Introduction to Biomicrofluidics
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Lecture - 16
Organ - on - a - chip

So, in our last module, we have discussed that how to do Cell Culture in Microfluidics platform with reference to 2-D cell culture and 3-Dimensional cell culture and at the same time, we have also discussed how to perform the different type of experimental setup like say different types of fluid flow, microscopy and then, we had gone through it that several experiments by using, you can utilize this fluid flow to decipher some fundamental behavior of cells in microfluidics system.

So, now we are coming how this microfluidics system could be used for application aspects like say biochemical analysis, say Organ on a chip. So, in this lecture I shall go through that how that organ on a chip is developed on the basis of cell culture principle in microfluidics and to some extent tissue engineering aspects to develop that miniature form of that organ on a chip. So, what do you mean by the organ on chip?

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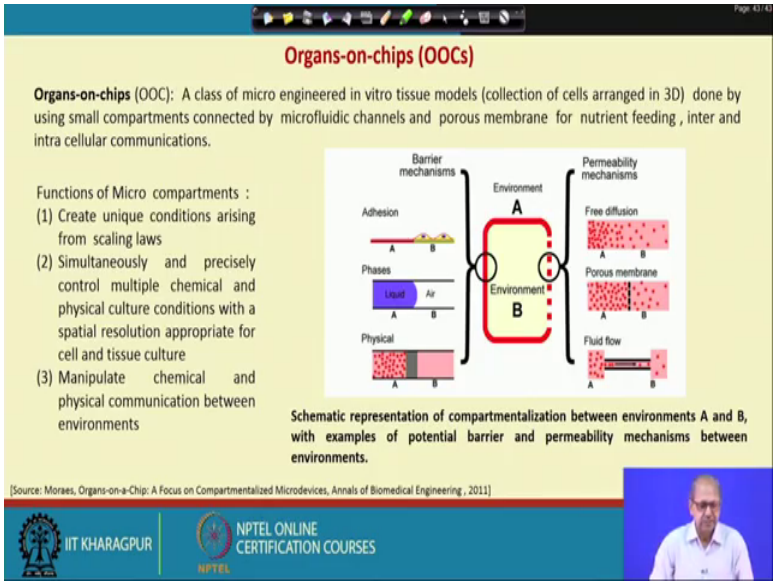

Organ on chip means a class of micro engineered in vitro tissue models done by using small compartments connected by the microfluidic channels and porous membrane for nutrient feeding inter and intra cellular communication. Now we are coming, what do

you mean by that micro compartments? We know that different types of cells comes together makes a tissue; tissues are organized to organ; then, organ system, then whole that living system.

Now, in a organ that tissues are arranged 3 dimensionally in a different types of micro environment and those environments are cross talking through that this permeable mechanism or barrier mechanism. So, permeable mechanisms like say free diffusion of the chemicals; may be porous membranes are involved means like say in extra solar matrix or vessel membranes, then fluid flowing micro fluidic channels. And if you look for the barrier mechanism of the environments say this is here that cells are attached and this is another environment, where cells are not attached means non adhering situations. May be different phases like say air and liquid like say alveolar sacs where the air and cells interface occurs.

Then, physical barrier like say blood brain barrier, where that physical barrier is that not all molecules will not go. In that way, that in a organ department different micro environments are created and they are cross talking with each other. So, that is why that micro pump compartments are described in that way. Create unique conditions arising from scaling laws. Simultaneous and precisely control multiple chemical and physical culture conditions with a spatial resolution appropriate for cell and tissue culture. And manipulate the chemical and physical communication between environments. What do you mean by the environments? This is micro environments inside that organs or tissues.

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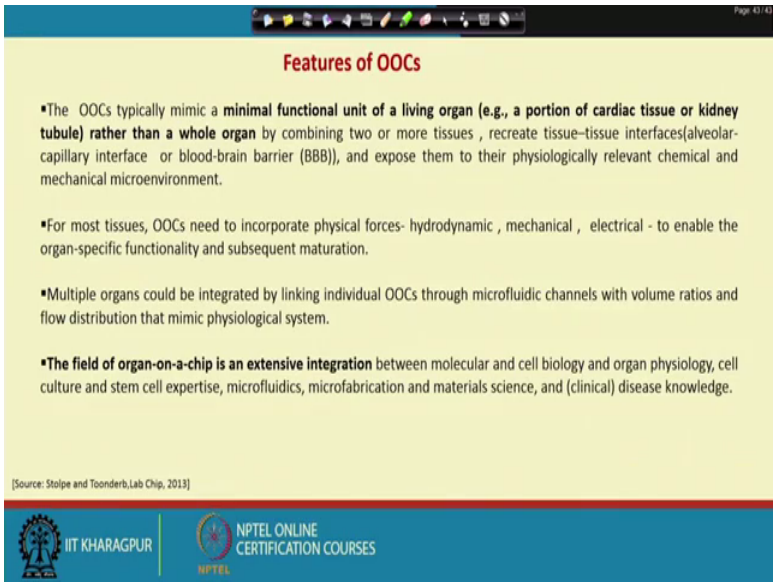

So, what is the features of organ-on-chips? We are coined as OOCs means Organ-on-chip. OOCs, I shall be coin that OOCs as a organ-on-chips. The OOCs typically mimic a minimal functional unit of a living organ, not a whole organ like say person of cardiac tissues or kidney tubule; like in kidney tubule is the unit of that kidney organ or an cardiac system that cardiac muscle or some bundle of fibers contractal fibers are unit. And this compartments or tissues will be organized to recreate tissue-tissue interface like alveolar capillary interface or blood brain barriers and expose them to their physiologically relevant, chemical and mechanical environments like say cyclic force or stretching force or fluid shear etcetera.

So, that is why for the development of most of that organ on chips, they need some mechanical force, electrical force or optical induction, electrical induction for their maturation at the same time to exhibit their physiological behavior. Multiple organs could be integrated by linking individual organ on chips through a microfluidic channel or through a porous membranes and most importantly this organ-on-a-chip is a highly multidisciplinary works. It is an extensive integration between molecular biology, cell biology, organ physiology, cell culture, stem cell expertise, microfluidics, microfabrications, material science and after all that clinical disease knowledge means physicians or clinicians should be involved to really develop that organ on chips.

