Electronic Systems for Cancer Diagnosis
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Lecture – 49
Lab: Introduction to Clean Room and Cleanroom Equipments
PDMS Moulding procedure

Hello everyone. Welcome to the next module of Electronic Systems for Cancer Diagnosis. Today, we will study an important part of micro fabrication which is PDMS Moulding. Much before that we have seen how a desiccator works and how it can be used for a vacuum chamber. Desiccator can only be used as a vacuum chamber another important application is, it is used for degassing which is nothing but removal of air bubbles from a polymer which is used for moulding. Let us study more about it.

Today, let us see an important part of bio chip fabrication which is PDMS moulding. What is PDMS? PDMS stands for Poly Di Methyl Siloxan and how does it become important and why is it a most preferred choice in biological research and in micro fabrication process. Like I said PDMS stands for poly di methyl siloxan, it is a polymer which is widely used for fabrication, prototyping of microfluidic chip or where micro channels are formed in graved or moulded on the chip which could either be on the polymer and it could be on a polymer which is nothing but PDMS, but the other alternatives are it could be formed on a silicon wafer or it could be formed on a glass platform.

So, today we will see how PDMS can be used to form micro channels and how it can be moulded with you know on a master which is nothing but a silicon on a silicon master. So, the microfluidic channels which are patterned by itself forms an environment, a micro environment where the physical, chemical and the biological parameters can actually be controlled. So, how does it be important when you can control the parameters with in a chip, with micro channels in that? Yes, this is a major advantage, what the microfluidic channels or the microfluidic device facilitates. You can do the entire performance; you can mimic the environment like you want through the PDMS channels which are moulded. Let us see why and how this can be done.

So, PDMS is a polymer of carbon and silicon. For fabricating microfluidic devices PDMS is formed by mixing 10 is to 1 ratio of the polymer with a cross linking agent that is you mix the silicone polymer with the cross linking agent and there is a change in chemical properties which we would see how it would benefit us. Once the cross linking agent has been added to the PDMS, it becomes hydrophobic in nature.

So, when the elastomer becomes hydrophobic, water struggles to wet the PDMS surface and this becomes very important for our application. Another important part of fabricating the PDMS mould is oxygen plasma; more about oxygen plasma can we discuss later. But let me tell you in short, what is the purpose of oxygen plasma? Plasma by itself gives the meaning a plasma chamber, where oxygen is supplied in order to change the surface parameters of the device which is exposed to the plasma.

Let us see how this will be used to change the surface chemistry of our device and how plasma bonding happens. This is mainly used for bonding two surfaces. It could be PDMS to a glass surface, it could be PDMS to a PDMS surface or it could be PDMS to another say you know silicon coated surface. The device is exposed to plasma. It forms the surface chemistry changes because there is covalent bond which it facilities for forming a covalent bond between the 2 surfaces which are exposed and hence forming a tight seal when they are put together.

Remember another important point which has to be noted is once they are exposed and removed out of the plasma, the surface chemistry that is the surface changes to hydrophobic; the surface change is hydrophilic and it can be bonded within the 30 minutes after exposure and once they are removed out. Hence, immediately after removal they are supposed to be bonded and only then, it forms are tight seal and between the two surfaces which are to be bonded.

So, what makes the choice of PDMS important and so attractive for research? Let me tell you a few advantages of using the elastomer which is nothing but PDMS. From technology point of view, it facilitates to make biochip in just few hours without needing sophisticated equipments. This is a major advantage when we are following research. Another important thing is PDMS is biocompatible that is when we say PDMS is biocompatible, it is available at low cost, transparent and it can be moulded with you know resolution which is down to a few micro meter to nanometre and this facilitates

single cell, when you say cell the biological cell analysis. And another important advantage of using PDMS is the elastomer is also permeable enough to gas that is it allows gas supply for on chip cell culture.

