mainly spent on writing down your observations. We also collaborate with a lot of other small labs to find the specific methods that they developed and that we need. Of course we also have special microscopes and other tools"

11. What is organoid genome editing? How does it work, what is it used for?

"Crispr which is the tool that is mainly used for genome editing is easy to use on simple cells. But on organoids its difficult to use Crisps-Cas. We are trying to improve at making single point mutations in cells. The use cases for Crisps-Cas with organoids is huge of course because then you can answer a lot of questions about which genes influence what exactly. What is an organoid farm?

"Organoid farms are that you automate these processes of pipetting and you track the growth and development of organoids automatically. The robots add new growth factors automatically and you have a system that stores the plates."

12. If we may ask what are you working on right now? Do you know what other researchers in the field are working on?

"Right now I am working on genome editing organoids. We have a good idea of what genes influence which things, but we don't know for sure. We hope to make more associations between your genes and your weight or your height."

13. What do you know about brain organoids?
    a. How promising do you find the research being done in respect to brain organoids?

"I don't work with brain organoids, so I don't know too much about it. But what I know is that you can grow neurons for the first time. But the structure of the vitro model isn't enough to simulate a real brain. The connections between the neurons are made randomly in brain organoids.

    b. If you feel like answering: what do you think about the morality or using brain organoids for research?

"Organoids are ethically seen pretty close to tissue. And we want to get as close to real tissues as possible. In general, we are trying to connect more different tissues to each other to get a web of tissues that is similar to the one we have in our bodies."

Note: All of Joep Beumer's answers are paraphrased.

6. Discussion

Although organoid research has been a relevant topic in biology for the past 50 years, it has made the most progress in the past 11 years after Hans Clevers first successfully pioneered it in 2009 out of his laboratory in Hubrecht, Netherlands. Human organoids have caused great advances in the research of homeostasis and organ development. One of its uses has also been to replicate viruses *in vitro*. This has led to the discovery of new regenerative therapies and the invention of patient-specific in vitro 3-D-cultures starting from both stem/progenitor cells (stem and progenitor cells share the same capability to differentiate into an alternate cell type). Organoids have a select few other uses as well including drug testing and there are many steps to be made in the future.[xxi]

One of the major roles that organoids could play in the future is the in the health industry. Steps are being made to take advantage of organoids for the battle of genetic/infectious diseases and has potential in the development of regenerative medicine. Besides the promise that they have shown for the previously mentioned future steps, organoids are important in the battle against cancer as well. Subsequent to the recent development of 3-D-culture technology, the procurement of more specifically applied organoids for more targeted therapies. Scientist have managed to foster numerous tumour organoids. These include some of the most common cancers like breast and prostate cancers (information on the discovery of all tumour organoids is referenced in source). These organoids are very similar to the

original organs that they are meant to replicate in terms of epigenetic characteristics, morphology, genetic specificity etc. This has created the opportunity to explore therapeutic options against these diseases.[xxii] [xxiii]

While it is clear that organoid research has many positive aspects in the health industry and developmental biology, it is not an ethically neutral topic. There are also a number of cons to organoids, one of them being the risk of commercialization. In view of the fact that organoids have very promising prospects and are quite modern, they can easily be patented. At the moment, the prices of organoids are gradually rising because of the possible profits for businesses such as pharmaceutical companies. This is seen as unmoral by a significant amount of people because organoids are derived from donated human tissue from the public. These donors do not receive and compensation for their contributions while large companies are able to generate large profits from them. Another concern is the weakness organoids have in drug testing. Although they are useful for the fact that with organoids animal testing can be avoided and the risks of unknown side effects can be reduced during clinical trials, they are also quite expensive and take a long time to procure. While organoids are good for testing therapies for the specific organ they came from, they do not account for the whole body so not all possible side effects can be observed. Another facet which is still being researched, is the possibility that donors feel a connection to the organoids that were generated by them[xxiv]

Another interesting topic of discussion are cerebral organoids (brain organoids). The main question is whether human consciousness, emotions or cognitive abilities can be mimicked. However, the research of this has controversy. For example, this specific type of organoid involves animal testing as well, which has already been a controversial topic since the seventeenth century. Mainly in the form of xenotransplantation (transplantation of organs or cells into other animals) of human cerebral organoids into rodents. This form of research raises the question of what dangers replicating human neural capabilities could have.[xxv] [xxvi] [xxvii]

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