Human Physiology
Dr. Sudip Mukherjee
School of Biomedical Engineering
IIT(BHU), Varanasi
Week - 05
Lecture - 02

Hello, welcome to another new class on human physiology. Hopefully, you are enjoying the different classes of human physiology. In the last few classes, we discussed different endocrine glands. We also discussed glucose homeostasis and further mentioned that if any irregularity in glucose homeostasis occurs due to various reasons, it can lead to hyperglycemia, which is a serious condition known as diabetes. We briefly discussed different therapies for diabetes and their advantages and disadvantages. In today's class, we will discuss a modern approach to therapy for diabetes, primarily for type 1 diabetes, such as cell-based therapy.

Briefly, we will also discuss how we can develop an artificial pancreas. So, this would be a very interesting class; let us stay with it. So, what would be the different contents that will be covered in this class? We will discuss what glucose homeostasis is. We will see if any irregularity or dysregulation happens in glucose homeostasis.

How diabetes develops will mostly focus on type 1 diabetes. Then we will briefly discuss the different limitations of the current therapies. After that, we will introduce you to cell-based therapy and discuss how cell-based therapies can be used for type 1 diabetes. Then we will introduce a new concept of fibrosis and also discuss what the challenges related to cell-based therapy due to fibrosis are. Then we will discuss immuno isolation and how alginate-based polymers or other natural biopolymers can be used for the immuno isolation of the cells.

Lastly, we will discuss an interesting component of vascularization and why vascularization might be helpful and needed for cell-based therapy. So, this would be a very interesting, maybe a little bit longer class. So, let us enjoy ourselves. So, you remember in the last few classes we discussed what glucose homeostasis is, and the primary thing is that our pancreas has mostly two important cells: one is the alpha cell and the second is the beta cell. What we said is that the beta cells secrete insulin, while the alpha cells secrete glucagon.

And how do they maintain glucose homeostasis? You know, whenever we eat food, it basically breaks down into carbohydrates and glucose after digestion. Eventually, the beta islet cells from the pancreas secrete insulin. They help in terms of carrying glucose with them and eventually distribute those glucose molecules to various cells and organs in our body. In case any excess glucose stays in the body, this insulin basically converts the glucose to glycogen, and it is stored in the liver. So, basically, any excess glucose is converted from glucose to glycogen and is stored in the liver.

And now, whenever there is a prolonged fasting state, it means the glucose level goes down; at that time, the alpha cells of the pancreas will secrete another hormone called glucagon. This glucagon will further convert glycogen back to glucose, and then this glucose will be distributed to the body. So, in this way, insulin and glucagon basically maintain the glucose homeostasis of our body. But what happens in case of any irregularity, such as if pancreatic islet cells are damaged by anything or if insulin resistance occurs, is that we experience a

persistent high glucose condition called hyperglycemia. I mean a prolonged hyperglycemia state that becomes a disease condition also called diabetes.

Now, diabetes, as you remember, we said there are two types or two categories of diabetes. One is type 1 and the second is type 2. Both of them were discussed in our last class as well, but in this class we will only focus on type 1 diabetes. So, as we said in the case of type 1 diabetes, our body generates an autoimmune reaction, and it mostly happens very early, at about less than 10 years of age in children, and our hyperactive immune cells, including hyperactive T cells, basically destroy all our pancreatic beta islet cells. So, all the pancreatic beta cells that are destroyed by the autoimmune system cause a deficiency in the formation of insulin.

So, basically there is almost no insulin production from those pancreatic beta islet cells in the type 1 diabetes patients. And as you can see, almost 5 lakh children who are less than 15 years of age are suffering from type 1 diabetes worldwide; about 11,000 are from India. And you see in the figure that in the case of this type of diabetes, because there is no insulin in the blood, they are unable to supply glucose inside our cells. So, basically, this glucose is completely cut off from different types of cells, including our brain cells, and as you know, if glucose is unable to be transported to various parts of our body, the blood glucose level will go high and will create a condition called hyperglycemia. Due to a lack of glucose in the brain tissue, the hypoglycemic condition can also generate in the brain, potentially causing instant death.