This becomes very important when you are studying real time analysis, when you want to create a micro environment and an provide it would serve as an incubator where your cells could actually be cultured and study their reaction towards various drugs or it could be any kind of research. Now, let us see how we can fabricate PDMS mould on a silicon master.

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So now, we have Suman who will helps us understand how a PDMS mould can actually be prepared. As you can see there is silicone elastomer which is nothing but the PDMS like I explained the poly di methyl siloxan and the one beside it is the curing agent or also known as the cross linking agent. When both of these are combined in the ratio 10 is to 1, the properties of it or the mixture actually changes and they become hydrophobic in nature.

So, now let us make a PDMS mould using 25 ml of silicone elastomer and 1 percent that is 2.5 ml of the cross linking agent will be taken.

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Always ensure you make gloves and you are wearing a face mask because you do not want them to contaminate your hands and always prevent in healing these because the mixture could release harmful gases in the air which you should not inhale. Hence, ensure you wear a face mask and keep them away while you are doing the process.

So, once he measures 25 ml, the cross linking agent which is 2.5 ml will be added. Another alternative is when you do not have a container with which has scaling like this, you could even use a weighing machine where you can use a cup and then, weigh them as per grams. Say you are using 5 gram; say 10 gram of PDMS. Then, you could add 1 gram of elastomer and then, measure them in weight and then mix them thoroughly. Make sure, once you use them close them back and put them back in the same place. Once 25 ml of elastomer is taken; 2.5 ml of cross linking agent will be added to the PDMS.

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So, when we add this in 10 is to 1 ratio, we have the PDMS and the cross linking agent in one container and then, they do not mix thoroughly by itself. Externally we need to apply you know apply some force and then, mix them thoroughly. What happens when you add, when the ratio is not 10 is to 1. Why is the ratio has why should be specifically maintained at 10 is to 1 ratio of PDMS with the cross linking agent. When you add more of the cross linking agent, it is seen that the entire mixture, once it is braked and then used, it forms a brittle structure and then it is not flexible and easily breaks which is not what you want.

Now, that we have taken 25 ml of PDMS and 2.5 ml of elastomer, we should thoroughly mix it like you can see. Now, you can see the PDMS and elastomer mixture. Now, make sure you thoroughly mix the entire mixture; it takes a I mean the lot of effort because this is a thick viscous mixture. It does not you know form a uniform mixture easily.

Hence, it nearly takes for this amount of quantity, it nearly take 15 minutes to form a proper mix and as you can see there lot of air bubbles which are being formed as esters through the mixture. As you can see he starts the mixture thoroughly and why he does that there are lot of air bubbles which are generated. All of this is a process through the process you can see the lot of hair bubbles keep getting formed and you have to continue this process for this amount of quantity for nearly 10 to 15 minutes for it to form a clear liquid.

Once a mixture is added, now you can see we have almost mixed it for 15 minutes thoroughly and if you could show how the viscous liquid now looks like. Like you could see closely, there are lot of bubbles which are formed.

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Now after a thorough mixture for 15 minutes, you could see how the thick viscous fluid which was initially added is now a mixture perfect mixture both the elastomer and the cross linking agent have thoroughly been mixed and you can see almost a lot of bubbles air bubbles which are formed, which got formed while we do the mixing process and this went on for almost 15 minutes. A thorough mixture is necessary to get a proper uniform mould.

Now, that this is done we would take this. Remove how do we remove the bubbles? Each of this bubble have to be eliminated from this mixture because we are working always remember we are working with channels which are micro and nano metre in length. Hence, hence even a small bubble could interfere with your device functioning and all of this has to be eliminated. Now, how do we remove this? We use a desiccator to remove the air bubbles from the mixture.