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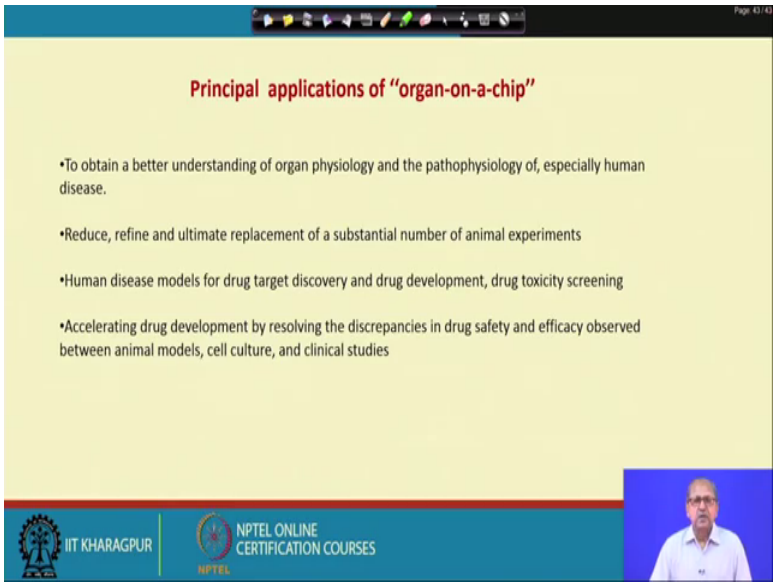

So, what are the applications of "organ-on-a-chip" means who are the stakeholders of this organ-on-a-chip? First thing is that using its organ on chips, we could have an understanding of organ physiology and pathophysiology of especially human disease means disease model could be established in a vitro, rather than based on animal models. Then, Reduce, refine and ultimate replacement of substantial number of animal experiments means this is the 3 R's basically. Generally, in the any development of a drugs, we are using animal as a model system. But nowadays ethics are coming very strong way, we cannot use animals for our purposes or drug targeting purposes or drug means clinical drugs.

So, for that this human organ on chips is very much essential and human disease model, already I have mentioned and accelerating the drug development by resolving the discrepancies in drug safety and efficacy observed between animal models, cell culture and clinical studies. Because when any drug is coming to the market, first preclinical data, clinical data; then, it is going to several phased clinical phased trials, then it is coming.

So, there is lot of discrepancy between the cell culture data to animal experiment data to clinical phase 1, phase 2, phase 3 clinical data. So, those should be rationalized on the basis of human organ-on-chip.

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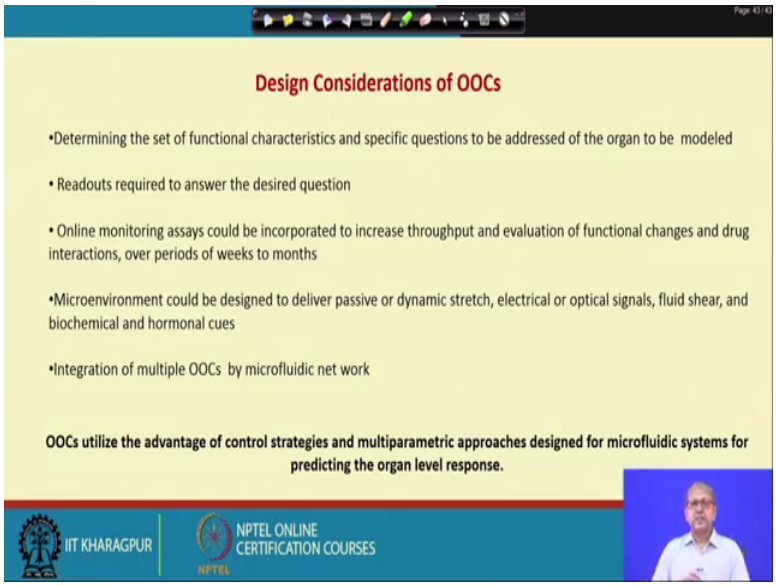

So, if we think that to develop a organ-on-chip, what should be the design criteria? First thing is that we have to determine the set of functional characteristics and specific question to be addressed in the organ to be modeled. As already I have mentioned that organ-on-chip, basically it is a miniature form of the organ not a whole organ. So, that is why specific questions should be asked, what we should be looking for from that organ-on-chip.

Next is what are the read outs required to answer the desired question like say if we look for that heart as a organ-on-chip; basically we shall be looking for that how that myocardial fibers are contracting and generating the force or how many bits are there's and how that electrical polarization will help for that mechanical contraction and so and so forth. Then, most importantly if we have a monitoring system which is online for the long term culture by which you can monitor that non invisibly that how that organ-on-chip behaves with respect of communication, with respect of their several philology oxygen of take ph and so on and so forth.

Then, manipulation of the microenvironment, passive or dynamic stretch, electrical and optical signals, fluid shear, and biochemical and hormonal cues means both biophysical and a biochemical cues which comprises the microenvironment and integration of the multiple organ-on-chips by microfluidic network which will mimic that different organ in our system like say heart, lung, liver, brain, kidney, gat in one platform by connecting

through fluidic networking and we can mimic that human physiology. And organ-on-chip utilizes the advantage of control strategies and multi parametric approaches design for microfluidic system for predicting the organ level response.

So, totally engineering approach should be utilized to harvest that potential of organ on chips.

