Fabrication Techniques for Mems-based Sensors: Clinical Perspective

Prof. Hardik J Pandya
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore

Module – 08
Lecture – 29
A Microfluidic Chip for Rapid Bacterial Antibiotic Susceptibility Testing

Hi! Welcome to this particular module, and here, we will be discussing about what do you mean by bacterial antibiotic resistance. Now, this is extremely hot area of research since, the effectiveness of the antibiotics on killing the bacteria is getting lower and lower day by day. The reason being that we take lot of antibiotics and the bacteria starts resisting to the antibiotic. It cannot be killed by a particular antibiotic. So, why need to design a engineering platform for understanding whether the bacteria is resistant to antibiotic or not? There are 2 cases, I will discuss. First case is urinary tract infection, and then we will take out of 2 case, 1 case we will take and we will see how we can use our engineering approach to solve this particular problem.

So, first is urinary tract infection. Urinary tract infection occurs because of the bacteria called E. coli, and the concentration of the bacteria E. coli is about 10 is to 5 colony forming units per ml. Now, this causes the infection and generally, when a person suffers from a infection, a bacterial infection he or she is prescribed tropical antibiotics. So, what do you do, we understand by tropical antibiotics? Topical antibiotics are 5 different antibiotics that is given, and the samples are taken. Well, this is a urine sample, and sends it to a path lab. In the path lab they grow the bacteria on the, using the growth media on the desk, and then, they use the antibiotic capsules or discs on this particular plate, and if the antibiotic is effective, the bacteria surrounding the antibiotic will die that is what we see. If the antibiotic is effective, then the clear area would be bigger or the radius would be larger right. I will show it to you what I mean and the whole process of growing the bacteria. That is, first capturing the bacteria and then to test several antibiotics, 4, 5 antibiotics takes about 48 hours. That varies on the type of bacteria you are testing.

So, once the report comes at out of 5 a particular antibiotic would be more effective for a particular bacteria that is, E. coli. Then after 2 days or 3 days, a patient is called back and out of 5 antibiotics, only 1 antibiotic he or she is asked to continue right. So, where is a problem? The problem is that, we do not have any engineering device that can rapidly capture and

understand whether the captured bacteria are resistant to antibiotics or not rapidly. Now, why to worry? Let us say, if I have a bacterial infection; if I go to a doctor, I can stay for 2 days with a 5 antibiotics. Third day I can get one and I can wait for two days. But, when we talk about neonates, neonates are 1 year, 2-year old babies. Their immune system is not as strong as ours, and waiting for about 2 days or 24 to 48 hours or more is not ok; because they die, and this bacterial infection that occurs in babies is also called sepsis s e p s i s; sepsis.

So, in the blood other than E. coli in there is an urine in blood. There can be other bacteria's such as Staph, Staphylococcus. There can be MRSA. So the point is, can we develop a technology that can on-chip capture the bacteria and understand the antibiotic resistance using electrical sensing rapidly right; that is our given problem. So, one way of doing it is a static way and second way of doing it is using micro fluidic. Here it is not about flowing the, a sample like all our other topics like immunotherapy or chemotherapy or a drug screening device right. We will talk about flowing the sample in the micro fluidic device. Here, we are not worried about flowing the samples. Here, we want to test the samples quickly. So, how it will work? Let us see in today's lecture.

