Fabrication Techniques for Mems-based Sensors: Clinical Perspective

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Lecture – 26

Hi, welcome to the second part of this module; what we learned in the first part is what exactly immunotherapy means right. And then we have seen how we can extract T cells from blood or from spleen. When we talk about spleen right we have to take spleen as you can as you have seen in the video right we are taking spleen, and then we are loading the spleen in a media and then the T cell isolation processes, that you take the spleen, crush it take the splenocytes; from splenocytes you can extract or isolate T cells using T cells isolation kit.

The kits are 2 types one is positive selection kid, one is negative selection kit, then these are your naive cells. So, you have to activate those cells and that activation can be done using dynamics. Now once you have this T cells what you will do with this T cell? You have to see what is the concentration, what is the ratio of CD 4 to CD 8 in the given number of cells that we obtain from spleen or we extract it from the blood right.

Now, this will be your T cells without interaction or without interacting with tumor micro environment. Then we have seen how the static process is done. Take a trans well the bottom of the well you can load this spheroids matrigel, load the well, trans well load the T cells and let the interaction occur between T cells and spheroid there will be some releasing of chemokines because of which there will be change in CD 4, CD 8 concentration.

Now, you treat those same cells with an immuno modulator or immunotherapy drug, and you block one of the immune checkpoint on T cells in this case we are talking about PD 1 and cd interaction right. So, now, you will be able to see the change in CD 4 CD 8 ratio when you talk about control that is cells, T cells which are not treated with any immune modulator and T cells which are treated with a particular immuno modulator.

So, we know whether the immunotherapy drug is working or not, we can understand the efficacy or we can evaluate the efficacy of immunotherapy drug. So, then where is the problem? The problem is that this system that we are using or that is currently being used

is static system. And like we mentioned in last module that our body is not static it is dynamics. Of course, I am sitting here without doing anything it is static, but still the body within it is dynamics right the blood is flowing heart is beating right blood is flowing. So, so when a patient has a cancer and we give a particular drug, drug will dissolve in the blood a blood circulates in the body and that is how the drug diffusion to a particular site would happen right. It is not that when you when you give a drug, drug will go to a particular site and stick there for 24 hours.

So, can we mimic this in vivo situation that is within the body on to a platform that is in vitro, and see the effect of immune modulators on T cell that is our question that is our interest and is there a difference when we do that? So, same experiment take out T cells now from where you can get spheroids for from where you can get this tumor you can get the cells from the patient grow the spheroid is in the lab using 96 well plate you we will we will talk about this 96 well plate cell culture in one of the one of the modules one of the lectures.

So, you take the cells from the patient from the site which is tumor extract the cells then grow spheroids in the laboratory, we have seen how spheroids looks like. We load this fluid in a microfluidic chip, flow T cells in the microfluidic chip next to the spheroids and see the interaction and see the CD 4 CD 8 concentration. Now you treat this T cell with immunotherapy drug again flow it in the microfluidic chip, have some interaction and see the change in concentration of CD 4 CD 8 cells.

By doing so, are we getting a difference in terms of result between a static experiments and dynamic experiments? If yes then, we should go for dynamic experiments rather than static. Another point about 68 percent of patient responses well for the immunotherapy drug; around 32 percent are not responding well. So, why? Why that 32 percent patients are not responding for a given therapy? Because the, it is already FDA approved drugs or these drugs that are given are already approved by a particular drug governing agency right approval agency; is all within the limits within the rules, with lot of results that you need to show that the particular drug is effective lot of clinical trials that you undergo.

So, finally, the drug comes to the market, then when a patient is taking the drug why drug is not effective. So, can we design a platform can we design a platform that is patient centric that is our quest right. So, if you see this screen.

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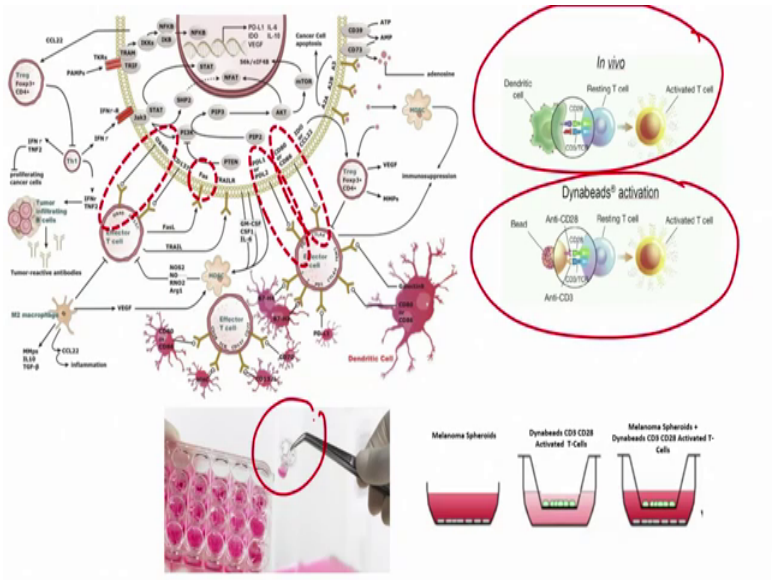

What we have seen? We have seen immune checkpoints, we have seen if we can if we block one of the checkpoint on T cell, what will happen right? We are assuming that CD 4 CD 8 concentration will be different; we have seen how the activate T cells are activated from the resting state in case of in vivo, and how dynamics could activate the T cell from his resting state, we have seen this is called trans well right. Now let us see.