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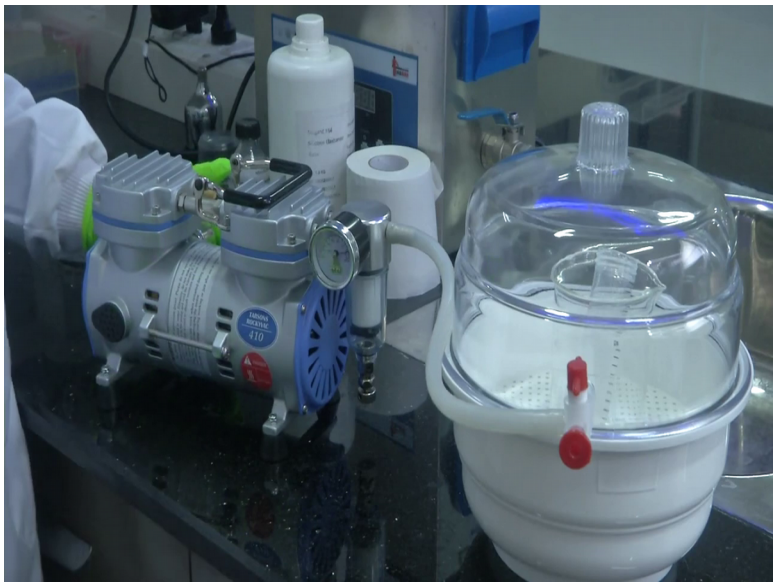

Now, let us take the mixture and put it for degassing in a desiccator. Now Suman takes a desiccator, places the mixture into the desiccator. Like I said even if you do not have tubing which has marking like this you can always use and throw container. Weigh them and put the mixture in the container and put it in the desiccator. Now, we placed them in the thoroughly mixed fluid inside the desiccator. Now that it sits properly. Let us close the lid and apply vacuum to the chamber. Again, we are doing this process to remove the air bubbles which are formed during the mixing process. Now that we put the mixture inside the desiccator, let us use the vacuum pump to create vacuum inside the desiccator.

Now, Suman would show you how the vacuum pump tubing would be connected to the desiccator. Make sure the fix is tight and it is leak proof. Once it is done let us turn on the vacuum pump; keep a note on the time, keep a note on time. Keep a note on time during the entire process. Now, approximately the degassing would take 5 to 10 minutes for removing the entire set of air bubbles. As you can see there is lot of vacuum which is being created and the pressure can be seen dial indicator like I had shown you in the previous modules.

So, the entire process of degassing would take around 10 to 15 minutes depending on the quantity, we have taken. If you can see there are lot of air bubbles which are getting formed on the top surface and there is clear liquid; the clear elastomer liquid remains at

the bottom and all of this air bubbles has time progress would be removed out of the chamber.

As you can see the air bubbles which are getting formed are slowly being degassed. Always ensure the container what you are using is clean and free from dust, if needed you could wash them thoroughly with water. You could even use acetone followed by IPA which is iso-profile alcohol and then, you do want any alcohol remains on the container which you would be using in future. Hence, you use nitrogen gas to remove any remains of the IPA. Each of this has to be done to ensure you are having a clean surface which would be used either to mix the mould or which could be used to store or make the PDMS devices. So, the entire process to cause almost 20 minutes for complete degassing and if you could see the mixture is now more clear and then, all the air bubbles have been degassed.

Now it is safe to remove from the desiccator. So, let us carefully release the vacuum that has been formed. So, this slowly open the valve and degas it. So, once the micro pump as been switched off; turn on the valve and then, slowly degas it. Now, let us closely see what happens? Yes, as you can see once the degassing is being done, you could if you can hear the noise, all of that is the air which is going inside. Make sure, you do not pull out the valve very quickly because the air which flows in can create a negative pressure and the fluid container which has been kept inside my tumble which is which is not what we want.

Hence, slowly degas overtime and once you hear no more noise that is no more air, the entire process is complete and safely open the lid. So, here we have a clear mixture of PDMS and the elastomer the curing agent and the PDMS have been thoroughly mixed. So, what do we do? Once the mixture has been completely mixed and degassed, we put this mixture; as you can see the clear mixture in Suman’s hand. Now, let us pour it into the silicon master what we already had.

