Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 12
Lecture: 60

W12L60_Xenotransplantation vs organ culture in practice

Hello, everyone. Welcome back to another class on regenerative biology. And today's topic is "xenotransplantation" versus organ culture in practice." So we will try to understand what xenotransplantation is, the scenario in which it is needed, and the different angles and aspects of organ culture in practice. So, xenotransplantation and organ culture go hand in hand. Okay, so in the sense, if I want to give a simple example, it's like ordering food online versus cooking.

Given a scenario, anyone will order food online so that they can get the food without any difficulty, but if no service is available or no restaurants are there, that's the time when you have to make food on your own. And you can have it, and one can say it is less tasty, or one can say, "Oh, it is less tasty," but it is of higher quality because with ordered food, I don't know how it is made, who made it, and whether it was made fresh or old; except that it is hot, we have no idea. But when you make it, you have good control over your lot of QC, something like that. So if X doesn't work, you do Y.

So that's how seno transplantation and organ culture go. Xenotransplantation is the transplanting of living cells, tissues, or organs between different species. That means you take from pig to human, or you take from rat to mouse, or from rabbit to elephant. This is a xenotransplantation. Organ culture, the process of cultivating and maintaining organs or tissues in a laboratory setting, is a field with significant potential to address the global shortage of transplantable organs.

Transplantable organs can be from a donor or transplantable organs can be from another species. Then this doesn't meet the demand; then comes the organ culture, while xenotransplantation aims to provide a new source of organs from animals like pigs. Organ culture offers a way to regenerate or repair damaged organs in vivo. Essentially, both do the same job, but in reality, the Xenotransplantation is definitely not a suitable solution. You can see in a little while.

Both approaches are actively being researched and developed using AI. These days, we use a lot of artificial intelligence tools that also play a role in enhancing their effectiveness and safety. Sometimes, you have to assess: can this person be given

immunosuppressants to retain this xenotransplanted organ? Sometimes the immunosuppressant makes the person more vulnerable. They can die from an infection. So you have to make a decision.

Xenotransplantation shows its potential; "xeno" means strange. Transplantation means putting one organ into another, so now transplantation could significantly increase the availability of organs for transplantation by utilizing animals like pigs, which have organs relatively similar to those of human organs in terms of size and functionality. And the challenges include significant challenges that still remain, including overcoming immune rejection, ensuring the safety of animal-derived organs, and addressing ethical and religious concerns. Sometimes it may happen due to an animal. When you take an organ, it has some viruses, but it was harmless in that case.

An organism, but once you get it, maybe that virus will kill you; you would have lived some more years with that defective organ. The moment you transplant that virus present in that xenotransplanted organ, it can kill you, so this is what one should be bothered about. A lot of tweaking has happened because of the artificial intelligence being used to optimize the process of creating genetically modified pigs, which reduces immunogenicity, predicts and manages rejection risk, and improves the efficiency of seno transplantation procedures. These days, it's a land of artificial intelligence that can handle many parameters and come up with logical conclusions. The best way to approach or adopt for treating this patient.

So one example includes CRISPR Cas9 gene editing being used to remove immunogenic molecules from pig organs, making them more compatible with human recipients; we usually refer to this as humanized. If there are five proteins that are present which can be immunogenic in humans, if you take that organ and put it in a human, what if in the pig itself you get rid of those proteins by editing or mutating them, and then you take that organ? Then they will never be expressed, and hence your immune system will not reject that pig organ. So the public perception of this kind of approach and the concerns about xenotransplantation include ethical, religious, and social factors that need to be addressed for its implementation. Organ culture, on the other hand, has huge potential, as it offers the possibility of regenerating damaged organs and creating artificial organs to replace diseased or damaged ones. So that is the simple approach to organ culture.