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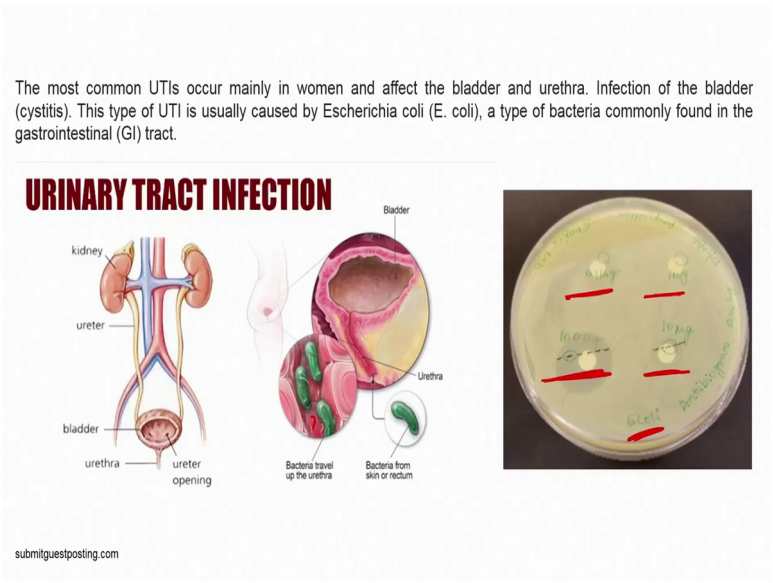
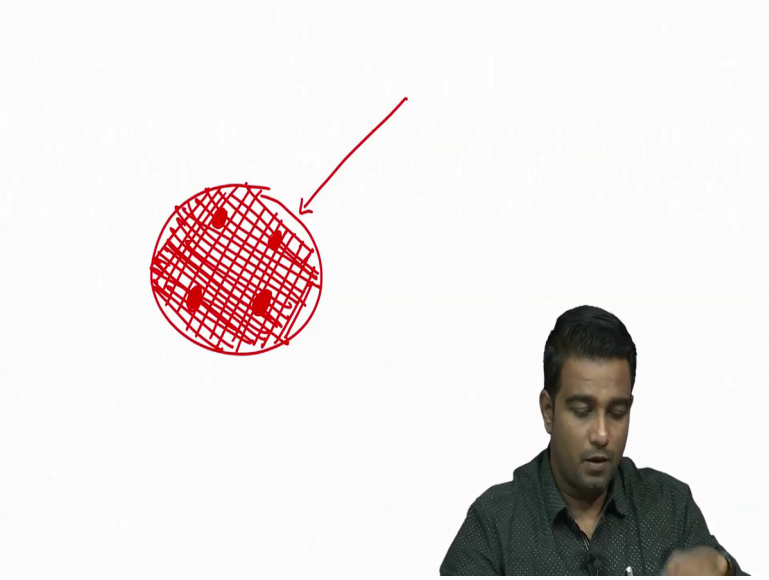


So, when you if you see the slide, what you see? The most common UTI's, a urinary tract infections occur mainly in women or women and affect the bladder and urethra. Infection of the bladder that is cystitis, this type of UTI is usually caused by E. coli. A type of bacteria commonly found in the gastro intestinal or GI tract. So, you can see bacteria travel up the

urethra, you can see here. The green color one. This is bacteria from skin or rectum from this area it can travel. You can see, right, and it travels all the way to the bladder. So, how to know whether a women or a woman is suffering from UTI or not? That is the problem. Here, what you can see is how the current gold standard techniques are used. There is a disk diffusion technique. It is also called antibiogram yes say.

And here we have, what you can see here is ampicillin which is one of the antibiotic for E. coli. We have used different concentration 0.1gram, 1 gram, 10 microgram and 100 microgram. Again 0.1 microgram, 1 microgram, 10 microgram and 100 microgram and you clearly see that the bacteria here has died right Means 10 microgram is more effective compared to, 100 microgram is more effective compared to 10 microgram, compared to point microgram and compared to 0.1 microgram right. But, there is something called minimally, minimum inhibitory concentration MIC that one has to take care when subscribing the antibiotic alright.

So, point is this is how it is done like, you can see here very clearly right. The effect of the disk diffusion technique and everywhere there is a bacteria which is your E. coli alright. (Refer Slide Time: 10:20).



So, for this, what we can do what we can do? What I was talking about is. Say when the sample is collected, it is first grown. It is the sample. The bacteria from the sample is taken, is extracted right or is captured and is grown on the, using the growth media right Then on this

sample once the bacteria is grown, we load 4 different antibiotics. If there are 5, there can be 5 antibiotics right

Now, if the antibiotic is effective then, what will happen? Then, the diameter will increase. So, that is what we have seen here. You can see this particular diagram. What do you see? The diameter has been increased right.