So, basically, type 1 diabetes is a deadly autoimmune disorder. What are some general therapies for type 1 diabetes? We already said, like, of course, the most common one, like, you can give insulin. So, insulin-based therapies can be used, but the problem is that continuous administration is needed because, in the case of type 2 diabetes, people or the patient still have some active pancreatic beta cells left. But in cases of type 1 diabetes, there are no active pancreatic beta islet cells left in the body. So, you can understand a kid who does not have any pancreatic beta islet cells; whenever he or she eats any food, he needs an insulin jab or insulin injection.

So, it becomes very painful when the parents have to continuously monitor their child's glucose level, and they probably need to inject insulin whenever required. And sometimes it happens that parents can judge it wrong, and if the insulin level or insulin injection increases in the body, the patient can also experience hypoglycemia or a brain coma. So, this is a significant issue, and persistent insulin injection can also slowly develop insulin resistance in the body. In that case, insulin will not be able to act properly and bind to the receptor. The second most important therapy that can occur is an organ transplant or a pancreatic transplant.

But the problem is how many donors are actually there, right? Even in the US, the government data says that no more than 10 to 20 pancreatic organ donations actually happen. So, basically, you can understand that although this can be effective, the numbers are very low. Apart from that, there is a risk of organ rejection due to the immune activity or the host immune response. So I will just introduce this term here: the host immune response, which is basically the immune response towards any foreign object. In this case, the transplanted pancreas is considered.

So, if the host immune response detects the transplanted pancreas as a harmful object or foreign body, it will basically destroy it. And to prevent against the foreign body response, doctors generally prescribe steroids or immunosuppressive agents. As you know, long-term use of immunosuppressants is not good for your body because it also compromises your body's immunity. So, as you can see, there are challenges with insulin therapy, and there are also

challenges with organ donation; what can be the alternative approach or modern therapy that is basically being explained, including in our lab? So, in this slide, we will discuss various aspects of basic physiology and also provide different research perspectives. So, it might be a little bit of an extended way; if you have any questions, please ask us, right? So, this will be kind of a research-oriented lecture today.

So, what can be a modern approach? Right, as you said, pancreatic islets can eventually be given to the patient, but the problem is you do not have sufficient pancreatic islets. Why? Because there is not enough awareness to donate our pancreas. So, either we are unable to completely get the whole pancreas or even some amount of pancreatic islet cells. Another problem is that human pancreatic islet cells are very difficult to culture. Basically, they do not regenerate.

But there are different strategies nowadays that have been developed to generate stem cell-derived pancreatic islet cells that can be cultured in laboratory conditions. But that is still highly costly. So, the approach that can be taken is the use of xenogenic. So, the use of xenogenic islets that can be taken from dogs, monkeys, or pigs. So, basically, we can take the pancreatic islets from other sources, right, other organisms, and then we can transplant them into the body, but there are certain issues, right? So, if we just take the pancreatic beta inlet cells from this body and inject them into our body, what will happen? So, in the last slide, I only said that our body will generate a host immune response and what will happen because these organisms are different from the human body.

Once these cells are transplanted or implanted in the body, our body will immediately detect them as foreign and harmful objects and trigger our immune response to destroy them. So, just injecting this genogenic type of pancreatic beta-ylate cells into a type 1 diabetic patient is not a solution because they will not be able to survive more than 24 to 48 hours in the body. So, what can be done is that we can create an immunoisolation or a kind of protective barrier. So, you can see that cells in cell-based therapy mostly act in therapeutic uses of cells, either human cells or different types of cells, and these cells are generally given inside a protective barrier. So, what does this protective barrier do? This protective barrier basically prevents the immune cells from coming inside.

So, basically, it prevents the immune cells from coming inside. It also has proper pores, or these have proper porosity. So that all the nutrients and oxygen can come in. So, basically, what does it allow inside? It allows oxygen and nutrients, but it doesn't allow the immune cells, right? And it is also because it has the beta-ylate cell, if you consider. So this beta-ylate cell will release what? Insulin.

So this insulin can also come out, and all different types of big complex proteins will not be able to go inside. So, basically we can create a productive barrier, and we can use human or different species' cells to create cell-based therapeutics that can be a modernized therapy for type 1 diabetes. But as I said, even if we create a protective barrier around these cells, this whole system, for example, if this is a capsule and we have the beta-islet cells inside, this whole system is still a foreign object, right? This whole system is still like a foreign body or object to our body. So, even though the immune cells, for example, like T cells and macrophages. So, this different type of immune cells you remember we discussed in our blood class and immune system class, even if this different type of immune cells cannot penetrate the capsules, even if they cannot go inside, what happens is that the body has another secondary immune response system, and that gets mostly triggered by the macrophage.