The challenges include maintaining organ viability and functionality in vitro, creating biocompatible scaffolds for organ regeneration, and addressing the challenges of vascularization and innervation, which are the major hurdles. Say you put in an organ, but if that organ is not getting the blood supply from the host or it's not receiving the nervous tissue command from the host, then that is not going to function the way it

should because each and every organ in your body functions because of the inputs coming from your nervous system. So your nervous system should be able to control that organ. Then it will only become a part of life. And here, artificial intelligence also plays a major role.

AI can be used to analyze intricate processes of organ regeneration, optimize culture conditions, and design biocompatible 3D printed organ scaffolds. So all these angles, the AI can come into picture. For example, AI can be used to predict the growth and development of cells in vitro, leading to more effective organ regeneration strategies. If you have a given cell or tissue in your hand, you can predict what the best medium is that can be used, how fast it can grow, etc. Based on many other parameters that are available to analyze for the AI.

And the ethical considerations also come into organ culture. The ethical implications of creating artificial organs and the potential for their misuse must be carefully considered because, like any technology, they can have side effects that include misuse. Evolving areas in heart transplantation. We'll take the heart as an example because it is one of the major organs that are being looked into for organ transplantation. Xenotransplantation refers to the grafting or transplanting of organs or tissues between members of different species.

This we have seen with increasing numbers of patients with end-stage heart failure and a shortage of donors, which means heart donors. There has been renewed interest in the possibility of cardiac xenotransplantation. Like we discussed, you can create a humanized pig heart, and you can transplant it. Advantages include an unlimited supply; no one will say that pigs are in short supply, hence we can have enough hearts. Since we can grow them as many as we want, there is an unlimited supply and elective availability of donor organs, expanding the candidate pool.

Sometimes you cannot choose, "Oh, I want only a 25-year-old person's heart," because I don't want someone who is 50 years old's heart. You cannot choose, but whereas if it is an animal, you can choose the age, what should be the age based on your demand. And you can also expand the candidate pool options and avoid the detrimental effects of brain death and donor organ function. This can be avoided. Although monkeys and baboons are most phylogenetically similar to humans, given the ecological and ethical restrictions, the pig is the only animal available to replace human tissue.

In various other angles, the pig is much closer to that of a human. The pig is physiologically similar to humans, and its organ size is compatible. It can be raised in a controlled environment and, more importantly, can be genetically modified so that there

will not be too much immune rejection from the human body. So the main limitations of xenotransplantation are rejection, which includes hyperacute, acute, and chronic vascular diseases, and the risk of transmitting zoonoses, which are various diseases called zoonotic diseases, such as many viruses that are present in the donor and can be transmitted to the host, potentially creating zoonosis. So the development of genetically modified pigs has allowed the expression of human antigens that can reduce the risk of rejection and provide hope for future feasibility.

That is called a humanized pig or a humanized organ. If you look at the table taken from this publication, you can see the current scorecard of the advantages and disadvantages of xenotransplantation. Unlimited organ supply and elective availability, expansion of the candidate pool, avoidance of the effects of brain death on cardiac function, potential for genetic manipulation, cost savings, etc. Advantages and disadvantages include size mismatch, ethical restrictions on primate use, ecological restrictions on the use of a given species, hyperacute, acute and chronic vascular rejection, coagulation dysfunction, and transmission of zoonoses, that is, zoonotic diseases. These are all the limitations; this is the schematic heart transplantation status.

So this is a central illustration, and it evolves areas of heart transplantation. This central illustration demonstrates three broad areas that are to be discussed. Area number one is the novel immunosuppressive agents to treat allograft rejection, which means your T cell-mediated response is the one that is killing the transplanted organ. So can you selectively kill rather than broadly suppress the immunity? And new modalities in imaging and genomic medicine to screen for the possibility of rejection, as we mentioned about the AI. You can predict the possibility of a rejection that can be calculated or evaluated beforehand.

And the future potential ways to increase the donor pool that you should be able to choose from. Like you're going to buy a shoe. You don't buy, okay, size eight is my shoe size, or give me any size eight. It will know that sometimes you have to wear it and feel it.