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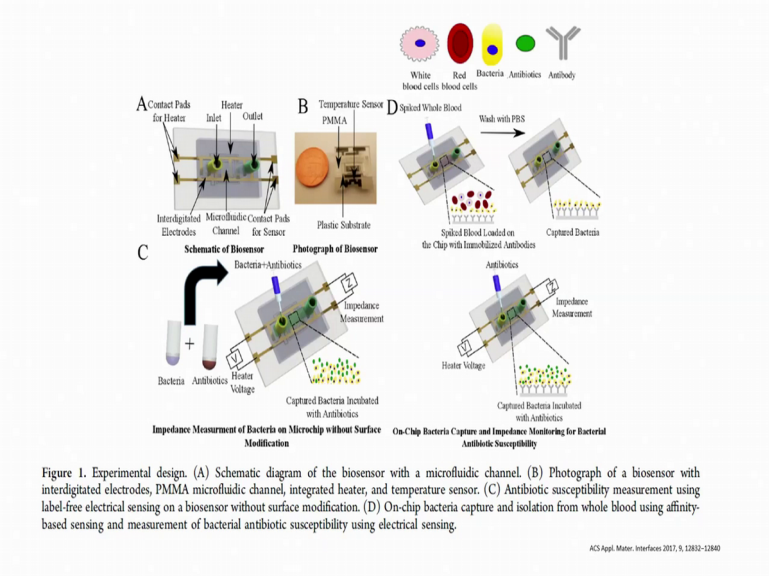

Figure 1. Experimental design. (A) Schematic diagram of the biosensor with a microfluidic channel. (B) Photograph of a biosensor with interdigitated electrodes, PMMA microfluidic channel, integrated heater, and temperature sensor. (C) Antibiotic susceptibility measurement using label-free electrical sensing on a biosensor without surface modification. (D) On-chip bacteria capture and isolation from whole blood using affinity-based sensing and measurement of bacterial antibiotic susceptibility using electrical sensing.

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Now, how we can perform the experiment? How we can come up with the technology that can be faster? That is rapid, and it should not be costly as well alright. So, if you see this particular device and then, you will see how we can fabricate this device; this device consists of a plastic substrate which is the substrate on which the device is fabricated. It has an indicated heater on which there is an insulator, on which there are interdigitated electrodes. Interdigitated electrodes you guys know right. What is interdigitated electrodes? Electrodes like this, correct? Interdigitated electrodes; they are all attached, which are not connected.

So, what is it having? It is having a heater right It is having a heater, then on the heater there is an insulator. On insulator there are interdigitated electrodes and there is a contact pad here. This is what you can see here. There is a contact pad for sensor. Sensor is nothing but, interdigitated electrode. Contact pad for heaters and then on that you have the channel. So, you have a PMMA sheet. PMMA which is cut and you create a channel, and you stick the channel on this particular chip so that, there can be a inlet and there can be a outlet.

So, what you do with that right. First is you need to create the micro heater. Now, in this technique since it is a plastic substrate, plastic substrate a screen-printing technique is used. You see until now, what we have learned? We have learned everything can be done using clean room, a macro fabrication can be done when you have a thermal evaporator or (Refer Time: 13:53) evaporator or a high end technology equipment like photolithography or you can talk about RIE, right or you can talk about (Refer Time: 14:01). But, what if your laboratory, your place does not have these high-end technologies? Can you still do research right without this technologies?

So, how can we how can we design the sensor in which even we are not surrounded by, where we do not have the access to set of technologies, can we come up with our novel solution. So, in this case what I am showing it you right now is a device, that is made using a technique called screen printing, screen printing. So, we can screen print a heater right, everybody knows screen printing. On that, so can you have a insulator on the heater, because you do not have technique for depositing insulating material like PECVD or like sputtering, you do not have any technique to deposit insulating layer on the heater.

So, what you can do? If you do not have this kind of technique what you can do right. So, I will show it to you. Now, let us consider this is a plastic substrate OHP sheet ok. On one side I have a heater, I do not have a technique that I can grow insulator on the top of the heater. What I will do? On the bottom side I will use the electrodes. So now my this hand which is my left hand right is the electrode on the substrate and my right hand is my heater ok. So, you do not have to worry about the insulating layer, because, the substrate itself is a insulator. It is a plastic sheet, and then, on interdigitated electrodes which is my left hand you have the channel. So, you can pass a liquid through the channel this channel is made up out of made up made out of PMMA alright, acrylic sheet PMMA and how can you cut the channel? You can cut the channel with the help of laser cutting.

So, these things you can have a sensor, you can easily have a sensor. Screen printing on one side of the substrate heater, screen printing on another side of the substrate, interdigitated electrodes and then on interdigitated electrodes you have the channels formed out of PMMA. Now, what you will do? So, if you see here on the slide we talked about this right, a chip with one side there is of OHP sheet, there is a heater. On another side there is a sensor or interdigitated electrodes you can see here right, and these are nothing but when you say contact pads for sensor, is nothing but contact pad for interdigitated electrodes and you have

your microfluidic channel. This is actual photograph, this is the actual photograph of the device and how the experiment is done right, how the experiment is done.