So, this secondary host immune response is triggered by the largest immune cells of our body, which are macrophages. So, initially in the primary immune system, if the T cells and NK cells cannot penetrate and destroy the inner cells, the macrophage cells will basically come close to this object, and if they detect that this is harmful, then of course, the object is so much bigger than the macrophage cells that they will not be able to chew it or destroy it. But what it can do is create a foreign body response, and by creating a foreign body response, it will basically create a response of inflammation and eventually fibrosis formation. What is fibrosis, basically? Fibrosis is basically like a combination of different fibroblast cells and collagen. So, what we said is that it will create a response of fibrosis, which basically creates a thick tissue layer made of collagen, fibroblast cells, and myofibroblast cells.

This thick tissue layer will eventually completely coat the whole system. So you can see in the system that these whole cells and the capsules will be completely covered by this fibrotic tissue layer. And as you can understand, if this complete thick layer of fibrosis covers the capsules along with the cells, what will happen? The nutrients and the oxygen will not be able to go. So, the oxygen will not be able to go inside, the nutrients will not be able to go inside, and even the beta cells will not be able to pass the hormones, like insulin or even the other toxic metabolites. So, in this way, the cells inside will die, right? So, even though we can create an immunoisolation layer with a proper polymer or encapsulation system, fibrosis can eventually hamper progress.

Hopefully, you understood that. Then there are various strategies to prevent fibrosis. So scientists and researchers have been studying for the last several years to see how to mitigate the fibrotic response. So, there are different physical properties; for example, we can change the surface topography, right? So, basically, we can create a rough surface or a smooth surface, and mostly, if we create a smooth surface, it has been seen that smooth surfaces trigger lower fibrosis. So, we can create a smooth surface to lower the fibrotic response. And then we can change the size and shape; in terms of size, it has been seen that if the size becomes too small, it creates a significant fibrotic response.

So, we can basically increase the material size as much as we can to lower the fibrotic response. Then there are also options for delivering different biological reagents. For example, different anti-inflammatories like cytokines. So, anti-inflammatory cytokines, like IL-10, are important. Then we can also give various steroids and immunosuppressants.

But as you know, all these reagents can actually reduce our overall immunity in the body. So, in the systemic run, these biological reagents are not good because they can create a lot of immune-related cascades or immune toxicity, and long-term immunity can be a challenge. So, the third handle or the third component, which is mostly interesting, is needed because this physical barrier or the change of physical property, although it can manipulate the fibrotic response, is not significant and long term. So, the challenge is that these are not significant and they cannot prevent long-term fibrosis. So, basically, they cannot prevent long-term fibrosis.

The biological factors, of course, can prevent long-term fibrosis, although the problem is, as I said, that they are biological factors; they can create different immune reactions, immune toxicity, and long-term use of immune suppressants is not good for our body. So, this third handle, which is in the middle, is highly interesting for use, like the chemical modification or attaching various chemical molecules to this, on top of these capsules or on top of this device. So, these molecules can be uterine molecules, or they can be small molecules. So, by coating

or conjugating the different types of capsules or different types of devices with the small molecules using chemical modification, we can actually reduce the response of the fibrosis. So, this is basically the use of chemical moieties to reduce fibrosis.

So that these capsules, which are carrying the therapeutic beta cells, can stay in the body for a longer term and can produce insulin that can correct hyperglycemic conditions. So, this is the process that is basically called immuno-isolation, where we can use either a small molecule conjugated like alginate or different other polymers that can basically isolate the therapeutic cells from the surrounding environment where active immune cells are present. Also, we have to ensure that the fibrotic response is reduced and mitigated so that our bodies, like a fibrous host immune response, cannot develop a thick tissue-based layer to cut off the whole system from the body. In this way, we can manipulate our body's immune system and create an immune-isolated air layer mostly using alginate polymer, which is already an FDA-approved polymer that has been used for several years to deliver therapeutic cells. As you can see, there are several papers that have shown that if you deliver therapeutic islet cells using alginate capsules, normal glycaemia levels can be maintained for almost up to 6 months, and diabetic disease can be temporarily cured.