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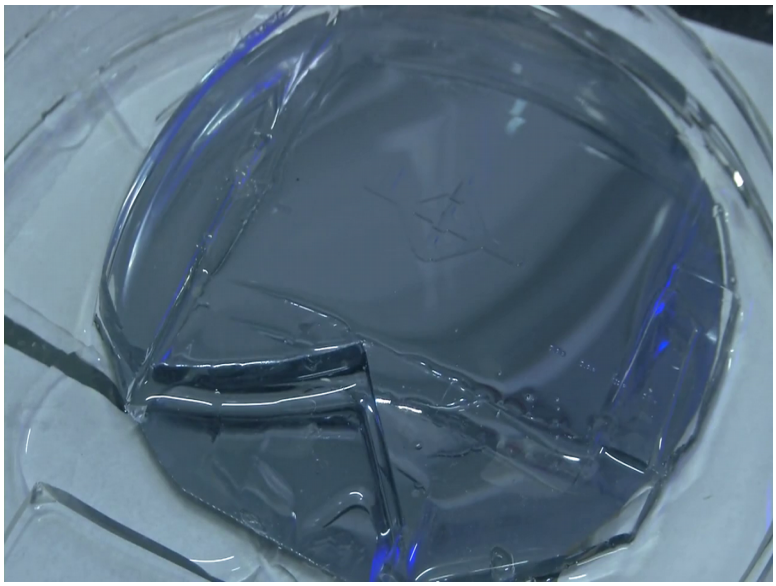

So, an experimental already been conducted and just to educate you guys how the mixture how PDMS moulding is done. Let us reuse this. As you can see the one in his hand is a silicon master mould with fine channels which has been engraved using lithography. So now, he pours in the clear PDMS mixture on to the mould. Now the clear mixture can be poured on to the silicon mould what we already have. So, make sure when you pour you are very close to the master mould because when you maintain a lot of this is easy when you are pouring it from a height, there is lot of distance between the liquid and a silicon wafer which is at the bottom. This interface when it is long could actually create bubbles. So, you do not want to invite more air bubbles into the PDMS mixture which has been created.

Now, that will safely put the entire mixture into the mould. Make sure there are no air bubbles which are formed. In case there are bubbles formed on the surface you can still remove them using a needle. As you can see, there are a few bubbles which are formed on the surface. Each of these can be removed using a micro needle. You could either bubble them out; blow them out or you could push them aside that a device apart from where the device is present.

As you can see how Suman is actually pushing away the bubbles which are just above the surface of the device because that part is what becomes important to us. Now, that we removed all the bubbles. This goes straight into an oven which is kept at 70 degree

Celsius and then, you have to heat it for almost 2 hours. Now, that it is clear without any air bubbles. Now, let us see how Suman uses the oven, puts the entire day into the oven which is set at 70 degree celsius.

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Now, let us use the oven which is set at 70 degree Celsius. Suman if you could please turn on the oven? As you can see, he gently places the tray into the oven.

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So, we need to bake it at 70 degree Celsius for 2 hours time and temperature accuracy is very much vital while you bake the PDMS mixture that is because when you over heated

or when the temperature goes beyond the limit, I mean it is seen that the mould is becomes more brittle and while you perform experience or even while you cut them off, it is seem that they easily trend to break through. This is not what you want you want a perfect elastomer highly elastic in nature and that is obtained when you use the optimal temperature and for accurate time which is 2 hours.

So, now that we have seen how the PDMS and the cross linking agent has been mixed thoroughly in 10 is to 1 ratio degas and then, put in the oven for 70 degree Celsius for baking. Now once that bakes for after 2 hours, you take them all safely and then you use a surgical knife to cut through and your device and you could see the channels which was there on the silicon master actually gets engraved on to your PDMS mould and then, you have the device which is ready can be bonded to any other surface and perform test.

Now, that we have to wait for another two hours for it to bake. Now, I will show you few other devices which we have fabricated before which can be used you can follow the similar procedures apart from the plasma bonding and then do the test.