You see here white blood cells, RBCs, bacteria, antibiotics and antibody. You should remember this ok, this design is white blood cells which is a gear like design and it is not actual representation how the white blood cell looks like, it is just a schematic representation to distinguish between the RBCs, WBCs, bacteria, antibiotics or antibody ok. So, now, what we will do is we will spike the whole blood. So we will take a blood and we will spike the blood with a known concentration of bacteria, let us say 10 is to 5 CFU per ml. I know what is the concentration of bacteria in my sample. So, that is called spiked right, known concentration adding in the blood is a (Refer Time: 18:25).

So, what would be where we will do? We want to we want to, first on a interdigitated electrodes you can see here right, there are antibodies right, antibodies which are immobilized antibodies, they cannot move immobilized antibody alright, so antibodies are like this of course like this actually, right. Immobilized antibody, and once we have immobilized antibody we will load spike whole blood onto the channel. So, it will look like the one in this particular figure, you got it. First step is to local to immobilize your antibody, Means you have to load the antibodies in the channel and incubate it for half an hour so that, it will be immobilized and here we are not performing any particular surface chemistry or surface modification. So our sensing would be the antibodies will be stick to the substrate using affinity. So, this is called affinity- based sensing ok, affinity-based sensing.

So, now you have the antibodies loaded on the chip what you will do? You load the whole blood; you load the whole blood onto the channel like you can see here in the schematic. After that, you have to incubate for 30 minutes and then wash with PBS. When you wash with PBS, what will happen? The bacteria in the blood will get captured on the antibodies. The bacteria that are present in the blood will get captured on the antibodies. Next step; what is the next step? Next step is, once the bacteria are captured you load antibiotics which is here right you load antibiotics with growth media, growth media plus antibiotics. When you do that what will happen? There is a growth media and antibiotics, what is the role of growth media? Growth media will increase the bacteria. It will help the bacteria to grow, growth media right.

Role of antibiotics, antibiotics is supposed to kill the bacteria right. Antibiotics are supposed to kill the bacteria, right. So, if the antibiotic is not effective either the bacteria will start growing or it will be stagnant, it will not die it will not grow, this is the case when antibiotic is not effective, you got it? If the antibiotic is effective the bacteria will start dying, in the presence of growth media if the antibiotic is effective then, bacteria would start dying. Now, whether bacteria will grow, or it will remain as it is, or it will die, this we can measure with the help of interdigitated electrodes, and how, by using the impedance analyzer. So, we can measure impedance, like you can see here right, of the interdigitated electrodes, when the bacteria are incubated with antibiotics in presence of growth media, right.

So, what you see here? There is a heater voltage and then there is a impedance measurement. Now, why there is a heater? There is a heater because, we need to keep the bacteria at 37 degree centigrade, we have to culture the bacteria at 37 degree centigrade. So, if you see figure 1, if you see this figure it is a experimental design, schematic A is a schematic right., diagram of biosensor with micro fluidic channel. B is photograph of a biosensor with interdigitated electrodes, PMMA microfluidic channel, integrated heater and temperature sensor. C will be antibiotics susceptibility measurement using label-free electrical sensing on a biosensor without surface modification. See label-free, it is very important right, we are not using any labeling techniques to or we are not staining the cells. So, It is a label free technique. D would be, D is this one right, on-chip bacteria capture and isolation from whole blood using affinity-based sensing and measurement of bacteria antibiotic susceptibility using electrical sensing correct. So, this is one way of testing the, first is capturing the bacteria and then testing the bacterial antibiotic susceptibility.