But the problem is that if these alginate capsules are not chemically conjugated to reduce their fibrosis, it eventually shows the fibrosis, and the cells inside the alginate eventually die. So, even though they are very good at maintaining the normal glycemic level for 6 months, these alginate polymers eventually require coating with some active chemical molecule after 6 months. So, after 6 months, the fibrosis response forms again, and all these implanted beta islet cells simply die. So, we have to create certain chemical modifications around the surface of the capsule. So that the body's host immune response or the fibrotic response can be reduced and the implant lifetime or the overall shelf life of the implant can be improved.

So, in this manner, there are certain classes of molecules that can be developed and that are developed by our lab. These are basically like triazole-type molecules. So, you can see this triple N. This is called a triazole, and these are different types of small molecules, right? These triazoles can be developed by a copper-catalyzed click chemistry reaction. So, this is called a click chemistry reaction, right? This happens through the cycloaddition between an alkyne and an azide.

So, basically, this is an azide and this is an alkyne. So, this is basically a cycloaddition reaction between an azide molecule and an alkyne molecule in the presence of a copper catalyst, eventually forming a cycloadded-like addition-based product, which is a 100% 1,4 isomer, which is also called triazole. And this is highly specific compared to Hiddleston's reaction. As you can see in the Hiddleston reaction, it forms a 1,5 isomer along with a 1,4 isomer. But in cases of clique chemistry reactions, it only forms a 1,4 isomer with a very high yield.

And as you remember, only 2 or 3 years ago, clique chemistry received Nobel awards, right? So, in our lab, what we developed is a molecular library of several triazole molecules, and in that molecular library, you can see there is one side which is the hydrophobic component and another side we created which is the hydrophilic component. So, why are we saying it is hydrophilic? You can see there are some PEG-type linkers. So, the PEG is generally hydrophilic in nature, and in the hydrophobic part, you can see there is this phenyl linker, which is mostly hydrophobic in nature, right? So, hydrophobic phenyl type or benzyl type. Right linker, and in this case, there are pegylated hydrophilic linkers. We took about 50 to 51 different alkyne combinations and had 5 different azide combinations.

With permutations and combinations, we developed about 211 novel alginates that are conjugated with this type of triazole molecules. And you will ask me why we develop both hydrophobic and hydrophilic types of systems. So, as you know, like alike. So, basically, if you want to use this type of small molecule coating in a soft polymer in a more hydrophilic type of system or polymer, we need a hydrophilic type of coating. But in case we want to use this small molecule for a hydrophobic system, for example, like a plastic-type system or a silicon-type system, we mostly need a hydrophobic type of coating.

So, basically, you just remember this thing like alloys. Like, okay. So, hydrophilic will kind of like a hydrophilic system, while hydrophobic will like a hydrophobic system. So, in the case of hydrophilic substrates, what different types of coatings can we use, for example, soft polymers like natural ones? It can mostly be natural polymers, and in the case of hydrophobic substrates, mostly plastics and synthetic polymers can be used. So, this time we developed different types of almost 211 new molecules.

Now, what we have to do is test and identify which of these 211 molecules are actually effective in preventing fibrosis. So, are you getting to the point that initially we developed a large chemical library and the goal is now to conduct a high-throughput screening process to identify which of the molecules is a lead molecule to prevent fibrosis? So, the eventual goal is to reduce the host immune response and the fibrotic process. So that the capsules can stay in the body for a longer term, surviving all those beta elite cells in the body. So, to do this screening, we approach it like a genetic barcoding method that I am not going to discuss in detail because this would then divert from the actual topic of why the genetic barcoding approach has been developed. However, just to give you a short idea, there is no proper screening approach in vitro for fibrosis.

For example, we first screen anti-cancer compounds or antibacterial compounds in vitro, which means in cultured cells or bacteria, and then, once they are cultured, we test the lead component or lead compound in animals and further in humans. But in the case of fibrosis, there are no such reliable models. And what happens is that as we have this large number of libraries of molecules, we need a large number of animals to screen this. So, unless you develop a high-throughput screening approach, screening this number of excess molecules cannot be done because nowadays all the institutes and regulatory agencies are not allowing us and all researchers to use too many animals. So, we developed a high-throughput screening approach using a genetic barcoding strategy where we can combine different molecules together, almost 20 to 40 different types of these conjugated alginate capsules that we can implant inside the mouse.

