Regeneration Biology
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Week: 12
Lecture: 62

W12L62_Future implications of Organ culture in patient care

Hello, everyone. Welcome back to another class on regenerative biology. And in today's class, we will learn about the future implications of organ culture in patient care, specifically discussing how we will advance organ culture in the context of regeneration and patient care. Organ culture offers significant future implications for patient care, potentially revolutionizing organ transplantation and disease research in general. We have seen why and how we know this, but we will still do a recap on creating a lab-grown organ and using that organ to culture and study various diseases. Can address the reasons for how the disease manifests in the host, and you will also know how it responds if you are putting in a new organ, how it is going to respond in the host.

Additionally, we will better understand the organ shortage issues and how we can minimize rejection risks and allow for personalized medicine. So, what are the factors that we can add to the cultured organ that can minimize rejection? Or mimic more of a humanized organ, so these are all the future angles. Furthermore, the organ culture can provide a more clinically relevant model for studying disease mechanisms and drug efficacy than traditional cell culture. Whatever we can learn from traditional cell culture, we can learn much, much more.

When you are creating an organ and studying the organ, note that every time you culture the organ, you do not have to use it for transplantation; you can study the organ's behavior in vitro as well. If you look at an update in the US, this is a statistic from a 2020 published paper, as it is mentioned here. The most needed organs on a transplant waitlist and their respective percentages in 2020 have been given in A, and in B, the number of individuals on the waitlist and the number of transplantations performed from 1991 to 2019, which means roughly around 30 years, that is what has been shown here; and in C at the bottom, the number of transplants performed in 2019 organized by. Based on whichever organ you are talking about. So you can see here that the majority of the trouble in the waitlist is for kidney transplants, 83%, and 11% is for liver transplants, and then you can see heart, lung, and other organs because these are all the main organs that are in demand as they deteriorate much more easily.

And if you look into donor waiting, donor availability, and also transplantation, if you

look at this panel, you can see that the number of transplants is going to be more or less the same. Hardly there was any change from 1991 to 2019. A marginal increase is present, and the waiting list has grown exponentially. You can see that the waiting list has gone way too high. The gap between demand and supply is very high; it has widened significantly, and the number of transplants performed in 2019 alone was around 23,400 in the U.

S. If you look at kidney transplants, that number is substantial, and for liver transplants, it is around 8,000. Just in 2019 alone, you can see how huge the number of transplants is. pancreas, intestine, lung, etc. So it says that the alarmingly large number of requirements is increasing. So, addressing the organ donor shortage, how do we tackle it? Lab-grown organs cultivated from the patient's own cells could eliminate the need for a donor organ.

You don't need to wait for a donor; you can make it. Solving the critical shortage problem. Being genetically identical and made from your own cells, these organs would significantly reduce the risk of rejection. And the major hurdle of the current transplantation is the risk of rejection. So you can get rid of that.

So, the personalized medicine leads to improved outcomes. So the current scenario is reaching a stage where you don't want to be a burden on others. So you have your own cells. And if you produce it, you have money, you have the opportunity to make an organ, and you transplant it. In vitro organs can be customized to individual genetic profiles, improving transplantation or transplant success rates and long-term outcomes.

Since it is your own organ, it will stay behind for a longer time. This approach aligns with the trend towards personalized medicine, tailoring treatment to each patient's specific needs. Since your own organ is being made, you don't need to be on immunosuppressants; hence, you will not have any more complexity because of the immunosuppressants. Enhance disease research and drug development. So the organ culture provides a more accurate model for studying disease mechanisms and drug effects than traditional cell cultures.

That is why, although in cell culture you can expand cells and transplant them into the diseased organ, if you can make the organ itself, all of them will be new, and the trouble that the patient is facing in that residual organ will not be there at all; you can replace the damaged organ with a fully functional one. Then, this tissue culture-based production can be avoided, especially when you are transplanting the cells; you can transplant the organ itself. Researchers can use diseased organs, even those removed during surgery, to investigate the causes of disease and identify potential treatments. You can also take an organ, then take the cells from it, culture them, and create a new organ to see if these

cells hold clues. Why did the disease occur at all? So there are multiple angles from which you can approach it, and then in vitro you can treat with different drugs and see which drug is helping, which drug is contributing to the repair, etc.

And overcoming the limitations of existing methods. The traditional cell cultures, like the production of cells, can lose key functions over time. Making them less reliable models, while organ culture preserves the integrity of cells and tissues within their natural environment, because when the cell is three-dimensional, of course, credit goes to the ECM, and then it will be more homely than when you make them into a two-dimensional flat surface. The organ-on-a-chip technology, which we have discussed in the past, is a form of organ culture that allows for studying fluid dynamics, nutrient support, and drug gradients, offering a more physiological approach. Organ culture is much closer to the functioning of the organ than just tissue culture.