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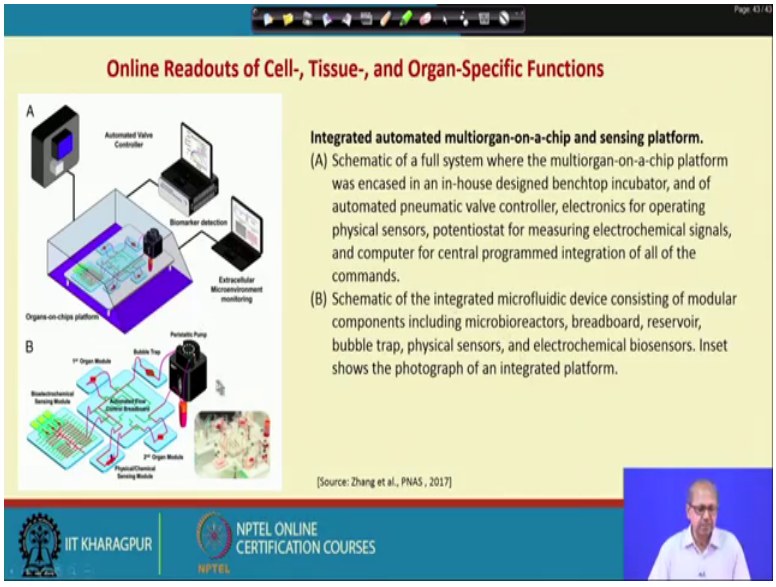

So, this an example how that online monitoring system is developed say like say there are 2 organs; this is a lever and this is a heart and they are connected through microfluidic platform and that online monitoring system is developed for say bioelectro chemical sensing module, then physical and chemical sensing module is automatically means without any destruction that fluids are monitored for their cellular behavior. In that way, we can monitor that whole the system like in our body, how it behaves in everydays or weeks after weeks, even months after months.

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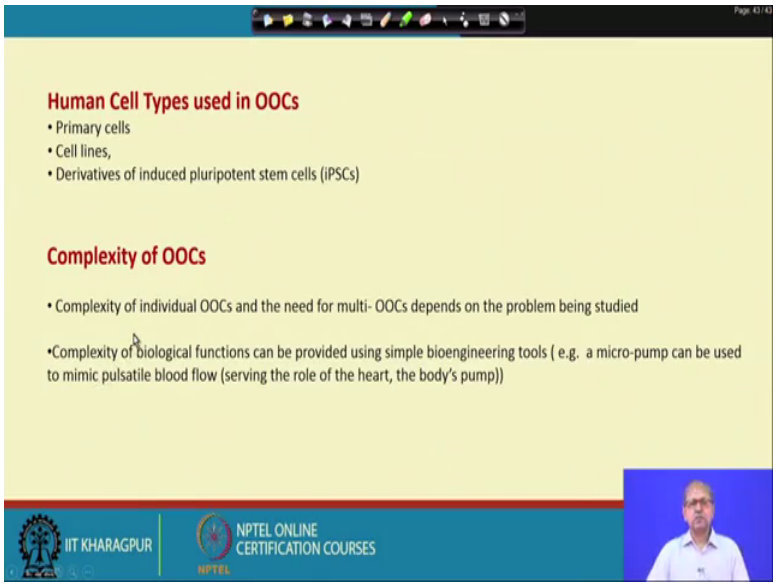

So, next question comes what are the cell to be used for organ-on-chip development and already we have discussed that for 2 dimensional cell culture or 3 dimensional cell culture, different types of cells like say primary cells or cell lines or some cancer cells should be we have already mentioned those cells are used. But in human organ-on-chip development we need cells of high density and always cells are not available. So, that is why induced pluripotent stem cells are used and which should be triggered to develop different types of cells and those cells would be used.

So, source of cells might you might be primary cells; cell lines and derivatives of induced pluripotent stem cells. Then, how much complexities would be in organ-on-chip? It may be a simple, it may be a complex; but complexity depends that how much what questions we are asking for that complexity. So, complexity of individual organ-on-chips and the need of multi organ-on-chips depends on the problem being studied.

say example say if you want to monitor that say mimic that microfluidic structure for heart beats say then, micro pump can be used to mimic that pulsatile blood flow that how that pulsatile flow can be could be means arranged in fluidic platform that already we have discussed in our earlier lectures.

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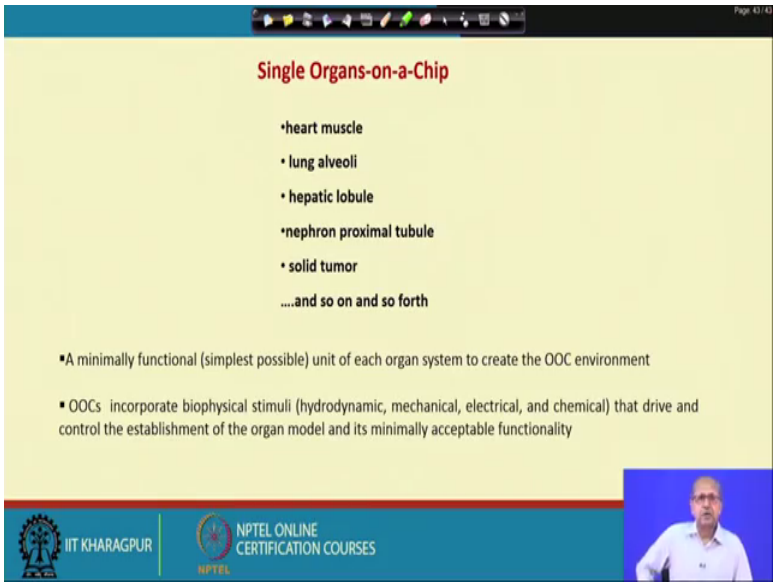

Now, we are coming how that single organs on chips are developed like say heart, lung, hepatic lobule, nephrons solid tumor and so on and so forth. So, a minimally functional simplest possible unit of each organ system to create organ-on-chip environment or so called Micro environment. And incorporation of biophysical stimuli like hydrodynamic, mechanical, electrical and chemical that drive and control the establishment of organ model and it is minimally acceptable physiological functionality. So, we shall be discussing some of that models like say first we are coming heart muscle for the organ-on-chip development.