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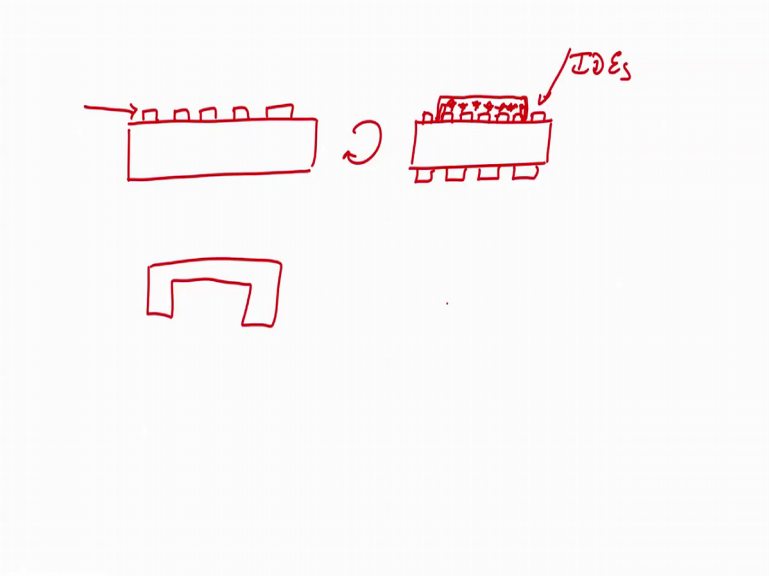

So, how to create the, this kind of chip? How to create this kind of chip? Like I said, if you have a substrate which is plastic substrate, you screen print you screen print heater on one side of substrate and then you reverse the substrate right. If you reverse the substrate, substrate will look like correct. Now, on the top you screen print interdigitated electrodes correct. What you have done. First step is you screen print heater on the plastic substrate, then you rotate the substrate, turn substrate to 180 degree and you screen print interdigitated electrodes on that, you create a channel, the cross-sectional view right.

So, how you can create channel? You create a channel in PMMA sheet like this right, so this is a channel that is created and then you load the, you first load the antibodies, then you load the blood, then you capture the bacteria. Way after you load the blood you have to incubate it, wash it with PBS, capture the bacteria and then add antibiotic with the growth media and look at the look at the change in the electrical property, that is the impedance.

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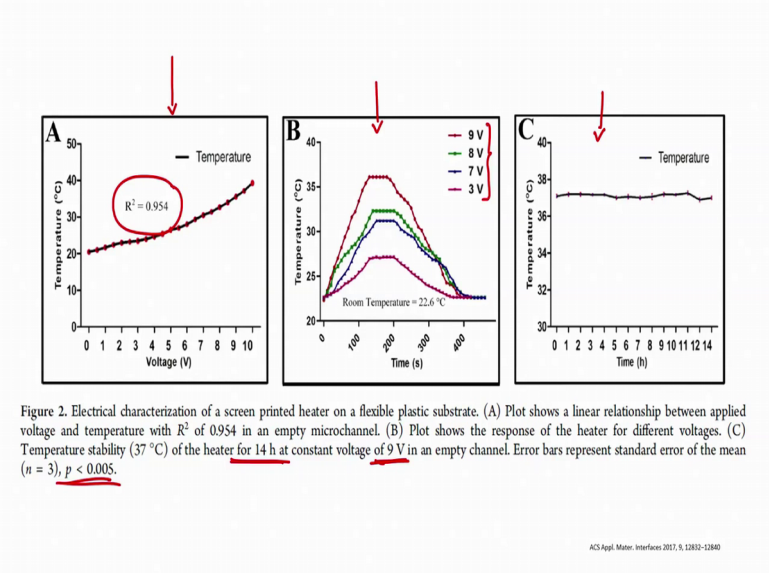

Figure 2. Electrical characterization of a screen printed heater on a flexible plastic substrate. (A) Plot shows a linear relationship between applied voltage and temperature with R^2 of 0.954 in an empty microchannel. (B) Plot shows the response of the heater for different voltages. (C) Temperature stability (37 °C) of the heater for 14 h at constant voltage of 9 V in an empty channel. Error bars represent standard error of the mean (n = 3), p < 0.005.

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So, the electrical characterization of a screen-printed heater or a flexible plastic substrate is shown here you can see here, as you increase the voltage the temperature of the heater also increases, and the R square value is close to 0.94 right.

So, this plot shows the A shows a linear relationship between applied voltage and temperature with R square of 0.954 in an empty microchannel. What does B shows? B shows a plot which shows the response of the heater for different voltages and you can see the response of the heater for different voltages here. Now, whether your heater is stable with respect to time for a particular temperature so that is what your C plot shows, that the temperature stability of the heater for 14 hours at, 14 hours at a constant voltage of 9 volts in an empty channel error bars. These are error bars here you can see in the red color right, represent standard error of mean for n equals to 3, we have performed 3-time experiment and p value is less than 0.005. So, we have performed the characterization of the screen-printed heater.