So if you look into the future directions, there are multiple options for organ culture. Bioprinting, the 3D bioprinting technology we have discussed in the past, is paving the way for creating complex organ structures, potentially leading to the fabrication of functional organs themselves. And then the stem cell therapy comes. So stem cells can regenerate damage to tissues and organs. Offering potential alternatives to organ transplantation, say myocardial infarction, you have created fibrosis, and because of which the heart is not contracting.

You can get rid of these fibroid tissues and colonize them with cardiomyocytes or stem cells that are capable of differentiating into cardiomyocytes. Now your fibroid tissue has functional heart muscles. So these are all the approaches to stem cell therapy. Then comes regenerative medicine. Research into regenerative medicine, including organ regeneration, could lead to the development of new therapies for organ failure.

We can understand the reasons and situations through which an organ fails. Let us see what the challenges and considerations are to be kept in mind. One is functionality. Ensuring that lab-grown organs function properly and can be scaled up for clinical use remains very challenging because, as the saying goes, "the cow in the book will not eat grass." Although it is an ideal organ you have made, if it has not been integrated functionally, it's not because of immune rejection; rather, its structure is not made properly so that the neuronal connection and the blood vascular connection are not ideally linked.

Then that organ will not have a future, and the regulatory network establishing clear regulatory frameworks for the development and use of bioprinted and other engineered organs is very, very important; otherwise, you can end up in a lot of trouble. Therefore,

the regulatory networks have to be established precisely so that there will not be any. Legal or non-legal or medical legal issues come, and then, lastly, the ethical considerations and ethical debates surrounding lab-grown organs and their potential implications for patient care need to be addressed. A rich man decides, "Oh, my liver is not up to the mark." The liver has no problem; he's thinking, "No, I want to live for another 50 more years.

" So my liver, let me get it fixed. My kidney is old. So let me get my kidney fixed. My lungs are very old. They have got some disease.

So let me get that fixed. Let me replace my individual organs, which are all vulnerable and will affect my longevity. A rich man then decides whether giving him a longer lifespan is ethical or not. So these are all the angles that will come into the picture. Approaches to engineering whole organs.

Let us see. There are two major approaches. One is a top-down approach. Another is a bottom-up approach. So let us see them in detail. There are two major approaches, as I told you.

One is the top-down construction, and the other is the bottom-up construction. So the concept of top-down engineering is to develop scaffolds on which cells can grow and form functional tissues. Macroscopic or even whole organ scaffolds are seeded with one or many cell types, where a combination of scaffold remodeling and self-assembly leads to the formation of complex 3D tissue or the whole organ itself. In contrast, what we see is that bottom-up engineering utilizes the smallest component elements of tissue, such as collagen molecules, as building blocks and combines them to assemble a larger construct. So you make pieces and then assemble them, or you already have a large scale, and then you allow the cells to colonize on it.

Similar to erecting a building by adding one brick at a time, the bottom-up engineering methods are designed to control and organize the interactions of cells with each other and their surroundings. This is how they work. Using stem cell engineering, it is very much possible to utilize a single building block, human pluripotent stem cells (HPSCs), to engineer constructs such that organoids and organ-specific functional tissue are created through the bottom-up approach. So, the top-down and bottom-up approaches can both go hand in hand when you are thinking about engineering a whole organ. So the comparison of top-down and bottom-up whole organ engineering strategies is mentioned here.

Top-down approach. Here we have the cells, and then you have the scaffolds, the stem

cells or differentiated cells, or you have a biologic or synthetic scaffold. You bring them together, seeding, repopulating, and self-organizing into complex tissues. That is what you are seeing in a top-down approach. In the bottom-up approach, what you're seeing, additive manufacturing, is basically 3D printing where you use cells, biomolecules, or substrates, put them together, and create a complex biological structure meant for replication. So both are done in a manner that benefits the patient.

But the approach and the strategy are different. So if you look further, if you want to conclude the way in which the regeneration, regenerative medicine, or organ culture is evolving, we know significant interest has been shown in whole organ engineering globally. Tissue engineering and regenerative medicine have grown exponentially over the past few decades, on a logarithmic scale, in a very promising way. It has grown because there are dedicated institutes. There are dedicated programs that have evolved to meet the demand for organs.