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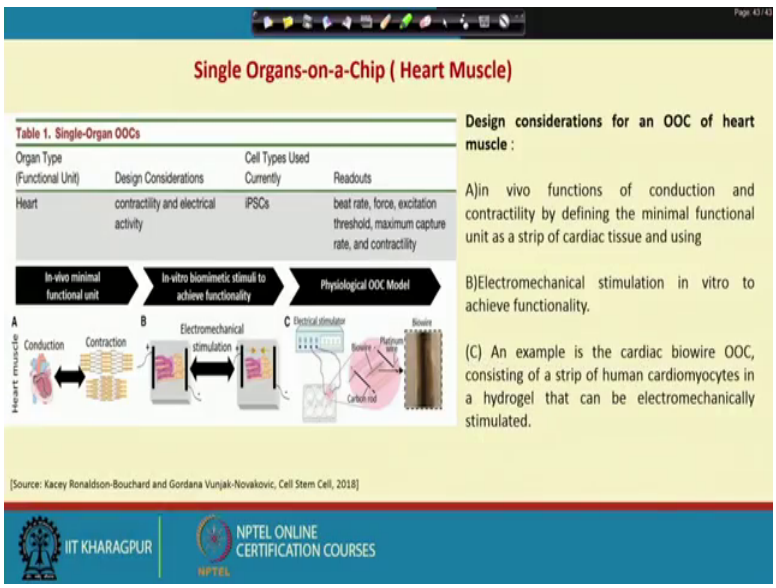

So, what are the design considerations for that heart, muscle? Contractility and electrical activity and what cell types should be used? Here, induced pluripotent stem cells derived cardiovascular monocytes are used and what are the readouts? Beat rate, force, excitation threshold, maximum capture rate and contractility. Say here that that minimal functional unit is that a bundle of contractile fibers or you can tell that myocytes means that when that is contraction occurs, then it generate force and that force are culminated or it is aggregated to make that heart beat basically.

And that contraction is induced by that electrical polarization that could be mimicked by that electrodes and in vivo system, this electrical conduction for the polarization electrical conduction are done by that press making cells or press making node at that senatorial node. That is mimicked by using this electrodes by depolarization and polarization, they will induce that stress fibers which will induce ultimately force generating that media.

So, this is Biowire means where that cardiomyocytes are embedded in a hydrogel wire format and that is connected with; that means electrical force by which that contraction will be there in the cardiomyocytes and generate that force and that could be monitored. So, this is an example of that heart muscle organ-on-chip.

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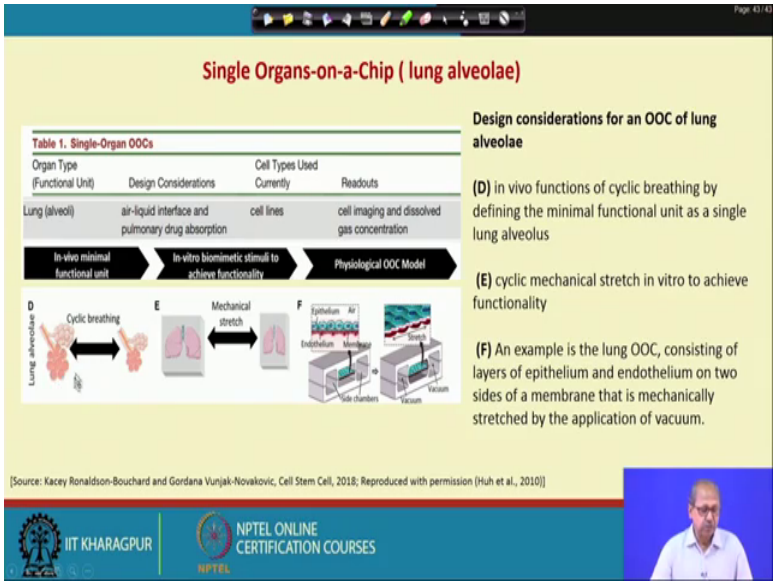

This a very beautiful model for that lung on a chip because unit of lung is alveolar sac these are the alveolar sac means air sac surrounded by that basolateral membrane, then endothelium cell means endothelium network and some extracellular matrix.

This is the unit of your alveolar sac. What is the function of alveolar sac that is there; there is a exchange of gas means air oxygen and interstitial. So, that is that function due to that cyclic stretching of lung means when you are breathing and exalting, then that cyclic stretching occurs. So, mimicking that cyclic structure in the microfluidic platform; it is a unit of your alveolar structure or lung alveolar. So, how this structure is formed? So, in microfluidic system, this is a membrane, porous membrane; biocompatible porous membrane, it is a stretchable membrane. In the upper side of the membrane that epithelial cells are cultured and the bottom side of the membrane that endothelial cells are cultured.

After that confluencing, then that media is replaced by air and that stretching force is given by using that side chambers vacuum and when that stretch stretching force is adopted, then cells are differentiated to like say alveolar or epithelial cells and that underneath is your interstitial flow and which behaves like your lung alveoli and here that gas exchange between that bottom chamber and upper chamber full of air that could be monitored. And here that microenvironments is your air cell interface and bottom is cell-cell interface through that membrane.

So, here cell lines are used mainly primary cell lines or some epithelial cells are available nowadays and here readouts are mainly by microscopically cell imaging and dissolves gas concentration by online, online monitoring system. So, this is a first system means first discover system or established system using that bio magnetic principle. Then, lot of systems are developed using this membrane and that gaseous exchange using that side or vacuums like in got in chip.

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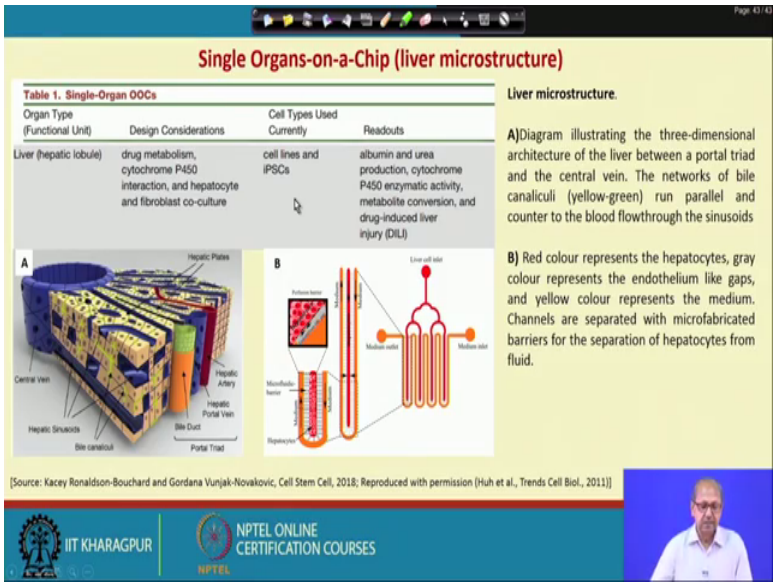

Liver is an organ that it is an chemical factory where that drugs are metabolized, lot of proteins are synthesized. So, what is the unit of liver? Unit of liver is liver lobule. So, lobular structures, this is an example of say representative example of liver lobules. They are arranged in such a way that this is that central vein and hepatic artery and these are the cells, where that hepatics cells are high density arranged and that venous and arterial systems giving that blood mean nutrient flow and then, exchange of that liver nutrients and other things.

