Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
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W10L49_Different aspects of organ culture

Hello, everyone. Welcome back to another class on regenerative biology. And in today's class, we will learn about different aspects of organ culture. In the previous class, we learned about how different scaffolds or materials can be used for culturing or growing them, etc. We'll also see some challenges in the next class. But today we will try to learn about different aspects of organ culture in terms of the pros and cons, what is the best, and what are the less effective or not-so-good approaches to organ culture.

So we can understand what the possible vistas of matrix production are, artificial matrices are used extensively due to their various biological properties, but they come with their own challenges. However, now people have shifted to using natural ECMs or natural matrices, which is The same matrix that an organ has, say for example, if you want a liver, use the matrix from the liver only. If you want a kidney, you should use the matrix from the kidney only. So that approach, which involves getting rid of cells from an organ and retaining the matrix, can come from anywhere; it can be from an animal, it can be from another cadaver, or it can be from some other sources.

A donor, of course, cannot be used because you cannot take an intact organ from a donor just for the sake of getting the matrix, so if it is mainly from a cadaver, if it is not... going to be or that organ is not going to be used or it is already dead; it has crossed its time of transplantation, then that organ can be used for the matrix. So that is called a decellularized extracellular matrix for organoid and engineered organ cultures.

So, the repair and regeneration of tissues and organs using engineered biomaterials have attracted great interest in tissue engineering and regenerative medicine. People have been doing that. But recent advances in organoids and engineered organ technologies have enabled many researchers to generate 3D tissue that recapitulates the structural and functional characteristics of native organs as they are. Say your kidney is 5.2 centimeters in diameter or length; you can make exactly that, not 5.

3, not 5.1, exactly 5.2. Opening up new avenues in regenerative medicine. Such an approach has cleared a lot of hurdles, allowed us to jump over many troubles, and helped us get through the problems.

So the matrix is one of the most important aspects of improvement. Organoids and engineered organ construction, we know, matrix is the backbone of an organ; however, the clinical application of these techniques remains a big challenge because the current commercial matrix does not represent the complexity of the native microenvironment. It is not because of the bioacceptability, biodegradability, etc.; it is not able to; we are not able to. Mimic the complexity of the matrix, which in turn is due to the complexity of the organ itself.

It limits the optimal regenerative capacities. Decellularized extracellular matrix is commonly referred to as dECM. We all know ECM. dECM, or decellularized extracellular matrix, is expected to maintain key native matrix molecules that are believed to hold enormous potential for regenerative medicine applications. dECM can be used as a matrix for improving organoid and engineered organ construction.

In this sense, you can get rid of the cells from an organ. What remains is nothing but ECM. So let us quickly see an overview of organ culture. As you can see in this picture, the repair and replacement of damaged tissue or organs is driving ECM. and expanding need worldwide.

A lot of people are in need of organs. However, there is a critical shortage of organs available for transplantation, and post-transplant immunological rejection still remains a significant concern despite the availability of immunosuppressant drugs. In the United States alone, a country where all these details are documented, the annual expenditure for diseases related to tissue defects and organ failure exceeds 400 billion. 1000 million is 1 billion. So, 400 billion is per annum.

You can see here that you have organs: lungs, liver, stomach, and kidneys. What you can do is make all these organs. If you start with a decellularized tissue or organ, you take it and decellularize it, then recellularize it using patient-derived cells, and you end up getting either organoids or an organ depending on what organ you are trying to make. Sometimes an organoid is enough; it will automatically mature into the organ itself. For the liver, etc.

, you don't need an intact, full-fledged liver if a small piece is enough. If you make the connections, everything will be fine, and that liver will grow. And some organs, such as a stomach, etc., you need full-fledged organs; a kidney—you need a full-fledged kidney—so organoids or organs can be made from this, you know, decellularized matrix. So why ECM? What is the so-called? We have discussed ECM, and dECM is a new concept right now.

ECM plays a critical role in cell signaling, function, phenotype, and morphology, providing a beneficial microenvironment for cellular homeostasis, growth, tissue formation, and repair. It is not structural support, although we compare it to that of beams and pillars of a building, which is equivalent to a matrix. However, beams and pillars do not provide energy; they do not give nutrition, but these matrix proteins provide nutrition and a favorable environment. They will feel at home when the matrix is present. So, each tissue or organ has its own unique ECM.

Lungs have a different ECM. The liver has a different ECM. The kidney has a different ECM. And the interaction between cells and the ECM is crucial in regulating cell behavior, function, and fate.

So everything decides. Everything is very crucial and detrimental when it comes to ECM. Cell interaction. Due to the complex biological properties and 3D ultrastructure of the native ECM, replicating them using traditional manufacturing methods and biomaterials is often challenging. Hence, the ECM you need for your research or organ production can come from an existing organ itself, and you can make de-ECM from that organ. Now, why dECM? Decellularized extracellular matrix materials refer to biomaterials formed by removing immunogenic cellular components.