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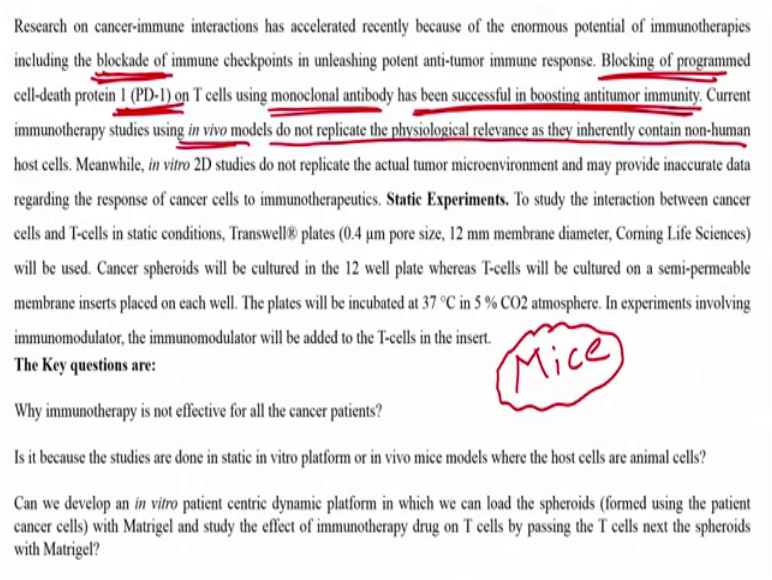

Now, you can read research on cancer immune interaction has accelerated recently because of the enormous potential of immunotherapies, including blockade of immune checkpoints right blockade. We can block immune checkpoints; in unleashing potent anti tumor immune response. Blocking of programmed cell death protein PD 1, PD 1 is on T cells right see on T cells.

So, blocking program cell death protein on T cells using monoclonal antibody has been successful in boosting anti tumor immunity see here. So, current immunotherapy studies using in vivo models, do not replicate the physiological relevance as they inherently contain non human host cell. So, the present studies that we go are in mice like animal models Irene Mice.

Now, the results that we get, we cannot say this is exactly replicating the human since the animal cells are the host cells right. Now meanwhile in vitro 2 D studies like we have seen right trans well do not replicate the actual tumor microenvironment and may provide may provide inaccurate data regarding the response of cancer cells to immunotherapeutics.

You see one thing is PD 1 blocking PD 1 can be effective. Second thing is in vivo models animal models will not replicate actual tumor environment because of the animal host cells or non human host cells. Third thing is 2 d in vitro model do not replicate the actual

tumor microenvironment and thus it is possible that they may provide inaccurate data regarding the response of cancer cells to immunotherapeutics.

So, what to do now? Static experiment; let us see how static experiments are done we have just discussed let us see here; to study the interaction between cancer cells and T cells in steady condition Transwell plates you see transwell plates point 4 micron pore size 12 millimeter diameter from corning life sciences, can be used or will be used right. Cancer cells will be culture in 12 well in 12 well plate, it can be culture interval plate where T cells can be cultured on a semi permeable membrane in such place on each cell.

The plates are incubated at 37 degree in 5 percent CO 2 atmosphere right. In the experiments involving immunomodulator, the immunomodulator will be added to the T cells into the insert that is what we have said what we have said? T you take you take this well.

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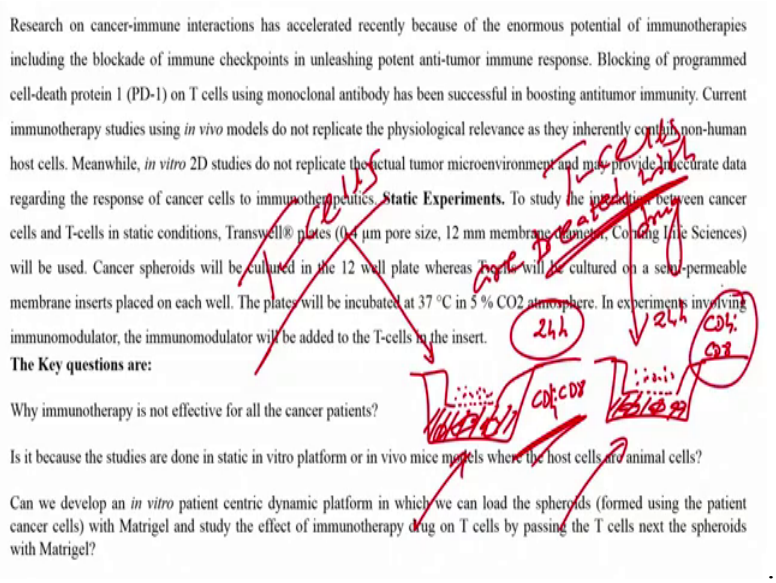

Load the T cells right and everywhere there is matrigel, there is a tumor spheroids here in the bottom, and T cells are here with media right. You have another well, you load T cells you have spheroids, here again have T cells right wait for 24 hours and see CD 4 CD 8 ratio right here wait for 24 hours see CD 4 CD 8 ratio.

The difference between this one and this one is in this case the T cells are treated with immunotherapy drug right. This is control just T cells; after 24 hours we see CD 4 CD 8

concentration T cells treated with immunotherapy drug after 24 hours we see CD 4 CD 8 concentration right and we compare this is what it is written here right and of course, when we when you do this you have to keep the plate in an incubator to maintain the 5 percent CO 2 and 37 degree centigrade atmosphere so, that the cells would not die cells would not die hm. And the T cells can be kept in an immune cult, which is the media for keeping the T cells alive.

So, if this is the method that is used and the results are obtained, there are some quick equations that arises. First question is why immunotherapy is not effective for all the cancer patients right? Is it because the studies are done in static in vitro platform or in vivo mice models where the host cells are animal cells? And can we developed an in vitro patient centric dynamic platform, in which we can load the spheroids with Matrigel and study the effect of immunotherapy drug on T cells by passing T cells next to the spheroids within the Matrigel right?

So, that is how we had to create that is why we had to create a dynamic platform that is a microfluidic chip based study will perform to understand the difference between static results and dynamics results.

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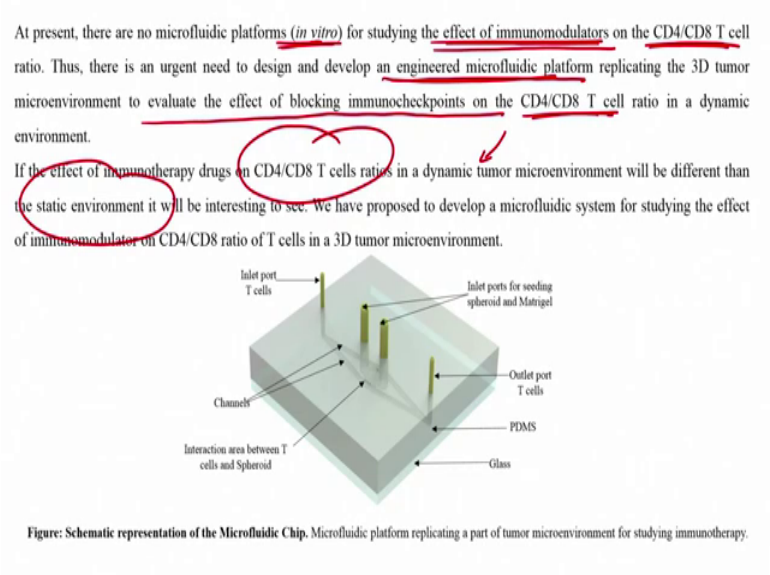

Figure: Schematic representation of the Microfluidic Chip. Microfluidic platform replicating a part of tumor microenvironment for studying immunotherapy.