So this has been especially true because end-stage organ failure continues to be a leading cause of morbidity and mortality around the world. Maybe because of your eating habits, maybe because of your living environment, maybe because of your stressful conditions, organ failures or human organs started failing more often than before in the recent era. So orthotopic organ transplantation is currently the only curative treatment option available for patients diagnosed with end-stage organ failure. As of now, no other alternative is available. However, significant advances in the bioengineering of whole organ constructions continue to advance rapidly, giving hope to those waiting for transplantation.

So, if you look further, the key technological advancements have supported whole organ engineering over the past few decades. These include, number one, the methods of whole organ decellularization and recellularization, which we discussed: how to take a pig's heart, get rid of the cells, and use only the matrix to colonize it. You can revisit. And then three-dimensional bioprinting, where you can create the organ itself.

And advanced stem cell technology. And fourth, the ability to genetically modify tissues and cells is important. These advancements give hope that organ engineering will become a commercial reality in the next decade. So that is what the scientists believe or what medical science believes. However, we must understand that several barriers to commercialization that need to be systematically addressed still exist as major obstacles to becoming a success. Engineering whole organ constructs with adequate cell populations is a significant technical barrier, as it is currently highly challenging to create an engineered organ with a homogeneous cell density close to the high cell density that native organs possess.

We must understand that each organ is very tiny in the embryonic stage when the organism is developing; the cells are proliferating, but they are not proliferating like a balloon. They are proliferating; some of them are dying, some of them are growing; again apoptosis, again proliferation. So there is a tug of war that exists because of the limited available space; when you allow an organ to grow, it becomes quite dense. But when you are culturing them in vitro, you will not be able to provide that environment. Like when my kidney is growing, it has pressure from the intestine, pressure from the body muscles and body fluids, the liver on top, and the lungs on the side.

These pressures are not present. When you are culturing, the cells will remain loose. So this is one challenge that organ culture faces in addressing this barrier. Stem cell research must be developed to improve cell invasion and the addition of organ constructs. We cannot tell cells to come close, come close. So you have to apply some pressure so that it will be dense enough.

In addition to improving the density of cells incorporated into engineered whole organs, the diversity of cells used must also be enhanced to better mimic the cell diversity found in native organ tissue. So, through the whole organ de-cell and re-cell approach, the de-cellularization and re-cellularization approach, and the cell bioprinting approach have demonstrated the ability to incorporate multiple cell types into engineered organ constructs. Now it is possible for you to incorporate multiple cell types into the cultured organ. This is often to replicate only a single part of an organ's function rather than replicating the entirety of the organ's function. It becomes a little difficult, a part you can deal with.

Also, though the variety of approaches to stem cell culture and differentiation, whole organ recellularization and decellularization, bioprinting, and genetic modification techniques being developed is conducive to research purposes. Standardization is vital for clinical implementation; that is another angle to consider. It's not that you can just take one patient who came in and made an organ. Okay, I don't know whether it will work or not. I made an organ, and you take it; if it worked, good for you; otherwise, it's not good for you.

It can't happen that way. One has to standardize and remember each patient is unique, so we cannot standardize for one person and expect the same result for another. So it is a challenging situation. Standardization necessitates comparing various methods in comprehensive studies to identify the safest and most effective approaches to engineering whole organs. So, as demonstrated by multiple studies performed by different research groups, this is the fact that we obtain from various other research studies. As an ultimate

goal of whole organ engineering, it is clinical translation and transplantation.

These standardized protocols must undergo a strict regulatory examination. So the regulatory pathways play a very major role. The regulatory pathways are an additional challenge facing the utilization of engineered whole organs as viable transplants and are principal among the non-technical barriers to commercial implementation, which basically means regulatory pathways should not hinder the use of cultured organs to the patient. So that has to be more conducive to the patient community. This is an important challenge, although it may sound very technical; the important challenge is that the regulatory pathways ensure the novel medical technologies can only be used commercially if they can properly demonstrate safety, with all potential risks and their respective likelihood of occurring being documented.

One simple example: you made a fantastic drug for cancer. And it cures every known type of cancer. However, that drug, when you want to solubilize it, has a slightly toxic solubilizing agent.

It is not going to kill you. It is slightly toxic. You will not get FDA approval or approval from any other agency. It is not going to kill you. Maybe there are more toxins in your food. But the moment that particular solvent comes into contact with a toxin or a not-so-friendly molecule, then you cannot.

Like urea, which is produced by your body. It is a toxin. So you cannot use urea as a solvent for consumption. You are not going to die because you had small quantities of it, as it is present in your body. But the same logic applies. These regulations have to come in an idealistic scenario.

