Regeneration Biology
Rajesh Ramachandran
Department of Biological Sciences
IISER Mohali
Week: 12
Lecture: 58

W12L58_Artificial, liver, kidney and urinary bladder for transplantation in patients

Hello, everyone. Welcome back to another class on regenerative biology. And in today's class, we will learn about the artificial liver, kidney, and urinary bladder used for transplantation in patients. And we'll also see how tweaking the scenario can help in fixing a damaged organ or one that is almost completely damaged, as such organs can be recovered; an overview of artificial organs includes artificial livers. Kidneys and various other organs are used to support patients with organ failure, often acting as a bridge to transplantation or until the body recovers; that is the whole purpose. Sometimes your organ has the potential to bounce back, and the concept of artificial organs is.

.. Not only are they terminally ill, but that situation also occurs when the liver or kidney is beyond recovery; then naturally you need a transplantation. However, sometimes you are derouting it, or you are taking the show into your own hands, so this is also an artificial way of sustaining the organism.

Artificial bladders are not yet as advanced, although some case studies of transplantation have been done. They are being explored for patients with urinary dysfunction. So let us see some examples. Artificial liver systems, such as the molecular absorbent recirculating system (MARS), are options that assist liver functions, particularly in patients with acute liver failure or chronic liver failure, so they can adopt this technique and have a MARS available for restoring their damaged organ function. These systems remove toxins from the body and may help.

With liver regeneration or a bridge to transplantation, before the transplantation, the organism should recover. If the liver is not functioning, then the organism is not ideal for transplantation, even if a liver is available. In that scenario, this kind of support system becomes very helpful; some examples include MARS, which is single-pass albumin dialysis, also called SPAD. Bio-artificial livers known as BALs use various methods, including adsorption, filtration, and cell-based systems, to mimic liver function. So this is basically to uplift the organ and the organism itself.

The benefits of an artificial liver system are that they can improve liver function, stabilize the patients, and potentially bridge them to transplantation or recovery.

Sometimes you need a transplant despite all these efforts. Sometimes they will recover. The purpose of artificial kidney systems, like those used in dialysis, is to provide well-established treatment for end-stage kidney disease; they are applied in scenarios where filtration doesn't occur in the body, so the functioning of this system filters the waste products and excess fluid from the blood, mimicking the functions of a healthy kidney. So the benefit of dialysis is that it can support patients with kidney failure until a kidney transplant becomes available or their kidney function improves.

So these are all the support systems, and in the same way, the urinary bladder is also used by people; while not as widely developed as artificial livers or kidneys, artificial bladders are being explored for patients with severe urinary dysfunction. The function of these devices includes helping with urine storage and emptying, offering an alternative to traditional bladder management techniques. And the examples include some research focused on using bioengineered bladders or other devices to restore bladder function. So the benefits include that artificial bladders could significantly improve the quality of life of individuals with severe bladder dysfunction because if the bladder is not functioning properly, it is not just a bladder problem; it is a problem with the kidneys as well, due to the pressure. If the regulation functioning of the bladder is not fine, then your kidneys are going to be affected very badly in a short duration, although their kidneys are perfectly fine.

The bladder is the culprit causing the damage to the kidney. The important consideration to be taken care of is the bridge to transplantation. Artificial organ systems can act as a bridge to transplantation, allowing patients to wait for a donor organ while their condition is managed. The limitations include potential complications and the need for long-term management, as people cannot live on dialysis forever, and it is cumbersome and too costly. But sometimes you have to stabilize the patient so that they are available for transplantation or other operative procedures.

The ongoing research continues to improve the effectiveness and safety of artificial organ systems with ongoing efforts to develop more robust and biocompatible devices. So now let us see a status update on artificial organs. Where do we stand? Artificial organs are engineered devices that can be implanted or integrated into the living body to replace a failing organ or to duplicate or augment one or more functions of the diseased organ. The development and optimization of artificial organs have progressed tremendously throughout the past 75 years to include fully implantable and biocompatible devices and materials. We have seen many examples, including putting a.

.. heart valves or putting an aortic tube, etc. There are so many such examples existing; they are all artificial organs. The first device feature we examine is mobility, and here, as with other properties, the technologies span a range from entirely stationary situations, one example being hemodialysis, to fully mobile situations, such as an artificial kidney. If you want to do hemodialysis, you cannot roam around and do it; whereas, if you have an artificial kidney, then it will do the job. You can roam around and do whatever you want in your day-to-day life.