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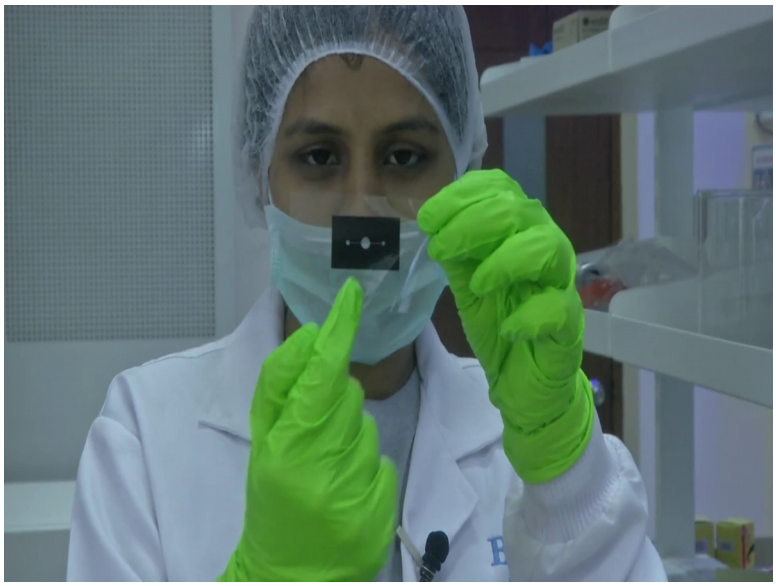

So, I clearly explain how this device has been fabricated. Initially this, what you see my hand is the mask. So, what we have here is a 5 mm dia inside channel and the inlet which is here and this is the inlet and this is the outlet port. Each of this is 2 mm in dia and this is a membrane a sync like structure which is 5 mm and this is the mask which can be

used for photolithography. This is the mass which can be useful in lithography in order to have this being developed on your PDMS you know the fluid.

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This is one such device which is in my hand. So, what are these? These are the inlet and the outlet ports. This is a tubing which is approximately 1 mm dia. This tubing goes into this PDMS mould which you can see and there is 100 micron channel which is actually difficult to you know see through naked eyes.

But there is a hundred micron channel which goes through the inlet and the outlet port and this PDMS channel has been bonded to this glass wafer what you can see here. This is a cover glass and this PDMS has been bonded to the cover glass and in between the 2 is the channels which have been engraved on to the PDMS surfaces which is facing towards the cover glass.

So, ensure even before you bond the two surfaces, both the surface once this device has been fabricated, you cut through the device using like the one which we put in the oven. You use a surgical blade to cut through your device and ones that is in your hand, you make sure you clean it thoroughly and how do you clean them? You watch them with acetone followed by isopropyl alcohol and ones acetone and IPA has been done, immediately use nitrogen gas, blow nitrogen air on to the surface. So, that you do not want the alcohol stains on your device.

So, once both in my case I am bonding this device to the cover glass. So, both of this will be cleaned for with the procedure what I have shown. So, once both these devices are cleaned. So, you have this plasma bonding which you use to bond to form a covalent bond between this and this. So, we use oxygen plasma chamber that both of these devices have been exposed to the oxygen plasma for around 30 you know for around 5 minutes and once that is done you pick them up and then even before few seconds after removal, make sure you born then thoroughly like I said mentioned before the surface properties changes and it retains only for a very short time once they are removed out of the plasma chamber.

Once the bonding is done thoroughly and then you have these tubing which are inserted and then you flow fluid through these channels. Like I said this is just 100 micron channel and then, you could imagine the amount of fluid which can be flown into this and this can be replicated to the biological cell because even our cells are micrometre in scale and you could use this to form a replica to mimic a biological environment.

So, in this case if I do not want to you know flow a liquid in a continuous fashion, I can as well just use a surgical just use this syringe which is in my hand and then, pump di water into this just to test your device you can flow it through one channel and then see the continuity of the channel and then you see liquid coming out of the outlet channel.