So, how these things could be mimicked in microfluidic platform. Here, cell lines are used means f j 2 and sometimes in this fluid pluripotent stem cells derived liver cells are used. So, here is that design of that microfluidic platform, this is the unit of that system that liver cells, liver cells are high density impregnated in that middle layer and that outside that is medium flow and these are the microfluidic barriers which mimic that structure of your venous and arterial connection to that hepatocytes. So, that cell density is here, near or almost equal to that in vivo system and here that total medium, medium inlet and medium outlet and medium is percussing so that where that hepatocytes presence in high density.

So, here the readouts are that how that hepatocytes secretes albumin, urea, cytochrome P450 enzymatic activity, metabolite conversion, and drug-induced liver injury means with this system some disease model also could be developed.

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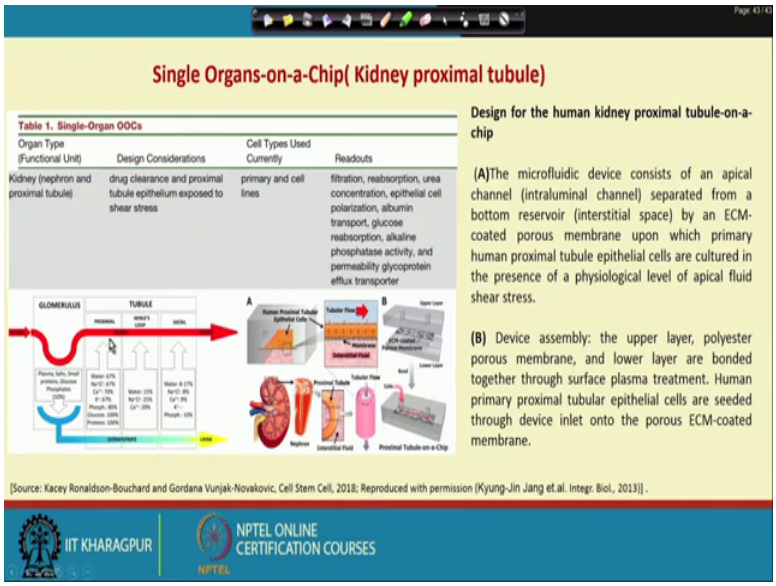

And this is kidney on chip that kidney function is that that glomerular filtration and it is that here blood is flowing through that channel of that glomerulus nephrons and these are these are the cells means where that plasma salts small proteins glucose phosphates are in. And that proximality will where that octave is there, then there means not absorbing urea and some salts, they are passing; ultimately, there excreting as a urine and some salts and urea.

So, this is the structure of kidney. This is your nephrons, unit nephron and how this could be mimicked? Because that epithelial cells in the nephron that highly polarized. So, for the polarization, we have to give that fluid flow. So, with the knowing this principle; so, that matrix is developed here that porous matrix above is that epithelial cells are growing and below that interstitial fluid means that interstitial flow is flowing. So, this is the tubular flow means, this is the tubular flow of blood and here is the interstitial fluid. So, if we give this fluid in this microfluidic chamber, then it is observed that epithelial cells are polarized epical and vessel and they express their functional means transport proteins.

So, in the experiments all types of epithelial cells are generated and they are used for that to mimic that whole that tubular structures at that level of Proximal, Henle loop and Distal sides. So, totally it can it could be mimicked that filtration structure of that kidney.

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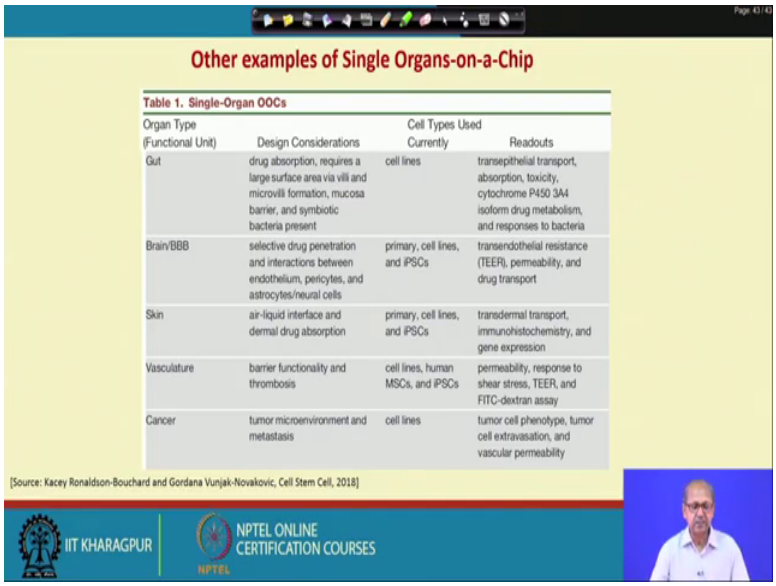

So, in that way lot of other organs are developed in organ and chip module. Like say Gut, Blood Brain Barrier, Skin, Vasculature and Cancer models. So, in the Gut design considerations, the drug absorption or the large surface area where the villi, microvilli formation and mucosa barrier, and symbiotic bacteria means micro biome what is present in your gut.

Then, cell lines are used say caco 2 cells are used and the readouts are transepithelial transport, absorption, toxicity, cytochrome P450 3A4 isoform drug metabolism, and responses to bacteria. In the same way for Blood Brain Barrier, the readouts are transendothelial resistance permeability and drug transport. For the skin, transdermal transport and gene expression; for a vasculature, permeability, response to shear stress and transendothelial resistance and for tumor, lot of things already we have discussed to some extent in our earlier lecture and for the tumor model that metastasis and how that with extra gadget from one place to other place.

So, after discussing that organ-on-chips, now we are coming how this organ on chips could be integrated to mimic that different organ system; how they are connected in our body.