You may wonder why cells have to be removed. First of all, the cells are not from this person. If the cells were from the same person, then there wouldn't be any immunorejection. Because a liver cell from person X and person Y, there is no main difference. But the liver cell from person X, when put into Y, is a foreign body.

His Y person's foreign body will reject it. That is why we don't want that cell, but in contrast, the matrix is not an issue because the matrix doesn't have the MHC complex protein present on the surface of those cells, which is unique to individual humans; so immunogenic cellular components are what you are trying to get rid of from humans, or if it is from another animal, say you took the kidney from the pig, everything is fine, but you don't want the pig cells, although the pig kidney is working perfectly fine. So, animal organs and tissues always cause severe immunological rejection. But if you decellularize it, then that matrix is not a significant antigenic molecule in your body. dECM minimizes changes to ultrastructure and composition and provides an excellent 3D microenvironment for subsequent cell seeding, opening new avenues for tissue engineering.

Because of the complex and unique nature of the ECM meshwork, when you seed the cells, they will recapitulate the same structure as it was in the original organ itself. In

clinical practice, dECM is being progressively adopted to support tissue repair and transplantation therapies. We will also see some examples. Let us see some examples of how and where we stand as of now. Various commercially available DECM products have been developed, including Alloderm, a registered trademark, and Graft Jacket, another product, which are derived from human dermis.

It is used for repairing skin wounds and tendon and ligament injuries; say you got some serious injury. It will be filled with fibroblasts. You may have seen surgical sutures. You can even see after 20 years, oh, there was a surgical suture that mark will stay because of the fibrosis. Because you damaged not just the epidermis, but also the dermis.

The moment the dermis is damaged, the basement membrane is affected. Hence, you will get fibrosis or fibroblast migration. But now, imagine a situation. You have the ECM. Of the skin, you are putting on top of that wound area; now the cells from the surrounding area will migrate onto this ECM, and it will restore the normally looking skin, so you have no way of knowing if there was a cut or an injury.

These are all very easily doable things; that is why Alloderm, grafts, jackets, etc. have come into the picture. And they are also used for several other wounds of the tendon, ligament, etc., which a sports person normally gets. Another company, Oasis, which sources porcine small intestinal submucosa, is utilized for skin wound healing.

While Prima Plus and CardioCell, two other companies derived from porcine and bovine cardiac tissues, are employed in cardiac valve repairs. The emergence of these products underscores the versatility and potential of dECM-based biomaterials for clinical applications. So we can use them to fix any of the damaged parts or organs themselves from these commercially available sources. Currently, where we stand is that tissue engineering materials derived from dECM have been widely applied in the fabrication of engineered tissues and organ-like constructs, including the gastrointestinal tract, kidneys, liver, lungs, pancreas, uterus, bone, and cartilage. heart, skin, brain, esophagus, oral cavity, and so on.

There are many other examples. So pretty much every organ, if the damage is moderate, you can take the help of these commercial firms to fix it. Now let us see a little bit about the organoids, their history, and how they have developed, etc. Single cells or small clusters of cells proliferate and reorganize to form complex structured cellular assemblies that mimic the functional attributes of specific organs termed organoids. Organoids are nothing but organs themselves, but they are miniature versions. Like you may have seen in some houses, you will have a huge miniature version of a guitar or a miniature version of a tabla, something like that.

But it is, you imagine, if they are functional as well. Sometimes some miniature versions will also be functional. It is just smaller. The study of organoids traces back as early as the 20th century. In 1907, Wilson demonstrated that dissociated sponge cells are capable of self-organizing and regenerating entire organisms themselves.

Sponge, the animal. The foundational work for organoid technology was laid in 1981 when Evans and Martin independently isolated pluripotent stem cell lines from mouse embryos, with Martin naming them embryonic stem cells. By 1998, Johns et al. had successfully isolated and cultured embryonic stem cell lines from human blastocysts, expanding the possibilities of using stem cells to culture organoids. Significant advancements were made in the years 2000, 2009, etc. When Professor Hans Clevers coined the term organoid, he cultivated the first intestinal organoid from Lgr5+ stem cells derived from mouse intestine.

Recent years have seen several rapid developments in organoid research, including Mendjan's generation of self-organized cardiac organoids from human pluripotent stem cells. In 2021, Clevers et al. The establishment of a human fetal hepatocyte organoid model was shown. So we are developing the organoid era like never before. Significant advancements in the reproductive system of organoids were made from 2018 to 2022.

So, this is a historical overview of organoid development. I will not go to each and every section. You can read about it from 1907 to 2022. If you see that there is rapid development in the field. You can see that in each year, some development or another has happened.