So, at present at present there are no microfluidic platform that is in vitro, for studying the effect of immunomodulators on CD 4 CD 8 T cells.

Thus there is an urgent need to design and develop an engineered microfluidic platform and application 3 D tumor micro environments to evaluate the effect of blocking immune checkpoints on CD 4 CD 8 T cells in a dynamic environment right. So, if you want to check effect of immunomodulators on CD 4 CD 8 T cells, we need a dynamics platform, but currently there are no microfluidic platforms that are used for this kind of study.

So, if the effect of immunotherapy drug using static platform on of course, CD 4 CD 8 T cell ratio is different than dynamics platform. If the effect of drugs on CD 4 CD 8 in static platform, if it is different than dynamics platform, it is very interesting to study further. For that you can see we are proposing to develop the microfluidic system for studying the effect of immunomodulator on CD 4 CD 8 T cells and what is this platform let us see.

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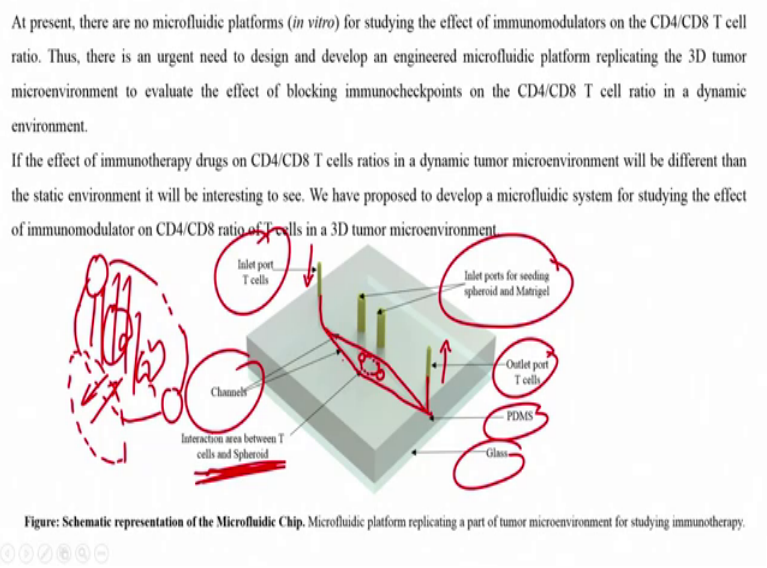

There is a inlet port right you can see there is a microfluidic chip that goes here and merges here, this goes from here, goes all the way. It merges here. This is the outlet, this is inlet this is outlet and here there are there is a circle where you can load the cancer spheroid in center. You can load in this region, you can load cancer spheroid, you can see here spheroid is unloaded here right. Seeding spheroid or matrigel these outlet for T cells, inlet for T cells; these are channels the surface is from PDMS the bottom is glass, and this is the interaction area where you can see here like this area right where you are loading the spheroid right spheroid is here with matrigel hm.

So, this area is where the interaction between T cells and spheroids would happen, there will be releasing of chemokines right and change in CD 4 CD 8 concentration would occur right. So, this kind of chip can be potentially used to study the effect of immunomodulators on T cell right.

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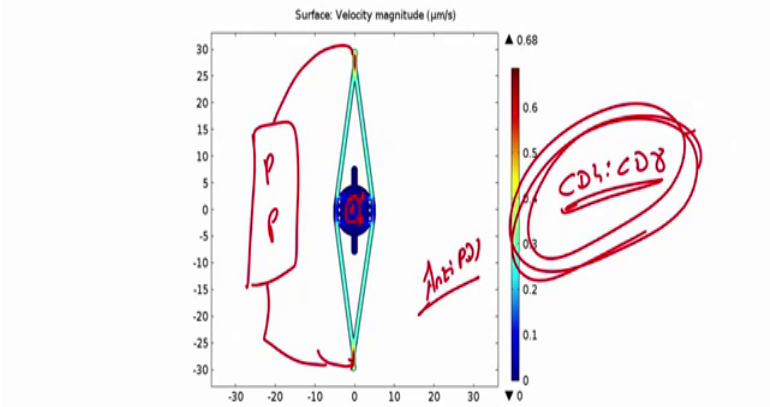

Figure: Simulation for Drug Flow through Microfluidic Device. (**a**) Shear stress plot of the microfluidic device. The shear stress is found to be <$1.1x10^{-10}$ Pa and uniform across the length of the channel. (**b**) Velocity plot of the microfluidic device. The Matrigel filled region is static and hence is shown at 0 m/s. The velocity of the T-cells near the interaction area is sufficiently low (0.35 μm/s) to realistically mimic the *in vivo* conditions. Velocity increases to around 0.7 μm/s near the inlet and outlet regions due to the tapered geometry.

Now, like I said we always need to perform a simulation before we actually fabricate a device ok.

So, before you go for any fabrication we have to always go for simulation. In microfluidic chip you need to perform analysis such as velocity stress, in the in the device whether the flow is smooth or not right. So, if you see in the screen you can see the simulation results when we are flowing a drug through the microfluidic chip, and you can see that the shear stress plot of the microphonic device is found to be less than 1.1 into 10 to the power minus 10 pascal and is uniform across the length, and we have not shown you the shear plot, but it was found that shear plot is the value is this.

While the velocity plot which is shown here the matrigel fill region in static and hence is shown at 0, this is at 0 because it is at static right while the velocity of T cells near the interaction area is sufficiently low to realistically mimic the in vivo condition. Velocity increases to 0.7 micron per second near the inlet and outlet regions due to tapered geometry.