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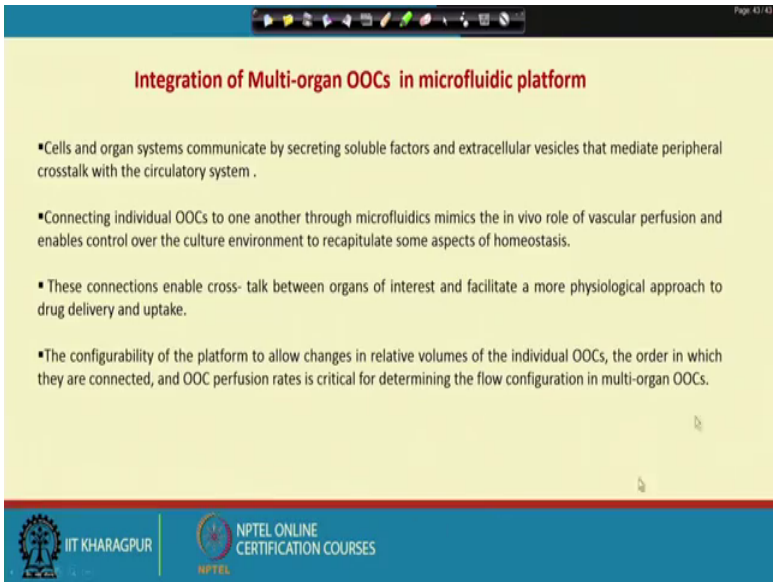

So, that is integration of multi organ, organ-on-chips through microfluidic platform. See had that basic principle is that communication between that organs through soluble factors and extracellular vesicles that mediate peripheral crosstalk with the circulated system. So, for that microfluidic mimics are used like say in our vascular system, perfusion system and enables it control over the culture environment and recapitulate some of the aspects of homeostasis and these connections like our in vivo system, helps to crosstalk; at the sometime feedback control mechanisms what is happening in our normal physiology.

Now, how this organ on chips will be integrated in different configuration means may be parallel serial or in one platform or in a continuous phenomena, continuous fashion or in a perfusion readouts or perfusion ways. So, one by one we are coming.

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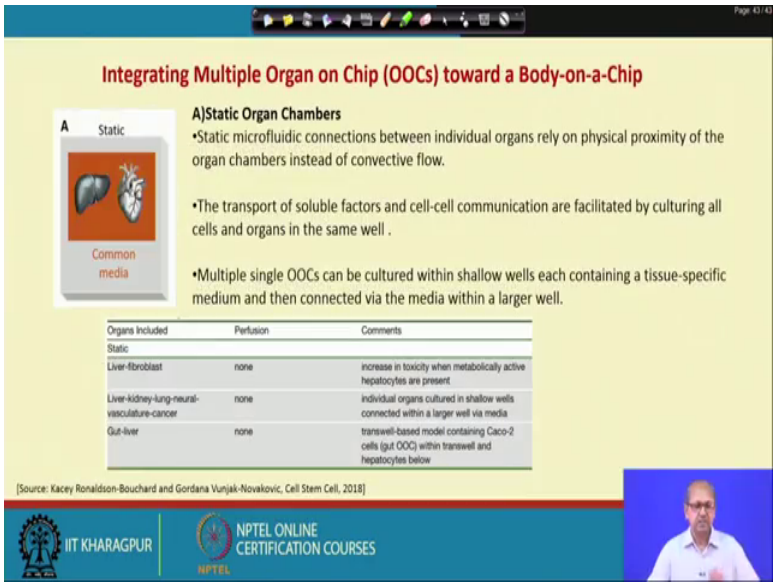

That first one is Integrating Multiple Organ-on-chips in a Static Organ Chamber. Means static organ chamber means there is no convective flow, in only in one chamber that different organ-on-chips are there or in a big a say peptides the small peptides are there, where that individual organ-on-chips and there within same your media. That is multiple single organ-on-chips can be cultured in a shallow wells each containing tissue specific media and then, connected via the media within the larger wells.

So, this is that first module means what are the drawbacks of this module is that there is no convective flow; what is happening in our body. So, in that way some of the examples are liver fibroblast. Here is no perfusion, increase in toxicity when metabolic active hepatocytes are present. Liver-kidney-lung-neural-vasculature and cancer means early 4 or 5 organ-on-chips are together. Then, Gut liver, gut liver in that transwell based model contaminates containing the caco 2 cells with transwell and hepatocytes.

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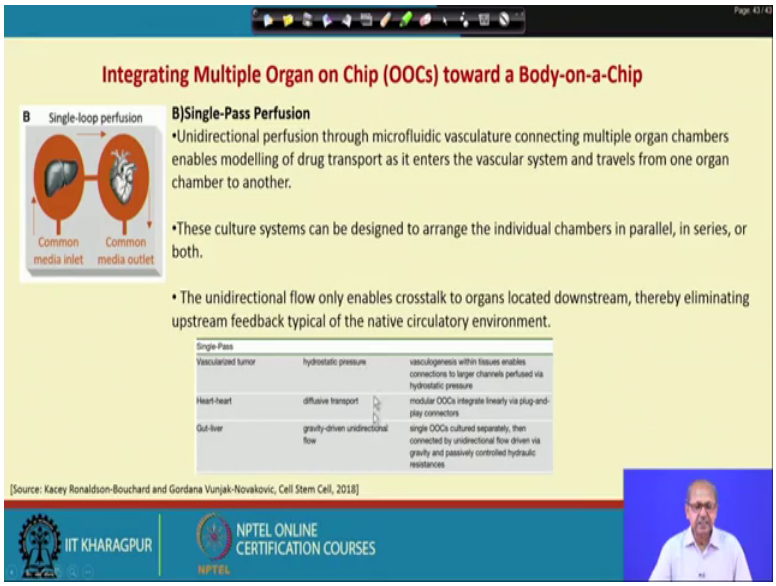

The Integrating Multiple Organ-on-chips by Single-Pass Perfusion means they are their conflictive flow is there. This is common media inlet and a common media outlet, means in that fashion this organ-on-chip could be connected by either parallelly or serial or in series or in both ways. But problem of this type of configuration is that that a downstream of that flow, they are no feedback; they are controlling only from that upstream because upstream organ on chips, they are not getting the feedback from the downstream.

So, examples in that system that single pass vascularized tumor hydrostatic pressure is used; heart and heart diffusive transport, gut and liver gravity driven unidirectional flow.