So, we implanted all those for 20 to 40 capsules, each having a specific genetic barcoding inside the mouse, and what we did, basically, was keep those for about a month inside those animals, and after the retrieval, we performed the microscopic imaging. So, as you can see, once we did the microscopic imaging, the maximum of those capsules had too much fibrosis; only very few capsules did not have any fibrosis. Now, we have to identify what these few capsules are right inside that came out from the mouse. So, how did we identify it? Because you remember I said that each of the capsules has a specific genetic barcoding. So, by identifying the genetic barcoding of which genes are present inside those capsules or which cells are present inside those capsules, we could backtrack and identify the lead molecule.

So, in this way, we identified three different lead molecules that can prevent fibrosis in the long term and reduce fibrosis. So, now that we have identified the reduction of fibrosis in the long term, there are actually a total of different leads we initially identified from a combination strategy, and all these leads, as shown in this last slide, were implanted in a capsule size of 1.5 millimeters. Now, you remember in a few slides before I said that if you reduce the size, the body generates a stronger immune response.

So, in this case, what we did after the 1.0 millimeter capsule size was reduce the capsule size to 0.1 to 0.2 millimeter. So as we reduce the size, what can we expect? As we reduce the size, we expect that the fibrotic response may increase, and we want to test all the top 10 leads to see if reducing the size can still prevent fibrosis or not. So, as you can see after screening this in the individual animal setting, we identified two different leads, G1A3 and G4A10; they do not have any fibroblast cells.

So, you can see that there are different stains we did. So, this is alpha SMA, which is like a myofibroblast cell. So, in the control alginate where there is no small molecule coating, you can see there are so many myofibroblast cells, and we also kind of strain for the T cells because T cells are also immune cells that can get attached if there is no small molecule coating. But in our lead, you can see there are no myofibroblasts, and there are no immune cells. We also estimated the collagen because I said that fibrosis is basically a combination of collagen and myofibroblasts, right? Combination of collagen and myofibroblasts. So, we also estimated collagen, and as you can see, compared to the control, all the leads have a very low amount of collagen.

So, basically, we identified the top two leads, right? And mostly, we selected this G4110 as a lead to perform the cell-based implantation in type 1 diabetes. So you can see in this figure what we did we took human eyelid and one thing to note that in this case we took a very high density eyelid right why because because a pancreas in our body contains lot of like almost like if I go few slides after if I go few slides after Yeah, so each pancreas contains about 1 million cells, right? So, you can imagine almost like 10 lakh cells. So, each pancreas has almost 1 million cells. So, if we have to, basically, in the case of type 1 diabetes, as you know, all the beta islet cells are destroyed. So, basically, we have to replace all those 1 million cells for proper function, right? To replace 1 million cells, a lot more capsules are required because you can imagine that if we can only use about 1000 cells per ml of capsules, how many liters of capsules will be needed, and they can be implanted in our intraperitoneal belly area, but we do not have that much volume in our belly area.

So, we cannot basically implant such a high number of capsules. And then, basically, what is the solution? The solution is that we have to increase the density of the islet. So you can see we introduce a high-density islet. These are all human islets. So we use like 4,000 cells per ml, 8,000 cells per ml, and 16,000 cells per ml. Until this work, the academic standard was to use only 1000 cells per mm, but that was too low; this was a very low density.

So, we implanted very high-density cells, and as you can see, up to 3 months, we did further observations for almost up to 4 to 6 months. For almost six months, they could maintain hyperglycemia at normal levels; they could correct the blood glucose. So, whenever these active beta-ylate cells—these red cells are all beta-ylate cells present inside the capsules—sense a high glucose level in the body, they secrete insulin into the blood, and this insulin basically helps in transporting the glucose. So, they meant they could maintain the normal

glycemia level for a very long time, and even after expansion, you can see that all the cells are alive.

All the cells are alive. There is almost no fibrosis in the explanted capsules. So, the lead in the last slide we showed you is correct. So, the lead Z1 A10 was very efficient in reducing the fibrosis in these implanted capsules and also in maintaining normoglycemia. Now the problem is, as we said, that it takes about 1 million islet cells to effectively implant or transplant in the body in order to have a proper long-term cure, because each pancreas has almost 1 million pancreatic beta islet cells. Now, to implant 1 million beta elite cells using a capsule-based system, it will require almost 4 to 5 liters of volume, right? So, it will require at least 4 to 5 liters of capsule volume, which is almost impossible to do and almost impossible to think of.