So, here the velocity increases little bit however, in this particular area the velocity is sufficiently low to mimic the actual flow inside the body. The flow within the from the capillaries to the tumor is close to 0.35 micron per second or less than 0.1 less than 1 micron per second.

So, realistically this is mimicking the in vivo situation that happens in the in the body. So, we can use we can use this particular device, and study further what is the effect of immunomodulator. So, let us see.

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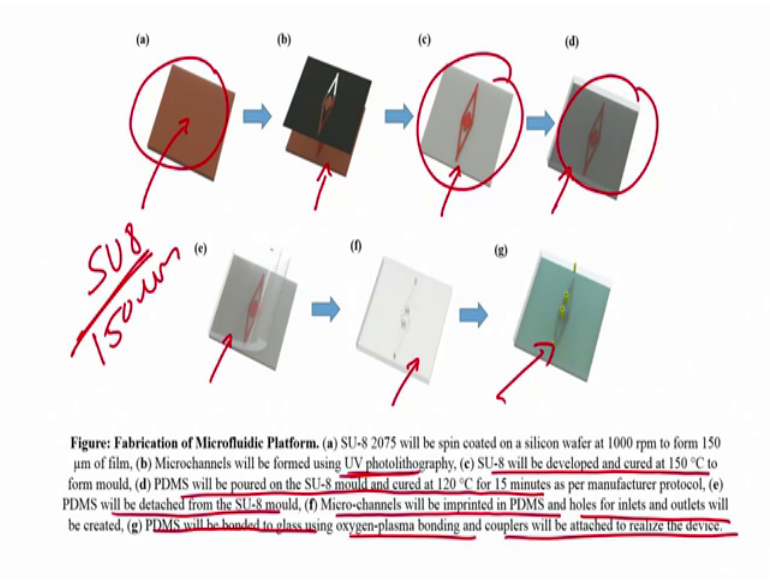

Figure: Fabrication of Microfluidic Platform. (a) SU-8 2075 will be spin coated on a silicon wafer at 1000 rpm to form 150 µm of film, (b) Microchannels will be formed using UV photolithography, (c) SU-8 will be developed and cured at 150 °C to form mould, (d) PDMS will be poured on the SU-8 mould and cured at 120 °C for 15 minutes as per manufacturer protocol, (e) PDMS will be detached from the SU-8 mould, (f) Micro-channels will be imprinted in PDMS and holes for inlets and outlets will be created, (g) PDMS will be bonded to glass using oxygen-plasma bonding and couplers will be attached to realize the device.

This is the process flow this is the process flow; since this is a process flow we need to understand how these things are done and this is one step process very easy one step single step process.

So, you see here a, is SU 8 2075 will be spleen coated on a silicon wafer at 1000 rpm to form 150 micron film. If you see this one this is SU 8 spleen coated on silicon wafer right and we have about 150 micron thick SU 8 film. Second is micro channel will be formed using UV lithography this is b; c is SU 8 will be developed and cured at 150 degree you can say it is cured. D is PDMS will be poured on SU 8 mold and cured 120 degree for 15 minutes; e would be PDMS will be detached from SU 8 mould; f would be micro channels will be imprinted in the SU 8 in the PDMS and holes for inlet and outlet will be created.

Finally you can see here PDMS will be bonded to glass using oxygen plasma morning and couplers will be attached to realize the device. Easy very easy right let us say. Let us see this thing how we can how we can fabricate the device. So, let us see here.

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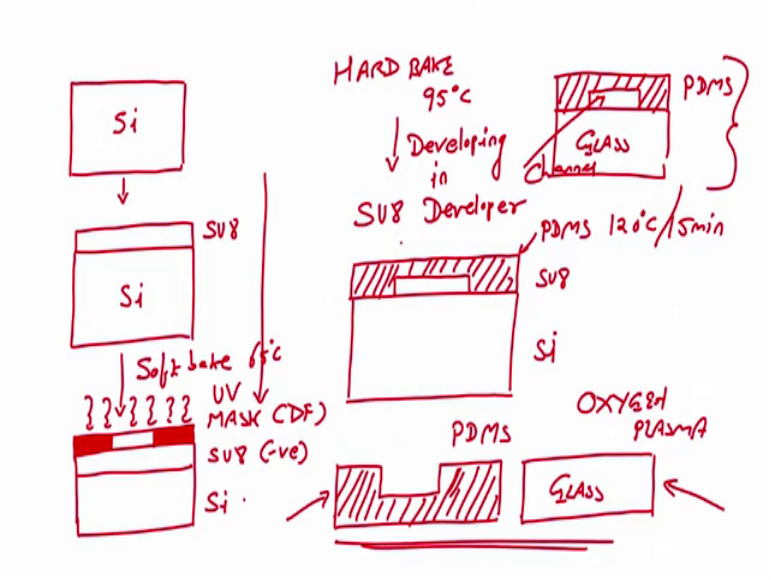

First point was you take the PD, you take this silicon wafer second point was you spleen code SU 8 on silicon. Third point is you load mask on SU 8 right and SU 8 is which kind of photo resist it is negative photoresist ok.

So, after SU 8 you code SU 8 you have to do soft bake; soft bake and then load mask right and you need to form structure which is which will help to create you channels right ok. This is your mask can be dark filled; now you expose your wafer with UV. After exposing next step is after exposing the next step would be to go for hard bake SU right is the little bit different. So, you have to perform hard bake before developing SU 8 hard bake.

Now, soft bake is generally done at 65 degrees centigrade hard bake is done at 95 degree centigrade, the time depends on the thickness of SU 8 material, it is already given in the manufacturer protocol ok. So, after soft bake and you load the mask and you expose the wafer, then you have to perform hard bake followed by developer developing in SU 8 developer.

When you do that what you get? You get SU 8 like this why? Because negative photoresist. The area which is exposed will become stronger; area which is not exposed will become weaker right. Now what you will do with this? On this SU 8 you pour PDMS and you cured PDMS at 120 degrees centigrade for 15 minutes after that you peel off the PDMS.

So, when I peel it off, it will look like this right correct now I take a glass and this PDMS and this glass, I will perform oxygen plasma I will perform plasma right around 100 watts, and about 10 minute or pressure. Once I perform oxygen plasma, I will bond glass and PDMS. So, this would be my channel right and this would be a microfluidic chip this would be a microfluidic chip right.