So, as a result. Manufacturing said organs is another challenge outside of developing transplantable whole organ technology. The current approaches for engineering whole organs involve processes with low production volumes and no indication of the ability to maintain consistent quality at a commercial scale. So, as a result, the current methods of whole organ engineering would not be able to make a difference in reducing the number of individuals who die from end-stage organ failure. Technology is there, but it is not powerful enough or sufficient to reach into a solution, like, don't worry. Partial solution; some people are benefiting, but it does not significantly reduce the number.

The patient's death has not significantly been... As such, developing novel manufacturing techniques in parallel with ongoing whole organ engineering research is necessary. So, despite the field of whole organ engineering being quite new, much attention has been focused on it. So it is, and the relevant technologies, such as stem cells, have been

developed to advance whole organ engineering. So ideally one has to find out whether transplanting a few stem cells is beneficial for this patient or if you need to transplant an entire organoid onto an entire organ itself.

So this has to be dealt with. You cannot say that again; like I mentioned, each patient is different. So in one patient, you put in a few cells, and he did not survive. He or she did not survive. It can raise questions. So there was an option of putting the organoid in the whole organ itself.

But instead, this doctor put in only cells, which were not powerful enough. You know, these are all the challenges that the implementation stage can encounter that can lead to serious trouble. Though there are many barriers to the clinical implementation of engineered organs, the critical need for the replacement of allogenic organ transplantation makes the whole organ engineering research vital in overcoming these barriers. So what we understand is that the replacement of an allogenic organ through transplantation makes the engineering of the whole organ very, very important and vital in overcoming any of these barriers. So ideally, we can conclude by saying that the technologies out there can make the organ and transplant it to the patient, and in some patients, it works effectively and efficiently, and there is no serious trouble that stops them from getting these benefits.

However, the long-term goal, once you put the organ in, is that just because it was not autologous, it is not made from their cells, or maybe the humanization of that big heart or kidney or liver did not work the way it should have worked, will all come back to a serious question for the medical science or the research community, that is why. When you are implementing any of this technology into a commercially scaled experimental level, it is working with terminally ill patients; people are doing this because doctors will say yes, they are not going to survive, they are not going to make it. Then you can say you are not experimenting; at least you are trying. Okay, this patient is not going to. Be alive, so why don't we try to necessitate their further movement or push their biology with the help of this transplanted organ that is available? That has been done, but it has not reached a stage where one can get across a counter and say, "Okay, hey, I want an organ, so let me get it transplanted.

" So this is what I had to explain about the future prospects of the organ culture. However, I would like to give an overview of this whole course in maybe the next two or three minutes. So this course was designed to elicit interest in the research community, which means that the student community and the research community can understand that there is a field called regenerative biology, and there is huge potential in the future; the demand for regenerative biology and experts in regenerative biology is going to go sky

high. And in many countries, developed countries have moved much further ahead. There are dedicated regeneration biology institutes, and there are many dedicated programs that exist to nurture and promote regeneration biology.

But in our country, we are still catching up, although we have some institutes dedicated to regeneration biology research, such as INSTEM Bangalore. But the potential for a country like India is much, much higher. The research community should also develop along with the demand; that is why courses like this in regeneration biology become more attractive and fascinating. In general, the best way to learn about regeneration biology is to study those organisms. Who can regenerate? So the question is very simple: if you have a damaged organ, why does it persist? Like if your skin is peeled off, it is not going to be an open wound forever; after one week, it heals.

Why doesn't your damaged organ heal? The reason is, why doesn't it fix itself? In some animals, they do that; they don't suffer, you know. If their organ fails, it's not that the rest of their life is with that failure. It doesn't survive like fish and frogs, which are examples we have studied. And why doesn't such a mechanism exist in animals like mammals or humans? That is the big question. So in this whole course, we were trying to understand how we deal with or how we learn.

From these various models of organisms and how they behave or interact with the environment, we learn what it means to be in a damaged condition and how they fix the damage in a given amount of time, what the trade-offs are between wound healing and regeneration, and who the decisive factors are. So these are all the things we learned from this course. And I would also ask you to read several textbooks and review articles to enhance your knowledge because this field has huge potential, and no one should hold back, even though some of you may not be actively studying students. Some of you may be taking this course, maybe I say as a part-time job, but try to kindle the information or knowledge that you gained. By expanding, it can be helpful for your family and other friends, relatives, etc.

So I would very strongly suggest that you enhance your knowledge by reading more and more about the latest developments in the field because that is good for the country, good for mankind, and good for the whole planet. OK, so I hope all of you had a nice training from this course and those who waited for this until this last lecture. You know, I really appreciate your patience and tenacity, and I wish all the best for all of you. Thank you.