So it doesn't restrict you to hospital life. Just even if it is for a few hours, it will become a cumbersome process for some people. But for others, if you have an artificial organ implanted, then you can do your routine things. The second device feature is biocompatibility, or the capacity of native tissue to tolerate the implant without rejection or thromboembolism; sometimes, when you put an artificial organ or tissue, it can trigger a thromboembolism, which means you can get clotting. Big clots can be found that may block your coronary arteries.

It's a very serious matter. So at times, doctors will give blood-thinning agents, etc. But this has to be taken seriously. The third technological feature is synthetic organ functionality, and devices exist as a continuum from the partial, that is, for example, an artificial pancreas, to full, that is, a total artificial heart. For example, functionality is being taken care of in all these approaches for designing an artificial organ.

The fourth range of device properties spans from artificial devices composed entirely of synthetic materials to biodegradable structures that integrate native tissue into functional performance. This means you are creating an artificial versus natural or an artificial plus natural hybrid organ, which has both artificial and natural components, and you can distinguish between them. These mechanical and functional differences provide the framework through which we explore the current status and future possibilities of abdominal and thoracic artificial organs because they are the major organs that are awaiting artificial organs. You don't care too much about artificial bones or artificial materials. Of course, they are important organs, but they are not important.

Always a life-threatening scenario, a list of organs is provided here, including the current and future status, so it can feature artificial organs and their capabilities. When you compare them, they have to be fully compatible, with the current status being none, and the future, for example, may include a pacemaker. Fully compatible and fully implantable, one status report indicates that a current pacemaker is available, and in the future, you may have an artificial pancreas and various other organs that can come in a fully implantable scenario, intracorporeal with external components. Many examples exist in the current scenario, such as the artificial pancreas, but in the future, we may end up getting an artificial kidney, and so on. Many organs are available in a usable scenario right now, but not all of them are mobile; many of them are very static and cumbersome, which limits the patients.

Movement. Let us see some examples. Artificial liver through culture and cell transplantation. Acute liver failure, so far, has been managed with artificial organ support. Now we are thinking about whether we can have some organoids, etc.

, that have been used. Acute liver failure is a high-mortality syndrome; in short form, it is referred to as ALF, for which liver transplantation is considered the only effective option in cases of acute liver failure. A shortage of donor organs, high costs of the surgical procedure, and surgical complications associated with the immune rejection of the transplanted organ constrain the therapeutic effects of liver transplantation because it is heterologous. Whoever is donating, you cannot donate your liver to yourself. If it is an organ transplant, it can come only from genetically similar or closer individuals. Recently, the mesenchymal stem cell (MSC) therapy was recognized as an alternative strategy for liver transplantation, where the damaged liver is restored to a better condition, and you are providing the MSCs so that they can help in fixing it.

The damaged liver, you may remember, when we studied the ethics of fetal stem cells, fixed a mother's liver problem; so that means stem cells have the potential to fix an existing liver problem as serious as that which required a transplantation, so that was the Boston case study which we discussed. You can go back and see those who want to revisit. Bone marrow mesenchymal stem cells have been used in clinical trials for several liver diseases due to their ease of acquisition. It's very easy to get it. No one will have difficulty getting bone marrow stem cells from any person.

Strong proliferation ability, multipotent differentiation, and homing to the lesion site are characteristics we saw in the previous class that the stem cells should be able to exhibit in order to recognize the damaged organ. Stem cells do have that ability, but they should respond to the chemokines coming from the injured area. Low immunogenicity, anti-inflammatory, and anti-fibrotic effects are seen from these hematopoietic stem cells or bone marrow stem cells that are used effectively for fixing liver problems. Homing and immunoregulation of MSCs are triggered by the injured liver. So in this picture, we should know if a liver is injured, what cytokines and chemokines are released, which will be sensed by the MSCs or bone marrow stem cells (BMSCs) that you are putting into the liver, which help in fixing the damaged liver.

Hepatocyte transplantation has been considered an alternative to organ transplantation, but it has been hampered by the lack of large cell quantities, expansion difficulties ex vivo, rejection of allografts and seno transplantation, and the rapid loss of liver properties in vitro. So that is why hepatocyte transplantation is a very good option because when you have the option of delivering hepatocytes to a damaged liver, life becomes easier. Than just giving stem cells. Stem cells, including fetal, biliary, tree stem cells, fetal liver stem cells, hematopoietic stem cells, endothelial progenitor cells, MSCs, induced pluripotent stem cells, and others, can transdifferentiate into hepatocyte-like cells to restore the damaged liver and respond to stimulation. Mesenchymal stem cell therapy has been extensively studied, and it shows great clinical promise due to its ease of acquisition, strong proliferation ability, multi-potential differentiation ability, homing to the lesion site, low immunogenicity, and anti-inflammatory and anti-fibrotic effects.