In case there are leaks, then you would see water flowing out of these. If the bonding is not proper between your PDMS and the cover glass, then you will see water coming the di water would leak through between the two surfaces. And hence, you could again remove them, again expose them to oxygen plasma and then bond them and then, do the same procedure to check for leak proof leak proof bonding between the two. So, this was one such device.

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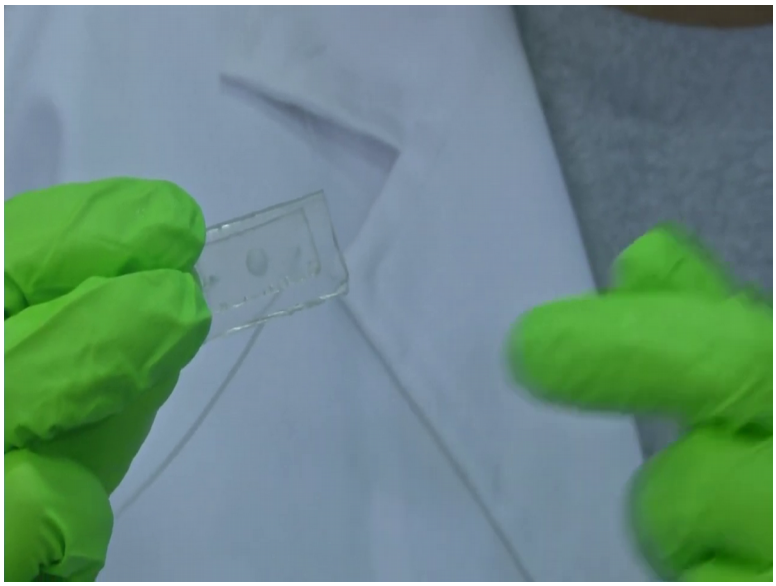

And this was the procedure. So, this is for a later use. So, I have made the PDMS. Once a PDMS device has been moulded, this is the step one. Once I take it out from the oven, I cut them to the surgical blade and immediately clean it with the procedure what I mentioned below and then what if I have to use it for a later purpose.

So, I after cleaning has been done, what I have done is I have used a scotch tape and closed and covered it thoroughly. This is the scotch tape let me remove it. So, this is the scotch tape and this is the device. Just you ensure you do not want tiny particles or contamination on your device and you can use it for later use. Cover it with the scotch tape, when you want to use them in future and then like I showed you I had an inlet outlet channel. Like I showed you there is the mask which I had an inlet, outlet membrane and then there is a central channel. This is actually a central dome which has been done and this is a PDMS to PDMS bonding.

Earlier, I should you how PDMS can be bonded to a cover glass. So, in this case this is PDMS which is again bonded to another PDMS layer and these are the tubings. If you observe closely, this was one of the trial procedures while fabricating the device. They you could see these bubbles which are formed at the bottom of this device. Like I mentioned what could be a drawback if you do not degas them properly, the process has to be done you know carefully considering it takes a lot of time. Make sure you degas

and there are no bubbles in your in your PDMS mixture, what has been in the PDMS mixture.

So, in order to avoid these bubbles, make sure you thoroughly mix and degas ensure there is no air bubble before you put it into the oven. What happens if these bubbles could actually come here interfere with the device channel? As you can see even viewing the micro channel is difficult to the naked eyes and in case there is air bubble which could abstract your channel, then there is no point in having a device with you know these micron channels where you cannot actually test. It would obstruct the flow of the fluid what you would be flowing.

Hence, taking all these examples; hence, considering all these examples like I showed you, we have seen how PDMS moulding can be done. Let us ensure we use a right temperatures as mentioned, use the correct time, make sure there are no air bubbles while you pour in and then take precautions even while you do the entire process. Make sure you do not do the mixing very close to your face or mouth because inhaling is harmful. So, let us consider all the points which have been taught through this module. Let us learn more in our next module.

Thank you.