Is it okay? Comfortable. So when the donor pool is larger, you will be able to choose the best. Novel immunosuppression is one approach. Different drugs act in different ways to protect the transplanted organs. Screening for the possibility of rejection that can be done and the potential for increasing the donor pool. That is what the three different cartoons that have been mentioned here are.

So stem cell therapy and tissue engineering are another promising approach. So the landmark scientific work has demonstrated that the resident cardiomyocytes can self-

renew. The annual cardiomyocyte turnover is around 1% in young adults and decreases to half a percent in elderly people. So promoting this endogenous repair process, your heart is being repaired on a small scale, just like your skin is getting repaired, but not at an adequate level. Promoting these endogenous repair processes can enhance the regenerative capacity and modify or reverse the adverse LV remodeling that is occurring because of cardiac malfunction or reduced blood supply, etc.

Regenerative cell-based therapies ultimately aim to restore normal myocardial function through both direct cell-mediated and indirect paracrine-mediated repair mechanisms. Sometimes you can stimulate that area with some growth factors that are paracrine factors, or you can have the cardiomyocyte-derived stem cells and deliver them to that area so that the damaged area can be fixed. Initial trials utilized first-generation cell-based therapies predominantly consisting of unselected progenitor cells, mesenchymal stem cells, and endothelial progenitor cells taken from the bone marrow. Although they seem easy, they carry some baggage. However, these populations may not differentiate into cardiac tissue despite having the potential to differentiate into cell types within the myocardium.

Two problems: one is that it may not effectively differentiate, or even if it effectively differentiates, there is a possibility that it may change into something else because it originally derived not from the heart but from the bone marrow, so this is the main issue. The next generation therapies include the isolation and expansion of cardiac resident stem cells or the creation of cardiopoietic stem cells not from bone marrow or any other tissues through the lineage-specific differentiation of the bone marrow-derived progenitors commonly called cardiopoiesis, so even if your source can be anything, you first make the stem cells that is. Specific to the cardiac tissue. So this is the first step. And then it is less likely that it will change its identity.

Cardiopathic stem cell therapies have demonstrated the promise of proof-of-concept studies, including C-CURE. And it's a cardiopathic stem cell therapy for heart failure. That is the full form of the trial. So, cardiac tissue engineering is another novel approach to regenerative therapy that uses engineered human myocardium.

That is EHM. The challenges of EHM are to create functional cardiomyocytes with the electromechanical properties of human myocardium. A recent study optimized the cellular chemical components that can be used for EHM generation, that is, engineered human myocardium, resulting in advanced cardiomyocyte maturation. Researchers have also transferred the cells into the nude rats; nude rats mean the immunity is compromised. Using an epicardial approach leads to the revascularization of the grafts. You took the cells, grafted them onto a recipient nude rat, and these cells got colonized and

differentiated, resulting in revascularization, which means the blood supply is restored.

However, the electromechanical coupling to the normal myocardium was not clearly seen. That means the contraction is needed, not just a structure. That was not properly coupled, and that was one of the drawbacks. Although not yet ready for clinical trials, EHM is a promising approach to cardiac repair that may soon become feasible.

So it's a question of time. You have to standardize it. Can I get it into electrocoupled effectively as well? So now comes the total artificial heart. The development of the total artificial heart known as TAH to replace the failing heart has been the focus of global research for several decades. The first successful use of TAH was performed by Dr.

Denton Cooley. In 1969, in a patient bridged to heart transplant, you connected the patient with the heart transplant. The only device currently approved by the US Food and Drug Administration is a 70cc Syncardia Temporary Total Artificial Heart. That is from a company in Arizona. The TAH-t is currently indicated as a bridge to organ transplantation for patients with serious heart issues. Means it is not a permanent solution, rather a makeshift solution until the organ transplantation is done, so this is a picture of the total cardiac replacement for irreversible biventricular heart failure.

This is the instrument that is used; that is the Carmat total artificial heart that you can see in panel A, and it has been used; it has two valves. Bio pro prosthetic valves also have sensors to detect issues and can prevent blood clot formation. This is another version by Wakorta, which takes a different approach. The advantages include magnetic bearings, compact size, and a single rotational component. Several mechanical properties are present, so either of them can be used as an artificial heart.