This approach has helped to illustrate a picture that shows the changes occurring to various cell types—mesenchymal stem cells, sinusoidal endothelial cells, and injured hepatocytes—all represented in different colors. What is important is that after damage, there will be too much of... Metalloproteases, interleukin 6, and various pro-inflammatory cytokines will be released from the injury site, which must be sensed by the mesenchymal stem cells or hepatocytes that you are artificially introducing from outside so that they can go and repair the damaged area.

So this is how the approach is done. Multipotent differentiation and functions of mesenchymal stem cells derived from various tissues. So this picture shows that MSCs are a heterogeneous population that can adhere to plastic and proliferate; they can proliferate ex vivo, form colonies with fibroblast-like morphology, differentiate into any cell type, including osteocytes, chondrocytes, adipocytes, and other mesodermal lineages, and have endodermic, and ectodermic differentiation potential exists in them, which means they are MSCs that are quite robust in that way. As you can see in this picture, MSCs can give rise to mesoderm cells, endoderm cells, and ectoderm cells. Several studies have shown that MSCs can differentiate into functional hepatocytes.

Cholangiocytes after growth factor induction ex vivo, means outside the organism. Intrasplenic transplantation of human-derived BMS into mice with fulminant liver failure. That means the mice model is experiencing liver failure, and they have been treated with a BMC cell that is isolated from humans, along with hepatocytes and immune cells. So they are humanized in the sense that these mice, although their liver is damaged, will not reject these human cells because they are humanized mice. And a recent investigation reported that MSCs can also self-assemble into three-dimensional, that is, 3D, human liver ex vivo by transdifferentiating into hepatocytes, sinusoidal endothelial cells, and hepatic stellate cells.

So it can give rise to many cell types that are present in the liver. Hence, transplanting MSCs or BMSCs will be a good approach to repair the damaged cells. The damaged liver. So this is an example of different tissues that can be used: placenta, umbilical cord, bone marrow, adipose tissue, etc.

for making mesenchymal stem cells. So now let us see acute kidney injury for therapy prospects and challenges. Acute kidney injury (AKI) is a heterogeneous syndrome comprising diverse etiologies of kidney insults that result in high mortality and morbidity if not properly managed. Although great efforts have been made to investigate the underlying pathogenic mechanisms of AKI, there are limited therapeutic strategies available. Extracellular vesicles are membrane-bound vesicles secreted by various cell types that can serve as a cell-free therapy by transferring bioactive molecules.

EVs can be isolated from your bloodstream. They are vesicles—extracellular vesicles that can be used for treatment. EVs in AKI have a high therapeutic potential and can be subdivided into stem cell and non-stem cell derived EVs. Several challenges and opportunities related to the clinical translation of animal studies exist. That means you can pick up EVs from a stem cell and deliver them into an organism so that its damaged organ can be fixed. So if you see an overview of this, by clinical definition, acute kidney injury is a syndrome of kidney damage resulting in a rapid decline in renal function and a decrease in urine amount, or both can occur together.

It is widely recognized that AKI is associated with an increased risk of morbidity and mortality. So this is a cartoon of EVs, which are extracellular vesicles. And that can be isolated, and you can do a multi-omic analysis to understand the contents of these EVs and their therapeutic application for fixing the kidney by delivering these EVs to the damaged organ in the organism. So, EVs are sources for human therapeutic uses. EVs are non-replicating phospholipid bilayer membrane-bound vesicles that are secreted by the cells.

EVs can be isolated from bodily fluids such as blood, plasma, serum, urine, milk, cerebrospinal fluid, etc. And also released from in vitro cultured cells. They can be released. Blood is particularly rich in EVs and is easily obtained. Thus, plasma, serum, blood, and platelet-derived EVs have been widely investigated as biomarkers and therapeutics in AKI to understand AKI and other diseases.

EVs derived from the hearts of post-myocardial infarction contain pro-inflammatory cargo naturally because myocardial infarction triggers an inflammatory response that exacerbates the injury in recipient mice, and donor age is also reflected in EV cargo and function. So if the EV is from an old person versus a young person, it will be reflected. So you pick the ideal EVs and deliver to the damaged organs. Since EVs have rich and complex cargo consisting of hundreds of different bioactives, microRNA, proteins, and lipids, they can act simultaneously on multiple pathways in different target cells, which

AKI can impact. For example, during ischemia or reperfusion-induced AKI, there is hypoxic and metabolic mitochondrial injury, plus additional necrosis and apoptosis of the TECs due to the trapping of erythrocytes and subsequent toxicity.