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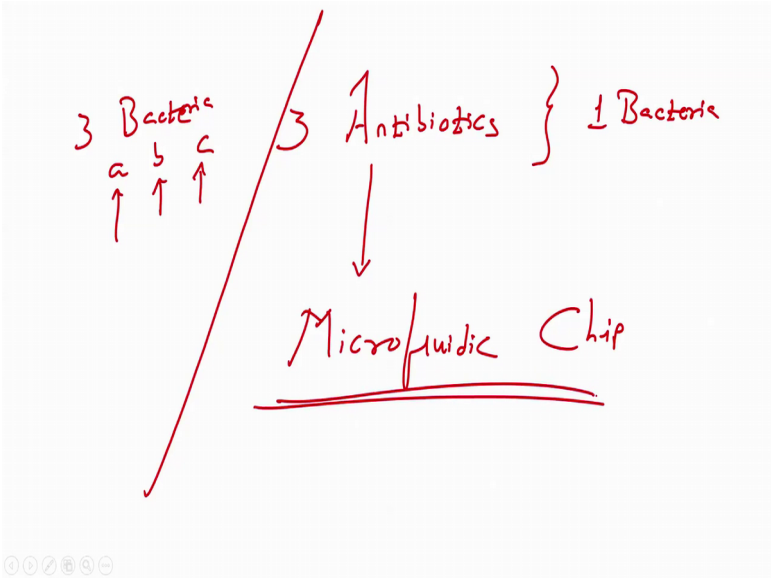

But like I said, if you want to test multiple antibiotics you need to have a microfluidic technology for testing 3 different antibiotics let us say, 3 different antibiotics at a time, you require micro fluidics or microfluidic chip. 3 antibiotics at a same time for 1 bacteria, 1 particular bacteria or 3 bacteria you capture, 3 different bacteria you can capture and test 3 different antibiotics and test antibiotics for individual bacteria right.

Let us say there is a bacteria a, b and c that you capture you can test antibiotic for a, Antibiotic for b, antibiotic for c at a same time using microfluidic chip. So, what I what do I mean by that, let us see.

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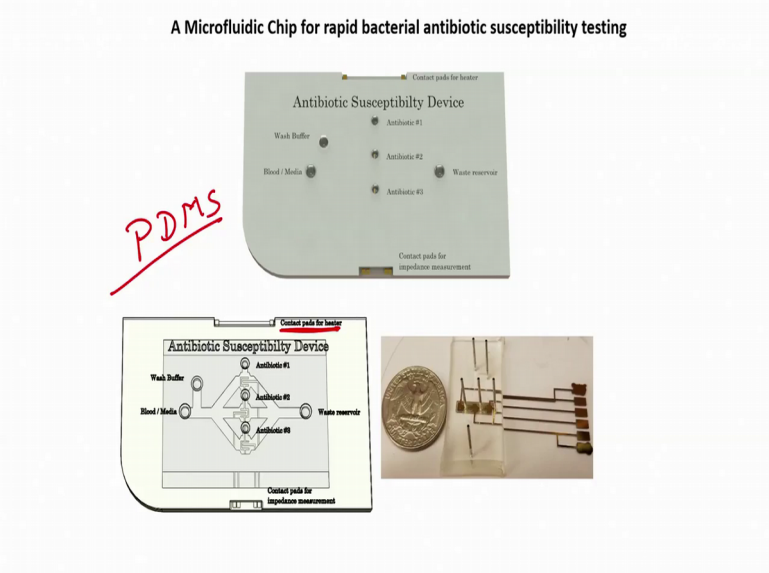

So, a microfluidic chip for rapid bacterial antibiotic susceptibility testing you can see here this is a channel right, this is a channel this one straight and one goes like this, correct, channels are there. Now, what do you see here? There is a loading chamber right and this is the chamber here, here and here and below that, there is a micro heater, at the bottom there is a micro heater hm. You cannot see in this one but, there is a micro heater. You can see here contact pads for heater so, and on the top we have designed the microchannels with the help of PDMS. So, we have performed soft lithography, we have performed a soft lithography.