So from here, if you conclude, it has been 15 years since Dr. Christian Barnard performed the heart transplantation from human to human back in South Africa and its advances in immunosuppression, donor and recipient selection, surgical techniques, and post-transplant care. All have improved over the years, and it has resulted in excellent long-term outcomes that are established these days. However, significant barriers still remain, including early and late risks of organ rejection and vasculopathy, such as when an organ may be rejected immediately. And if it does not reject, you can go smoothly for a few years.

And that is not the end of the show because you can receive a rejection much later. So you can't be tension free. You cannot be tension-free at all. And it can also bring the risk of infection and malignancy because of the immunosuppressors you are taking. Although the donor pool is cautiously being expanded, it is unlikely to fill the needs of the

continuously growing sick patient population.

More hearts are needed. So the future depends on the progress of stem cell research and tissue engineering, as well as the progress in xenotransplantation, while optimizing post-transplant health through improved immunosuppressant strategies to avoid acute and chronic rejection. So the reversal of heart failure to avoid the need for organ transplantation is also as important as the organ culture itself. So human organ cultures. In human studies, the criteria for developing new diagnostics and therapeutics are limited by ethical and logistical issues. The preclinical animal studies are often poor predictors of human responses; that is also another problem.

The standard human cell culture can address some of these concerns, but the absence of a normal tissue microenvironment can alter cellular responses; hence, you may not end up getting the same organoid or whatever you are looking for. Three-dimensional culture, also known as 3D culture, positions the cells on synthetic matrices or organoids, or organ-on-a-chip cultures, in which different cells spontaneously organize and contact other cells in a natural matrix only partly. Help in overcoming these limitations so that human organoid cultures known as HOCs more faithfully preserve the in vivo tissue architecture and better represent the disease-associated changes so that they can help in a much better way. HOCs can be combined with the traditional and more. Modern morphological techniques reveal how anatomic location can alter cellular responses at a molecular level and permit the comparison of different cell types and shapes.

Because if an organ is put in place, it should be supported by the blood flow, blood pressure, etc. Sometimes, if the pressure is high in a given area, the organ may not sustain. So this is also one that has to be taken care of. HOCs are prominent not only for transplantation, but they are also very significant in studying inflammation, cancer, and stem cell biology.

HOCs and how they are used in organ transplantation. In normal kidneys, ECs of glomerular capillaries, one example from the kidney, and peritubular capillaries (PTCs) express a higher level of TNF receptor 1, which is tumor necrosis factor receptor 1. Associated with inactive apoptosis, the signal-regulating kinase known as ASK1, and the absence of activated ASK1. So these are all scenarios that you can normally see in healthy kidneys. In the renal canal, the types of TNFR1, ASK1, and TNFR2 are only minimally expressed in any cell type in a given normal kidney. So this expression pattern of TNFRs differentiates the renal cells in situ from the cultured renal cells.

That is very important because when you culture, if these are not there, receptors are not there; they cannot mimic normal renal tissue where both receptors are typically expressed

in the same cell. It also differs from the patterns of rejecting renal allografts or ischemia profusion injury, where the resident renal cells lose TNFR1 expression, and the ECs of the PTCs and tubular epithelial cells express TNFR2. Effects of TNF signaling, that is, tumor necrosis factor signaling, on different renal populations can be analyzed in the organ culture. Because these receptors are expressed, it is very easy to analyze. Treatment of normal kidney organ culture with the TNFR1 selective mutine.

Results in the activation of ASK1 and the induction of cell death in the ECs, as well as in the glomerular and PTCs, while the TNFR2 selective mutant upregulates TNFR2, resulting in the phosphorylation of endothelial to epithelial tyrosine kinase and initiating the cell cycle entry of these ECs of the PTCs. and is also present in the TECs. So TNF does not affect the expression of either receptor in cultured cells. That is because of the unique nature of the culturing conditions.