You can go through it. Those who are interested can read this article too. Decellularization of the extracellular matrix is performed. Although we are telling dECM how easy or how difficult it is. We should try to understand that. dECM is a promising natural biomaterial prepared from human or animal tissues or from organs themselves through decellularization.

The idea is very simple; there is no complexity to understand. Take a liver, let it be from human cadaver or maybe from an animal, get rid of the cells, retain the liver as it is, just remove the cells. That is a simple concept. dECM retains the 3D structure and biological properties of ECM, exhibiting bioactivity, biocompatibility, and non-immunogenicity; the most critical part is that it is not immunogenic and preserves numerous cell growth factors such as fibroblast growth factor, transforming growth factor, and hepatocyte growth factor if it is for the liver. which can enhance seeded cell growth, migration, proliferation, differentiation, and angiogenesis.

We discussed it. If you made a properly functioning tissue in a tissue culture, but it doesn't have blood vessels inside, then how will the host supply nutrition, oxygen, and glucose to those tissues? Impossible. So angiogenesis, the blood vasculature is more important than the tissue itself. Otherwise, it will be like the operation was successful, but the patient died. You don't want a scenario like that.

Oh, beautiful kidneys. I made it, but it doesn't have blood vessels. So it will look like a toy model, like we saw in the previous class. It will be like that. It doesn't make much sense. This real-time interaction with the seeded cells can reshape the structure of tissues and organs, playing a crucial role in tissue and organ regeneration and their functional repair.

Therefore, various scaffold materials based on dECM have garnered increasing attention in recent years because they work. Hence, people will go after it. The origin of ECM is unclear. ECM can be derived from three primary sources. Human tissues, cell cultures, and animal tissues are used in research.

This we know. These are all the sources of ECM. ECM from human sources significantly reduces the risk of immune rejection associated with xenogenic ECM. Although we say this dECM ECM doesn't have immunogenicity, etc. But if it is from a completely different animal, there is a possibility that the ECM protein itself can have some antigenic material. There is a possibility, such as the response to alpha-gal epitopes. Sometimes, some animals can have unique epitopes in their ECM proteins.

Which can be immunogenic. Hence your organ can get rejected. Human-derived ECM best mimics the structural and functional properties of native tissues and organs that don't have an immunogenic epitope for another human being, offering superior biocompatibility and clinical efficacy. Ideally, tissue from young, healthy donors is the best source, but human tissues are relatively scarce, and their availability is limited, especially for younger, healthier tissues that are difficult to obtain; cadaveric tissue can be used as donors, but availability and acquisition are limited for cadaver skin. Sold as Alloderm and Graft Jacket, it is used clinically for wound treatment in modern days. Tissues associated with birth, such as the placenta, umbilical cord, and fetal membrane, are often discarded after delivery, making them more abundant and accessible. Hospitals, after birth, if they are throwing it, if you can collect it, that will be a great thing to have.

Studies have shown that ECM derived from placental tissues possesses favorable immunogenic and regenerative properties, containing various growth factors. That supports the growth and differentiation of diverse cell types. Because it is from a fetus, it

is pro-regenerative in any way. So those ECM will be superior to an adult ECM.

For instance, research by Murchison et al. demonstrated that placental ECM hydrogels can serve as a suitable substrate for hiPSCs differentiation into three embryonic layers. You can make ectoderm, endoderm, and mesoderm. Wang et al. utilized placental dECM hydrogels to construct spinal cord-like organoids that are more stable and mature than those developed using simple Matrigel. So we should understand that the embryonic leftover tissues have huge potential.

Anyway, we are throwing it. So let us see what the decellularization methods are. This is an overview of common decellularization techniques and post-treatment processes, as you can see here. Decellularization of target tissues is effectively achieved through chemical, physical, or enzymatic methods, with combinations of these approaches often applied first. To a given tissue based on which organ, the strength, the tissue density, and cellular density will vary accordingly.

You have to come up with a strategy. The harvested decellularized extracellular matrix (DECM) materials are sterilized to eliminate potential microbial contamination, reduce toxicity, and enhance biocompatibility. Inevitably, these processes can impact the structure and functionality of the dECM, which is frequently addressed by deep employing solubilization. And cross-linking methods for improvement. As you can see here, chemical treatments are given, biological treatments are given, sterilization is applied, dissociation and cross-linking are applied, and physical treatments are applied. So once you harvest the organ, you have to undergo extensive processes.

Biological treatment can be trypsinization, nucleus treatment, and physical methods, such as applying pressure, pulling, pushing, etc., so there are different approaches, as you can see in this cartoon, but they have to be applied to achieve a proper result. DECM is prepared. So this is an overview of the application and research progress in organoid and engineered organ based on DECM materials. So the utilization of stem cells and organoids to populate the whole organ decellularized scaffold needs to be done if you want an organ.