So, I had to use only one mask process you see only one time we have used masks single mask process. So, now, if you want to see you can see here once again what you are looking at? You are looking at SU 8 right spleen coated on silicon then we perform photolithography to form channels on silicon to form the pattern of SU 8 on silicon.

Then we cured the SU 8, then we after curing the SU 8 we get the mould which is right over here this is our mould right after mould we pour PDMS we pour PDMS on the mould. After pouring PDMS we have to cure PDMS for at 120 degree centigrade for about 15 minutes following that we will peel off or we will detach PDMS from SU 8 mould which you can see in e. After detaching you have to you have to insert holes in this PDMS right in this particular 4 section through holes.

So, holes for inlet outlet and for loading matrigel with spheroid could be created and finally, this is PDMS. So, we will perform oxygen plasma bonding; once oxygen plasma bonding is done we will connect the couplers we will attach the couplers right.

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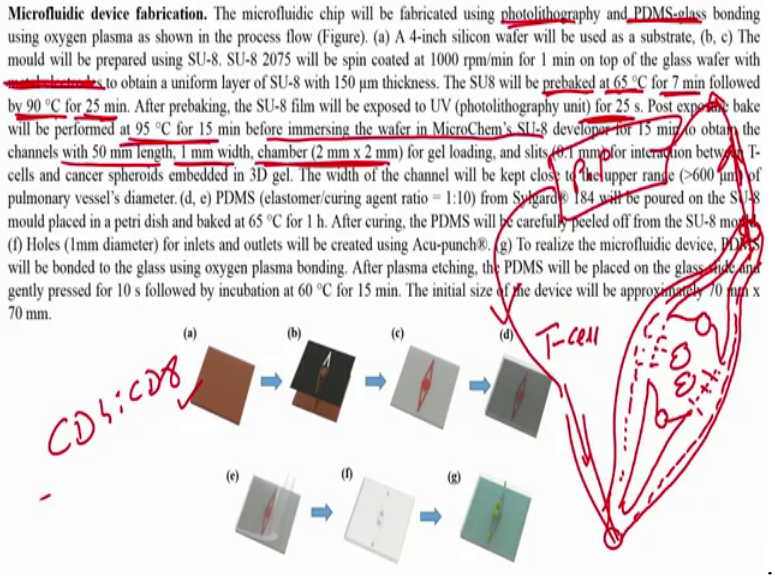

So, if you see here the device fabrication, this is in detail device fabrication process. And as we have discussed the microfluidic chip will be fabricated using photolithography and PDMS glass bonding; a 4 inch silicon wafer will be used as substrate which is here b and c the mould will be prepared using SU 8 SU 8 2075 will be spin coated at 1000 rpm for 1 minute on top of glass.

Wafer with metal electrodes to obtain uniform layer of SU 8. The SU 8 will be prebaked a 65 degree for 7 minutes followed by 90 degree, this is not metal electrodes we do not require metal electrodes here. SU 8 will be prepared pre baked at 65 degree and like I said 3 baking is at 65 degree, now this for 150 micron of SU 8, we require 7 minutes of pre baking time. Followed by 90 degree for 25 minutes after prebaking the SU 8 will be exposed to UV lithography for 25 seconds, post exposure bake will be performed at 95 degree centigrade for 15 minutes before immersing the wafer in microchem SU 8 developer right.

So, once we do that, we can obtain channels with 50 millimeter length 1 millimeter width chamber size of 2 millimeter chamber size is what. So, let us draw it once again. So, you understand in detail we have something like this ok. And here we have inlet, here we have outlet and here we can load spheroids ok.

So, T cells let us say T cells are flown from here and they are takeout from here, there is a peristatic pump. So, T cell goes inside and flows back here right is a circulation from here to here to peristatic pump. Now T cells will flow in this area like this and in this area like this alright guys it is very easy and then it will interact you see T cells can diffuse through this region; it can diffuse through this region if there are chemo attractants and after 24 hours of flow, we will measure CD 4 CD 8 ratio that is our idea.

So, for this kind of work, we have now fabricated a microfluidic chip right microfluidic chip.

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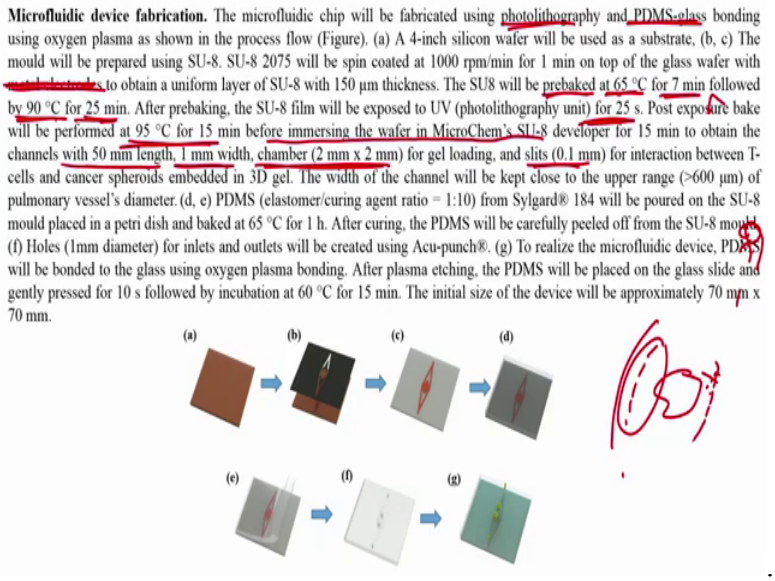

So, slits that we are talking about slits is this area right where the spheroids are there and the T cells will flow. These are the slits, these are slits right both side they are the slits. This slits will be 0.1 millimeter about 100 microns, for interaction between T cells and cancer spheroids in 3D cells.

The width of channel will be kept close to upper range, which is about 600 microns of the pulmonary vessel diameter right. So, the channel is about 600 micron, PDMS elastomer ratio is 1 is to 10 we know it after curing PDMS it will be peeled off to realize the device PDMS will be bonded to glass right and of course, before that we had to create the holes using Acu-punch right here to create holes using Acu-punch.

So, once the device is ready then what you will do? Once the device is ready we are to perform experiments like we are we were discussing.