Because you can see, in cases of any complication, what will happen; basically, these capsules can get stuck in different parts of our body, and eventually, it will become very difficult to retrieve those capsules. So, what would be the primary goal? Eventually, the main goal would be to develop the macro device or artificial pancreas. So, it would be like a macro-sized object; there we can put this 1 million cells inside, and also in case of any complications, we can easily retrieve the device and further load it with new allied pancreatic islet cells. So, initially in this field, when the type 1 diabetes therapy was started, the Edmonton protocol was used, where the cells were directly injected, but as we said, the host immune response would basically kill the cells. After that, this capsule-based system is used as an immunoisolation object, and small molecule coating can also be used, as we showed in the last paper in our work, where you can basically reduce the fibrosis response and effectively cure type 1 diabetes for 6 months to 1 year.

But what we just said is that there are a few challenges; the first is the difficulty of loading high-density islets, and even if you load high-density islets, the volume becomes too much. So, in case of any complications, you cannot retrieve that many capsules, and the space is not enough to implant that many capsules. The eventual goal is to create a macro device, but with a non-vascularized macro device, the device is unable to get proper nutrient support. So the final goal of creating an artificial pancreas is to develop a vascularized macro device where there is a nice vascular invasion or network between the device, and this blood network basically carries blood, which, as you know, carries oxygen and nutrients. So, as the blood carries all these required substances for the cells, the cells will stay alive for the long term, and this macro device should also be properly immuno-isolated and, if needed, coated with the small molecule, so that our body's host immune response or the host-like kind of fibrotic response cannot be triggered.

There are different approaches for vascularization, but the most effective one is like transplanting vascular cells. So, the eventual goal is to create a certain design system where we have a core shell type of structure, and in the inner core part, we will put the beta islet cells. In the outer layer, we will include a vascularized device that will have a specific patterning, and this patterning will attract faster vascularization. So, you can see that in the outer shell layer, we can have different architectural patterns; it can be serpentine, or it can even be parallel lumen. It can be like different octahedral types of structures, hexagonal and octahedral structures; it can be a honeycomb structure.

So, all this patterned architecture actually stimulates faster vascularization. So, any case of pattern architecture stimulates faster vascularization, and faster vascularization basically helps in terms of the delivery of oxygen and nutrients. So, as you can see, a typical type of macro

device or artificial pancreas can be developed with a serpentine structure that will have a lot of beta pancreatic islet cells inside, and after some time, this device will actually form a lot of vascularization around it, and this vascularization or blood supply will basically carry oxygen, glucose, and other ions. And all these inside islet cells will be able to stay alive and happy for a longer time. So, hopefully, by using this type of macro device, an artificial pancreas can be developed that can eventually cure type 1 diabetes for almost 2 to 3 years. And in case of any complications, you can easily retrieve the macro device and further replace all the dead islet cells with new fresh cells and implant them back into the body.

So, hopefully you like how the artificial pancreas can be developed. There are other ways of managing diabetes, such as an artificial pancreas, which includes hybrid closed-loop, fully closed-loop, or dual hormonal systems. These are basically like mechanical devices that have a continuous glucose monitoring system, and with the hybrid kind of injection of insulin and glucagon depending on the glucose level of the body, this type of injectable system or artificial pancreas can also be created. But as I said, there are a lot of challenges, including high costs; apart from that, there can be insulin resistance, and all the injections of insulin can cause pain to the body, especially for the kid. So, ideally, the target would be to develop a cell-based therapy with a vascularized macro device where we can implant the cells in the body, and the whole device should be immunoisolated so that our immune system cannot recognize and destroy it. So, this is some of the modern approaches that researchers are taking; hopefully, you enjoy the class.

So, let us think about what vascularization is, why it is important for cell-based therapy, what you mean by angiogenesis, and, in general, can you tell me what cell-based therapy is. You can also refer to different books, and please go through all the references that have been mentioned in the slide. Hopefully, you are enjoying the class on human physiology. If you have any further questions, please drop us the question in an email; you can also ask us during the live sessions. Thank you again for attending this very interesting class on cell-based therapy and the artificial pancreas.

Hopefully, you are enjoying the class. Let us meet with another new class very soon. Thank you.