Following the cultivation of bioreactors, it has significantly advanced the field of whole organ bioengineering. So, with the rapid progress of these technologies, the researchers demonstrate the immense potential of this application in regenerative medicine and organ transplantation. It offers promising avenues for addressing current challenges in these fields. So you can see here, there is an injury, cancer, failure, etc.

In the patient, there is a shortage of donors. It's not available, and hence the

transplantation is not possible. The whole organ bioengineering is one option, and the bioengineering of organoids is another option. And we have to decide on the strategies of bioengineering based on the availability of the dECM.

That is what you are seeing. It can be from humans, cadavers, pigs, etc. And you can decide which ECM materials can be used. So ECM materials exhibit tissue and organ specificity and can be enhanced with relevant proteins and cellular molecules to promote the development and construction of organoid models closely mimicking those of the natural organ itself. So now another example is the application of dECM materials in gastrointestinal organoids and engineered organ culture. One example of how you deal with this is a complex picture, but don't worry about that.

Those who are interested can read this article, as a reference is given. First, A is the decellularization and characterization of human small intestine and colon scaffolds. You can see here that it's a part of the gastrointestinal tract. And B is a schematic representation of the strategy for in vitro preparation of functional human jejunal mucosal graft. Using a bioreactor that simply gets rid of the cells, you inoculate them, providing them the nutrition so that they colonize and see the bright field images of the gastrointestinal organoids cultured in the decellularized stomach-derived ECM; it is also known as SEM (stomach-derived extracellular matrix) or decellularized intestine-derived ECM (IEM).

And hydrogels and Matrigels are also compared to that of this dECM. And panel D is a schematic diagram illustrating the application of dECM hydrogels carrying GI organoids as grafts for treating acute epithelial injuries in mouse models. Of the stomach and intestine, you can use mouse models to prove the principle with these animals, and that is what you are seeing here in panel D. People use GFP cells to show that the newly formed organ has no cells, or vice versa. If you took the dECM from a wild type, you can use GFP-expressing cells to show that, yes, my cells are staying back.

Either way, one can do it. And if you look further, That's where we stand. If you look closely once again. The organoids that are produced when taken from a donor with a transgenic reporter line allow us to trace the fate of those cells. Sometimes it may happen that the host-derived cells may intercalate into these organs, newly placed organs, and this organoid can become a fully functional organ. So, with that tracing work, you will end up getting a chimeric organ. So organoid transplantation, once it is done, must be followed precisely by observing how the donor cells acclimatized or how the host-derived cells got acclimatized.

It can also happen sometimes that the wandering neural crest or other wandering stem

cell populations can contribute. Such studies are extensively done using various animal models. We cannot do that in humans. So, looking into the conclusions and outlooks of this dECM approach. dECM is a designated approach to mainly remove the immunogenic cells while preserving the original tissue architecture and composition.

We know by now that the purpose of the dECM is to have the proper identity of that organ or organoid, whatever you are making. Organoids have to become proper organs. Or if it is part of an organ, then you don't need to fix the entire organ.

Only part of the organ is bad. Then you only need an organoid. You don't want the full organ. It's like if your car's tire has only gone bad, you don't need to have a new tire. So the tire is an organoid, part of the car. Due to its inherent structure, enhanced bioactivity, reduced immunogenicity, and favorable biodegradability, the dECM has generated widespread attention in the fields of tissue engineering and organ culture for biomedical applications. The prevention of decellularization techniques or the use of techniques to prevent decellularization are the prevalent methods most commonly applied; they use physical techniques, chemical techniques, and enzymatic tools, which can be employed individually or in combination, depending upon which organ you are dealing with.

Currently, dECM-derived materials have been increasingly applied in the preparation of organoids, engineering, and other engineered organs. Further research into the specific impact of ECM in different tissues and various disease sources on dECM and subsequent bioengineering needs to be done to enhance organ models, physiological complexity, and clinical relevance. Although we know proof of principle is done, this approach may not be foolproof in every available scenario; that is why one has to work on this approach to improve the biological acceptability and how easily, just like you manufacture a phone, there is a conveyor belt and an assembly line, so that it should reach everyone and they can benefit from it. Despite the challenges in exploring dECM for organoids and engineered organ fabrication, ongoing research and technological advancements bring us very close to creating viable transplantable tissues and organs.

We are reaching that stage. These developments include optimizing decellularization processes, refining organoid construction methods, scaling up production, and enhancing the matrix biological cues to guide cell behaviors. The continuous improvement of these technologies holds great promise for future organoid engineering and engineered organ fabrication. So the future of organ culture and organoids is on a very promising track, but many researchers have to take action to benefit humanity. We will learn more about regenerative biology in the next class. Thank you. Thank you.