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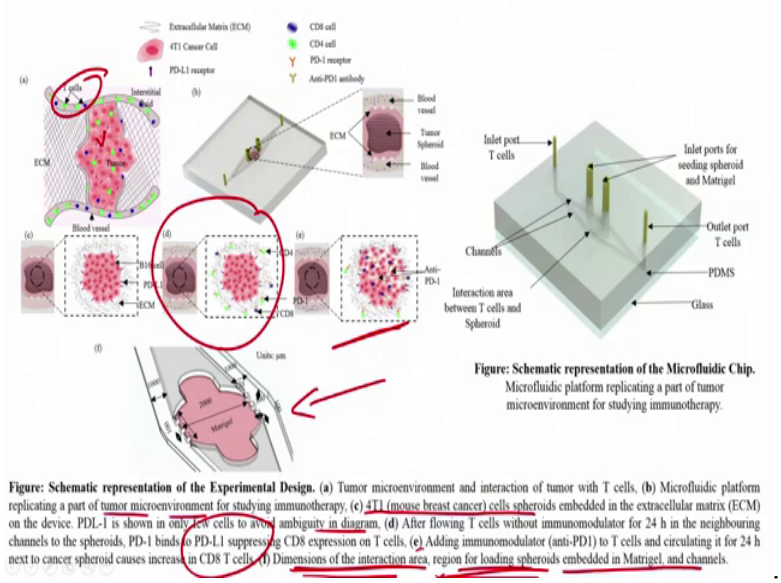

Figure: Schematic representation of the Experimental Design. (**a**) Tumor microenvironment and interaction of tumor with T cells, (**b**) Microfluidic platform replicating a part of tumor microenvironment for studying immunotherapy, (**c**) 4T1 (mouse breast cancer) cells spheroids embedded in the extracellular matrix (ECM) on the device. PDL-1 is shown in only few cells to avoid ambiguity in diagram, (**d**) After flowing T cells without immunomodulator for 24 h in the neighbouring channels to the spheroids, PD-1 binds to PD-L1 suppressing CD8 expression on T cells, (**e**) Adding immunomodulator (anti-PD1) to T cells and circulating it for 24 h next to cancer spheroid causes increase in CD8 T cells, (**f**) Dimensions of the interaction area, region for loading spheroids embedded in Matrigel, and channels.

So, you can see here in detail what we are doing? So, there is a tumor right there are T cells in the tumor right there are T cells on the in the vessels flowing next to the tumor, there is a extracellular matrix surrounding the tumor right similar situation we have to mimic on our microfluidic chip.

So, you can see here very clearly right this is the area center area, where we can load we can load spheroid these are the channels right through which the T cells will flow. So, now, what we are doing? We have to. So, this particular situation we are trying to mimic in this microfluidic platform and in the center of the channel, there is a matrigel with tumor spheroid which will work as this particular section. And the channels through which the blood will flow right here we are flowing T cells with media right.

So, we are trying to mimicking we make this particular blood vessel by creating the channels. So, this is again the same device, there is a glass the bottom there is a PDMS, is outlet port for T cells there are inlet port for sealing matrigel and spheroid, there is an inlet port for T cells channels are there interaction area between T cells and spheroid.

So, what does figure shows? The a shows tumor microenvironment interaction of tumor with T cells b cells microfluidic platform replicating a tumor micro environment c will

show 4 T1 mouse breast cancer cells spheroids embedded into the extracellular matrix PDL 1 is shown in only few cells to avoid ambiguity in the diagram, d will show after flowing T cells without immunomodulator here for 24 hours in neighboring channels to spheroids PD 1 binds to PDL 1 suppressing CD 8 expression.

While adding immunomodulator to T cells is shown in e representative diagram and it shows that adding immunomodulator to T cells circulating it for 24 hours. Next to cancer spheroids causes increase in CD 8 T cells right and finally, the f this figure shows the dimension of the interaction area, region for loading spheroids and embedded in matrigel and channels. So, this is what the diagram shows right.

Now, if we have this particular device with us, we making the in vivo situation on to in vitro platform what you will do? What you will do we will perform experiments we will perform experiments.

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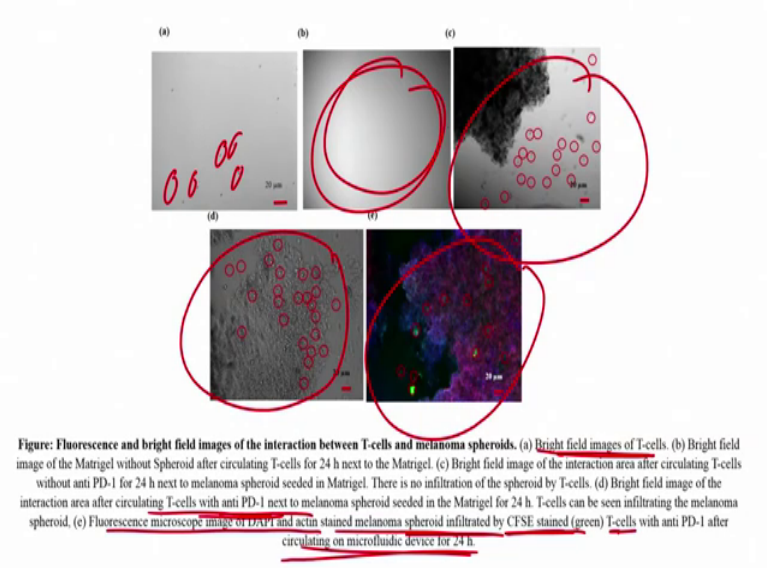

Figure: Fluorescence and bright field images of the interaction between T-cells and melanoma spheroids. (a) Bright field images of T-cells. (b) Bright field image of the Matrigel without Spheroid after circulating T-cells for 24 h next to the Matrigel. (c) Bright field image of the interaction area after circulating T-cells without anti PD-1 for 24 h next to melanoma spheroid seeded in Matrigel. There is no infiltration of the spheroid by T-cells. (d) Bright field image of the interaction area after circulating T-cells with anti PD-1 next to melanoma spheroid seeded in the Matrigel for 24 h. T-cells can be seen infiltrating the melanoma spheroid. (e) Fluorescence microscope image of DAPI and actin stained melanoma spheroid infiltrated by CFSE stained (green) T-cells with anti PD-1 after circulating on microfluidic device for 24 h.