The major mechanisms of EV action relevant to AKI therapy include the acute protection of the parenchymal cells of the kidney, reducing their apoptosis, stimulating their proliferation, modulating inflammation, reducing inflammation by these delivered EVs, and immune cell recruitment. Promotion of endothelial cell angiogenesis and modulation of matrix remodeling and fibrosis by fibroblasts. So this can be fixed so that the ongoing damage can be controlled. So EV delivery and uptake by the kidneys manifest their activity. The classically designed mechanism is that EVs must reach the target site, interact with the desired cell membrane, and then deliver their cargo in sufficient concentrations to alter the cell responses to injury trajectory.

So the precise mechanism of EV uptake and cargo delivery is very complex. In brief, there is evidence that EVs can fuse with the target cell membrane, delivering cargo directly into the cytoplasm, or they can bind to cell surface ligands, which can be endocytosed, at which point they may fuse with the endosomal membrane and deliver the cargo into the cytoplasm, or they can be subjected to degradation or recycling as well. So, cells such as macrophages, key players in the response to AKI, are also capable of engulfing EVs by phagocytosis. Lastly, there is evidence that EVs can stimulate intracellular pathways by binding to cell surface proteins without the need for internalization. They can do that by acting on the cell surface proteins as well.

Thus, assessing the delivery is a complex issue, and most studies take the whole organ approach, and they do not specifically measure extracellular or intracellular compartments. Cell-specific uptake or organelle-specific uptake is sufficient for repairing the damaged organ when you are undergoing EV treatment. There's a schematic diagram showing the isolation of the therapeutic cell-derived EVs and their cargos. So you can isolate the EVs from the cells, centrifugation is used to separate the EVs. You deliver to the damaged organ, and then you expect uptake and activity; EVs can get in, and you end up getting a recovery.

So multiple mechanisms of EV activity are being studied in kidney tubular epithelial cells, as mentioned here in this picture, including endocytosis, membrane fusion, etc. So indirect activity via macrophage polarization is also possible in the case of EV. EVs can probably be engineered in the future as well. So the reported mechanism of the therapeutic action is based on the available evidence, but its future potential is enormous.

So tissue engineers create autologous bladders. Now we understand that urinary bladder cytoplasty is possible for patients; we saw the liver, we saw the kidney, and now we are looking into the bladder. Patients with end-stage bladder disease can be treated with cystoplasty using gastrointestinal segments. That is the only existing option. Such segments in the urinary tract have been associated with many complications; although it can be done, it also has many complications. So the researchers have explored an alternative approach using autologous engineered bladder tissues for reconstruction.

So the urothelial and muscle cells were grown in culture and seeded on a biodegradable bladder-shaped scaffold made of collagen or a composite of collagen and polyglycolic acid. And about seven weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and were implanted. So the engineered bladder tissues were created with autologous cells seeded on collagen polyglycolic acid scaffolds.

It's a combination of both. One is artificial; one is natural. Collagen is natural; polyglycolic acid is artificial. Can be used effectively in patients who need cystoplasty. So that means you are creating a bladder in an external environment outside the body. Those who are interested can read this Lancet paper. So this is a picture from the same paper on the construction of an engineered bladder.

You can see here a scaffold seeded with cells in A and an engineered bladder analogous to the native bladder with four to zero glycolytic structures in B, and the implant covered with fibrin glue and omentum is seen in C, which is ready for transplantation. To summarize, what we know is that a major challenge in engineering tissues or organs for clinical use has been to develop biodegradable three-dimensional constructs that can accommodate adequate amounts of cells. For functional tissue formation, without which the organ production will not maintain its integrity. The scaffold should have the appropriate biomechanical and structural properties to preserve tissue integrity for the long term.

You don't want an organ to be working for five or three years. You want it for the rest of the person's life. Using a composite scaffold composed of PGA, polyglycolic acid, which supports structural integrity, and the next component of the scaffold is collagen, which supports cell growth and survival, proved optimal for bladder tissue engineering, as we saw in this paper. Another challenge for engineering tissues and organs involves the need for constructs to be sufficiently vascularized to support and maintain the transplanted cells, which means angiogenesis. Although the cells are being formed, the urinary bladder is a less complex tissue; that doesn't mean that you do not need a blood supply. So tissue engineering techniques can generate bladders implanted in patients that require cytoplasty.

That means it is a very serious condition. Although follow-up of longer than five years is reported in these transplanted patients, additional studies will be needed before this procedure can be used widely. But, in principle, bladder transplantation is a big success. But more studies need to be done to understand the exact effectiveness for each patient. So, organ culture and organ transplantation are vast fields.

We cannot cover all of them in a short time. Those who are interested can read more articles. We'll study more in the next class. Thank you.