It has to be induced if you want to place it into the host. So if you look at a comparison of different cultures and culturing methods, a wide range of 3D in vitro models is emerging to better mimic human physiology and, at the same time, reduce animal experiments. 3D cultures use different types of porous scaffolds derived from naturally engineered substrates. And gene expression profiles of cancer cells often differ in the cells that are grown in 3D as compared to the 2D cultures. In particular, genes involved in angiogenesis, proliferation, invasion, migration, and chemosensitivity. The 3D cultures have been used to determine the effects of cytokines and cells seeded into 3D cardiac ECM scaffolds that have been shown to increase calcium signaling and kinetics that promote stem cell maturation, but we should understand that, in contrast to HOC human organ culture ECM scaffolds, the 3D cultures fail to emulate.

The natural biochemical and physical properties of the ECM are recognized as independent factors that influence cell activity. Furthermore, we should note that the basement membrane-derived scaffolds may contain undecided components like viruses and growth factors. So if you look further, other matrices permit cell attachment, but not easy detachment of cell mimics, as development is difficult. And even with the generation of 3D biological scaffolds via organ decellularization, which we discussed a few classes ago, the current system fails to offer complex in vivo tissue vasculature for the supply of nutrients and oxygenation, as well as for the removal of waste material necessary for promoting.

Attachment, differentiation, and the proliferation of the cells. Moreover, the clonal variation across various strains of cell lines and the 3D culture techniques used in different laboratories may also influence this kind of experiment. Unlike human organ culture, cell lines used in 3D cultures lose many of their native in vivo characteristics

once removed from primary tissue. Most recent research has shown that in vitro models ignore the origin of cell lines and histological characteristics because of the unavailability of this data. That is the gene expression data. For example, one gene is SKOV3, an epithelial ovarian cancer cell line that is widely used in a 3D in vitro model for studying ovarian cancer, which bears a striking histological resemblance to clear cell ovarian cancer, the least common histological subtype of an invasive disease.

So in some situations, a cultured scenario will also mimic exactly like the normal scenario. So, the 3D bioprinting system incorporates biology and tissue engineering to develop biological substrates and restore, improve, and maintain tissue function. The three most common bioprinting mechanisms that we discussed in detail in the previous class include inkjet, laser, and extrusion bioprinting. You can refer back to our previous class. So this system offers additional biocompatibility and capacity for uniform cell incorporation.

This system has shown promise in gene and drug delivery with precise placement during tissue construction. However, compared to human organ culture, HOCs, the limitations of this system include difficulties in managing single cells, overdrying leading to the failure of biological systems, and cell damage and altered phenotype caused by the printing processes themselves. Bioprinting requires fine-tuning matrices for optimal simulation conditions and deposition of cells and scaffolds. Thus, the precise delivery of cells and biological factors to the desired 3D cultures remains unresolved. So if you look into the organoids, the system stands at the forefront of 3D approaches as a more accurate recapitulation of the in vivo characteristics of the original individual tissue because the ECM and cell-cell interactions are intrinsic in nature to the culture.

Organoids have been successfully generated from healthy and diseased human tissues and pluripotent stem cells. Organoids from the primary tissue sustain a basement membrane extract that is a hallmark of the original tissue in terms of its architecture, cell type composition, cell renewal properties, etc. Patient-specific organoids have allowed for functional genomic studies that can be done to diagnose the future. However, in comparison to HOCs, organoids lack the histological diversity and sequential differentiation that occur in adult tissues.

So a lot of secondary alternatives also have to be taken into consideration. To conclude, in contrast to other experimental cultures commonly employed for basic research and clinical drug testing, human systems and HOCs have their unique place. The increasing data also suggests that they exhibit reduced or altered key functions. HOCs preserve the integrity of the cells and matrices during organ culture. And HOCs can be modeled in a

wide range of applications that permit many functional mechanistic studies. We will study more about organ regeneration in the next class. Thank you.