So, you can see here the channel area a shows the bright field image of T cells, you can see few T cells here right b bright field image of matrigel this matrigel without any T cells, c shows bright field image of interaction area here after circulating t cells.

D shows bright field image here of interaction after T cells circulating T cells with anti PD 1 next to melanoma. So, what we see is, that the T cells enter the melanoma if you use anti PD 1 right. You block the immune checkpoint on T cells will cause T cells to go

nearby tumor and starts killing the tumor finally, what we see? E is fluorescence microscope image of dapi and actin stained melanoma spheroid infiltrated by CFSE stained which is T cells with anti PD 1 after circulating on the microfludic device right. You can see T cells here infiltrating tumor microenvironment and some of the live videos that you will be able to see on YouTube of T cells infiltrating tumor, you will be able to see T cell starts eating the cancer cells literally eating the cancer cells.

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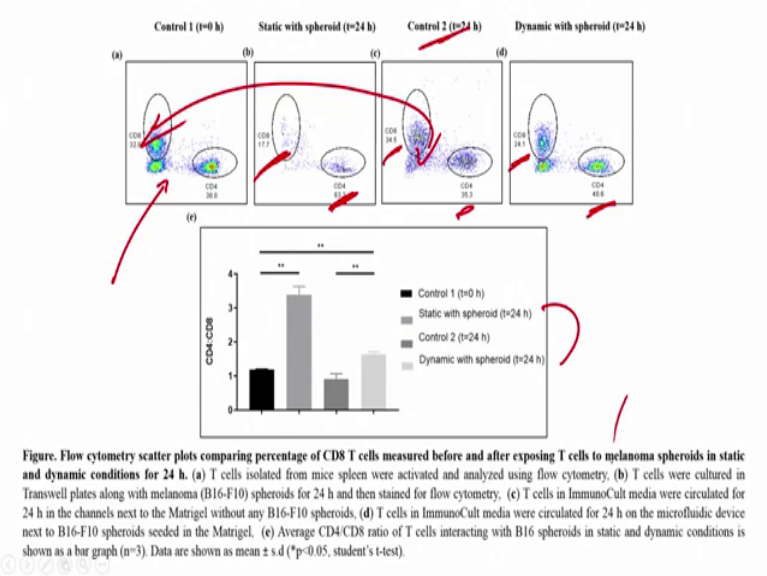

Figure. Flow cytometry scatter plots comparing percentage of CD8 T cells measured before and after exposing T cells to melanoma spheroids in static and dynamic conditions for 24 h. (**a**) T cells isolated from mice spleen were activated and analyzed using flow cytometry, (**b**) T cells were cultured in Transwell plates along with melanoma (B16-F10) spheroids for 24 h and then stained for flow cytometry. (**c**) T cells in ImmunoCult media were circulated for 24 h in the channels next to the Matrigel without any B16-F10 spheroids, (**d**) T cells in ImmunoCult media were circulated for 24 h on the microfluidic device next to B16-F10 spheroids seeded in the Matrigel. (**e**) Average CD4/CD8 ratio of T cells interacting with B16 spheroids in static and dynamic conditions is shown as a bar graph (n=3). Data are shown as mean ± s.d (*p<0.05, student's t-test).

So, if you see the results, we can clearly see that when we have controls that the T cells that is activated at when it is taken out from live to activated cells at t equals to 0, we have CD 8 to CD 4 concentration as 32 dot 38; when we have static with spheroid at t equals 24, the CD 8 concentration to CD 4 concentration is 17.7 to 63.3. When we have at 24 hours not doing anything we have 35.3 and 35.4 which is similar to this right not too much different. So, this control is and when we have dynamics with spheroid dynamics experiment, this CD 4 CD 8 concentration is different than CD 4 concentration you can clearly see here.

Thus we can see that compared to control either as 0 or at 24, the results for static and dynamics is to statistically significant, while if you just consider dynamics experiment with static right, then also you can clearly see that the results are statistically significant. That means, that the, we get different results from static platform compared to the results, that we get from a dynamic platform and that is why we should go for dynamic

platform for understanding the immunotherapy or evaluating the efficacy of immunotherapy, rather than using the static platform which is used right now right.

So, the these are all flow cytometry studies flow cytometry scatter plots comparison of percentage of CD 8 T cells with respect to measured before an and after exposing T cells to melanoma spheroids in static and dynamics condition for 24 hours. So, thus what we understand is that the results obtained from static experiments are different than the results obtained from dynamics experiments. I am not claiming that static is better than dynamic or we should go for dynamic, but now what we can say is that, if you get the cells from the patient, if you get the cells from the patient right then using the cells we can grow this kind of spheroid right. Using the cells we can grow a spheroid which will look like this.

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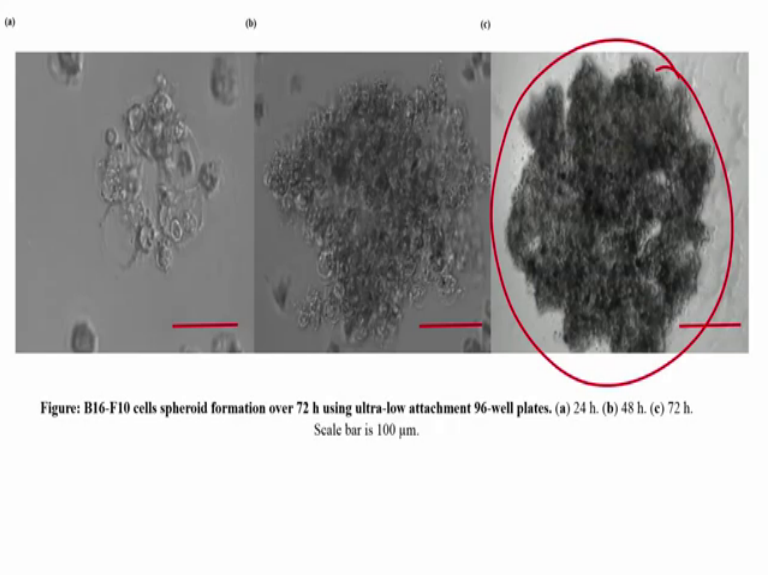

Figure: B16-F10 cells spheroid formation over 72 h using ultra-low attachment 96-well plates. (a) 24 h. (b) 48 h. (c) 72 h. Scale bar is 100 μm.