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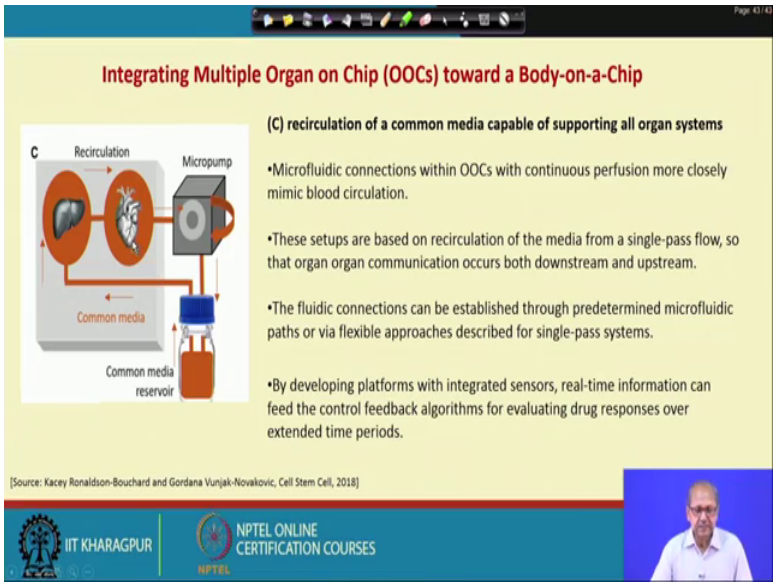

And this is an chip, where that recirculation is there means like our normal vascular system that common media is passing through that each of the organ-on-chips and again circulating. This is like a circulating system in our body. So, in that platform that every organs they are getting feedback from their other organ there is no downstream of upstream. So, they are getting in that way.

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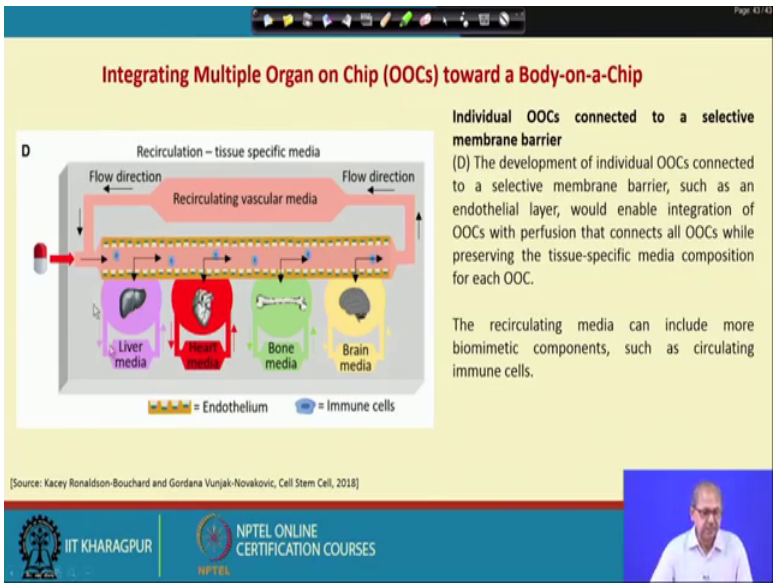

Then, last module is that where that each organs are connected by that perfusion modules, here is circulation is there and each organ means each organ-on-chips, they

have individually cultured in their individually media. And then, they are connected through a means common channel with endothelia and where that immune cells will passing on and this is the circulating flow direction by which all cells are connected keeping their individuality.

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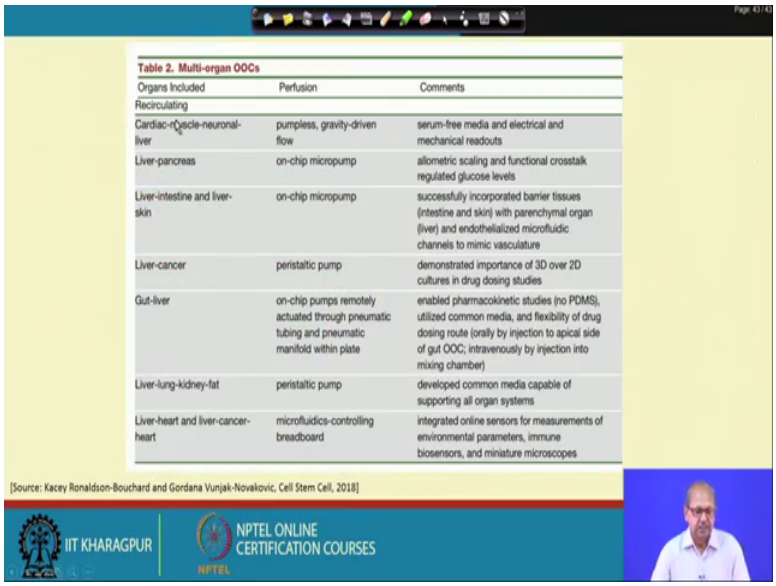

So, these are the some of the examples where that perfusion flow are induced for that recirculation purposes; Liver-lung-kidney, Liver-heart-liver-cancer and etcetera.

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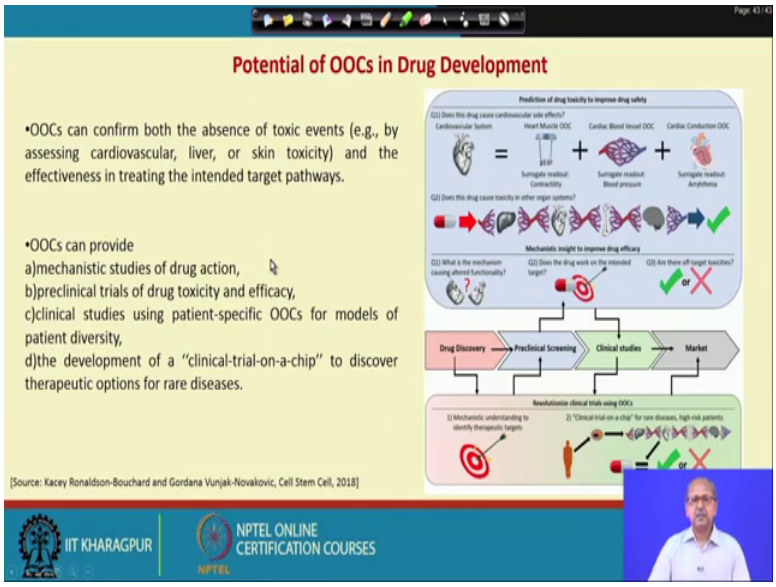

So, now that utilizing this organ-on-chip in a connected way; how these systems are used in drug development? If we look for that drug development project, they are passing through different edges. Say first drug discovery means where it works drug discovery means which is the target then, preclinical screening like say in cell culture level or animal model level. If then pass, then it is going to clinical studies. Then, phase 1, phase 2, phase 3 clinical studies; then it is coming to the market. But real sense, most of the drugs they are not passing after the preclinical stage because most of the it is drug development situations are done in cell culture model or in animal model because there is a huge amount of discrepancy between these and human system.

Again, after that two or first clinical trial or second clinical trial, these are failure due to the different level of populations. So, how this could be adjust using these organ-on-chip model? So, like say this an example; say we are looking for a drug for cardiovascular disease, first question we are asking, does this drug causes cardiovascular side effects? Means first is target identification, means say in the heart whether it may be a heartbeat is effecting or blood pressure is effecting. If target is identified properly, then if there is a in that target level there is a any toxicity means if this drug works on heart muscles contractility or it effects your blood pressure or that uncontrolled heart beating means arrhythmia means this the side effect in that target level.

Then, if you look for that side effects in other organ level means then, does this drug causes toxicity to other organs? Means this is a cardiovascular drug whether it effects liver or whether effects bone marrow; whether effects this brain means a blood brain barrier? So, this intra and inter toxic drug toxicity could be as said in a microfluidic platform using different organ-on-chips models. Then, if that mechanism causing that altered functionality or does this drug work on the indented target, if it is pass through all these things. Then, possibly we can go for that clinical study.

So, if it is not possessed through these studies like in organ-on-chip model, then it should not proceed further; means this will help drug development project to proceed or not to proceed in earlier stage and it will decrease that cost of that drug development. Even if that it is possible to do that drug trial in patient basis say because in most of the cases clinical trial that terminal patients or who are means highly disease situation they are not included because due to that they should not be trail with that drug trial.

So, most of the drug trials are done by that population level with some normalization; they did not consider the death intensity genetics of that humans or something like that. So, those should be considered in the personnel basis in an organ-on-chip model. So, organ-on-chip can provide mechanistic studies on drug actions, preclinical trials, clinical studies using patients specific organ on chips and most and most the last that "clinical-trial-on-chip" to discover therapeutic options and for rare diseases means organ-on-chip has a huge application to that in the coming fields, but there are lot of challenges.

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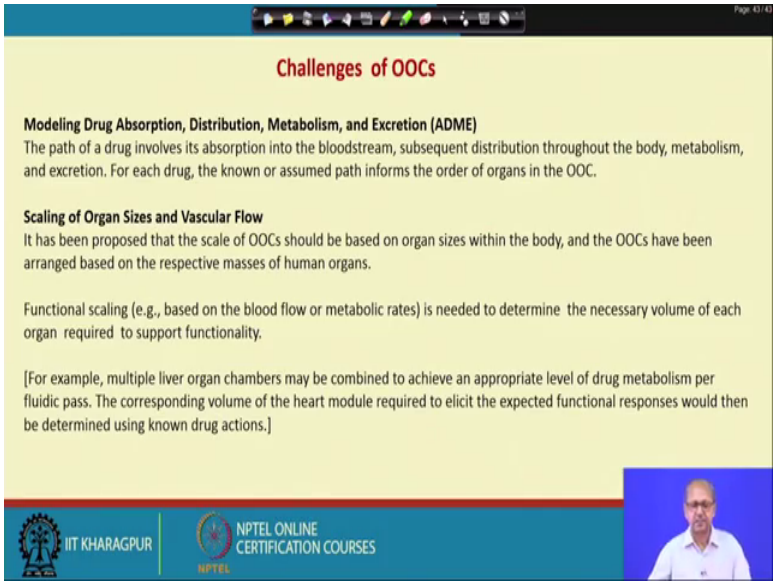

What are the challenges that Modeling of Drug Absorption, Distribution, Metabolism and Excretion, ADME; then, scaling of organ size and vascular flow because if needs lot of means simulation and engineering aspects to scaling the organ size and vascular flow.

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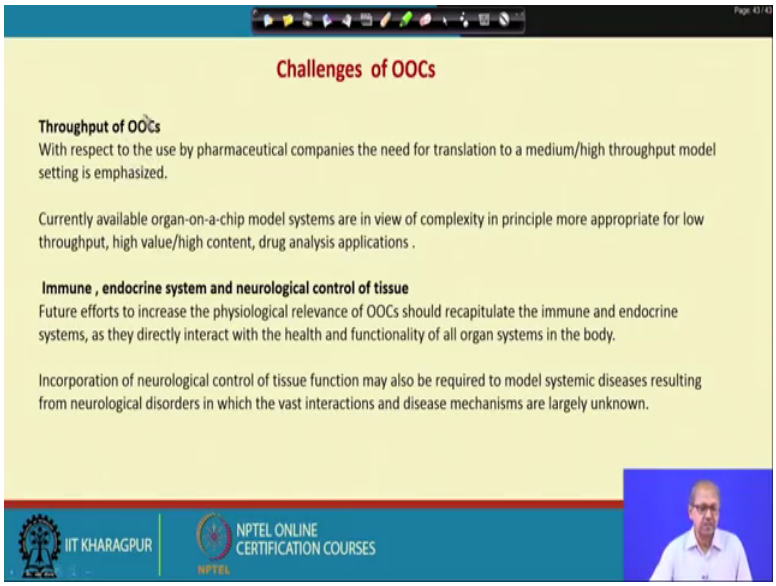

Then, throughput of that organ on chips; right now, what organ on chips are available? Their throughput is not very much what pharmaceutical companies wants? So, high level or high throughput level organ on chips are should be developed and last of all so far, there is no organ-on-chips where that immune system, endocrine system and neurological systems are integrated. Because we know that each and every organs then high level neurological controls are there's. So, those type of integration is necessary to mimic that real organ on chips which will mimic that in vivo physiology or in vivo system.