So, take the cancer cell from the patient grow spheroid in the laboratory. Now you can extract T cells from the blood of the patient right then take these T cells and this spheroid load into this device, load the spheroid here right and then take your T cells and take the patient T cells and extract and flow the T cells next to spheroid using peristatic pump and using the microfluidic chip right, then after 48 hours or 24 hours you can just of course, it depends on you have to optimize the time, just see CD 4 CD 8 concentration right. Then take the same T cells and treat the T cells with a particular drug, let us say anti PD

1; flow the T cells again next to the spheroids for 24 to 48 hours and against check the CD 4 CD 8 concentration.

Then if the drug is effective you will see the CD 4 CD 8 concentration very different when compared to when the drug is not effective for that particular patient. Thus we can use this device as a patient centric platform since we can try to evaluate the different available drugs for particular patient. And from the results that we obtain let us we should get some idea that which drug to give to a patient or from given number of drugs, a particular drug is more effective for a particular patient. Thus this device can act as a patient centric device after we get lot of results right.

So, what we learn what we learn in this modules? What we learn is, what we learn here is what is immunotherapy right how T cells looks like, how can T cells be isolated from these from this plane of the mice right.

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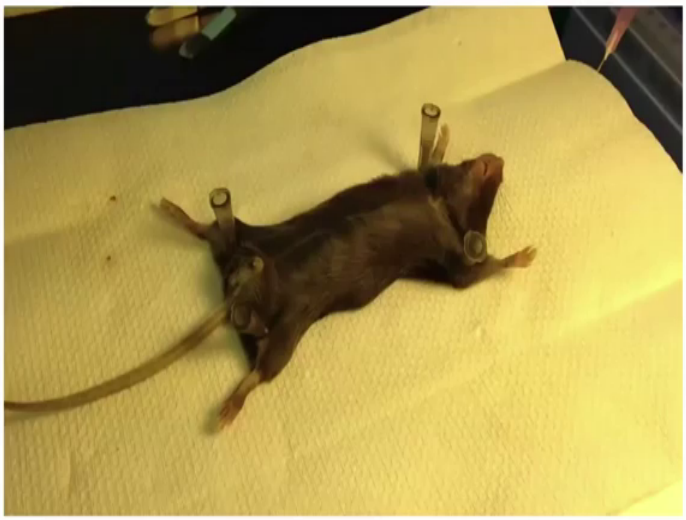

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This is how T cell looks like these are 20 x image so, that is why you can see it is bigger.

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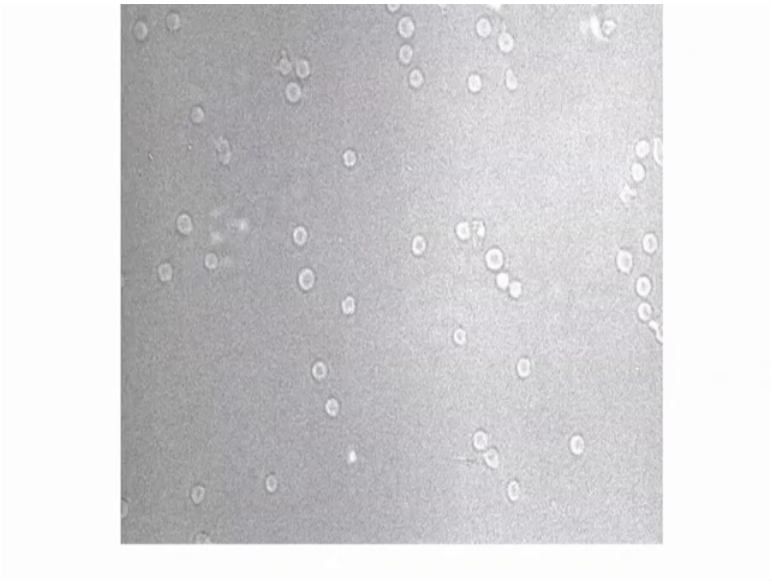

ah How we can create, how we can form spheroids and what is static way of doing the experiments right? How can we activate the T cells, then what are the key questions that 1 has to address when we are looking at immunotherapy.

Now, immunotherapy is extremely vast, we are just addressing a very small section of immunotherapy alright. What kind of microfluidic chip we can design, what kind of simulations we can do right we can do shear stress you can do velocity we have shown you the velocity plot here, I have shown you velocity plot in this particular module. How we can fabricate this device using 1 mask step single mask step how you can design the channel, what is what are the process flow for device fabrication right and then we have seen how the microfluidic chip can be utilized and for understanding the understanding the effect of anti PD 1 that is our immunomodulator right.

So, thus go through it its extremely interesting and burning topic in the area of research particularly for those who are interested in applying their engineering knowledge particularly micro engineering knowledge, micro technology or mems whatever you want to talk about it the photolithography that we have learned. Now you see we can utilize it here. Now when you have a mould and you keep on getting the design, it can be considered as a soft lithography.

Since you can create lot of channels by just pouring PDMS and curing pouring PDMS on the mould that is your SU 8 mould and curing the SU curing the PDMS and just striping it off. So, you get a channel in PDMS right. So, you can get lot of PDMS lot of channels in PDMS a lot of PDMS based microfluidic chips using a single mould, and that is why this can be consider as a soft lithography right we are not really performing photolithography mask and everything once we have the mould.

So, this is a beauty that we can create a lot of devices, when we go through this particular route right. I hope you understand the importance of immunotherapy I hope you understand how you can use microfluidic chip to understand or to evaluate the efficacy of given therapies particularly we are talking about immunotherapy drugs right; lot of things to learn.

So, I always believe that if our teacher gives you 10 percent, a student has to put 90 percent emphasis to learn more. And if you are stuck somewhere feel free to ask me I will respond to your question to the best of my understanding, and let us create something new let us create a novel technologies, that can be translated to clinics and hopefully we can save few patients life by understanding this particular technology, and implementing it and designing a new kind of devices.

So, I hope you will go through it once again not so, easy to understand, that is why I have tried my best to keep it as simple as possible right. However, if you are confused feel free to ask me through the beautiful NPTEL forum that you guys have and I will respond to your question.

Thank you, take care; I will see you in the next class bye.