So, you can see the device here and this device this is the outlet, this is the inlet, these are this is for the or you can say this is the inlet let us say, because we have the design in this particular format. This is the outlet, and we have here wash buffer right. This 3 things, 1,2 and 3, are for loading antibodies and antibiotics with growth media alright. These 3 points or this 3 in inlets are for either antibiotics, antibodies or antibiotics plus growth media, first you have to load antibodies and then you have to go for antibiotics plus growth media. So, how does it work, how does it work? First, let us see how does it look-like. I have the chip in my hand, so if you can just look at my hand and you can see the device, I will show you the device, if you can see the device in my hand, right. This is the device right now and what you can see here, let me just put it here yeah this is better, ok.

So, what you can see here is the chip that I have right and I can just see you show you in the this particular format, because I want to show you the PDMS layer right. This PDMS and

then you can see a channel right, you can see a channel, you can see interdigitated electrodes and there is a heater there is a heater here, it is a heater and this everything is on the front side everything is on the front side the reason is that because, we need to have, we can have an insulating material on the heater right, for the device that I am holding there is a heater on which there is an insulator, on which there are interdigitated electrodes and then we have the channels form in PDMS and we have attached this PDMS onto this glass substrate and what are the channels in PDMS, what is the design, we have just seen in the slide right.

So, this is the device. Now, let us see how we can fabricate this device and then we will see how we can test this device. So, let us see how we can fabricate I will show it to you here.

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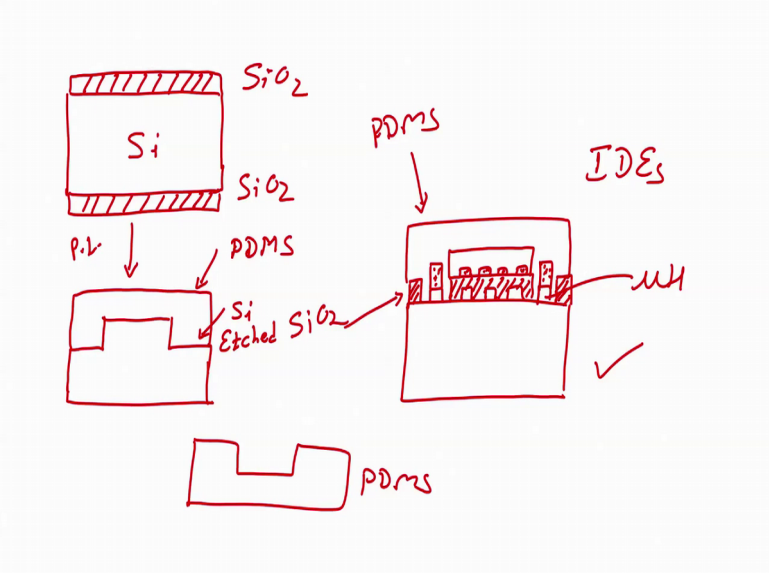

The fabrication first thing we have to do is to design and fabricate a heater right, so there is a glass substrate so we start with a glass substrate on that glass substrate we will deposit, we will deposit a metal for micro heater right, metal for micro heater. On this, we will perform photolithography. Now, I am not going into depth of photolithography, you guys know how to do perform photo lithography right, so when you perform photolithography you will have you will have heater correct, you can have a micro heater.

Next step would be, next step would be to deposit silicon dioxide on the micro heater, on the micro heater. After we deposit silicon dioxide what we will do? We will remove silicon dioxide from the windows for the contact and the remaining area would have silicon dioxide, remaining area would have silicon dioxide. So, what you can see? That there is a micro heater

and there is a silicon dioxide and this is also silicon dioxide you can say it is a glass, right. Now, on this what we will have? If you just see, let us see the screen please. So, after this what we will have? We will deposit we will deposit a metal, on this will deposit a metal as I have drawn here and then we will perform again photolithography. So, when we perform photolithography what we will have? We will have heater, we have insulator, contact, heater coils, interdigitated electrodes, right and this is our insulator correct.

So, what we have? we have interdigitated electrodes, we have a micro heater right, I have written here micro heater as MH let us use a micro heater like this um and we have silicon dioxide correct. Now, what we will do? Now, we have this particular substrate right, we have this substrate. So, this substrate is having a micro heater, it is having an interdigitated electrode and a insulating layer separating the interdigital electrodes and the microheater. Now we want to create a channel in PDMS. What I will do? I will take a silicon wafer right, grow oxide um then, I will perform photolithography so that, I can etch silicon dioxide from the unwanted region right, then I will perform deep reactive ion etching, DRIE. When I perform DRIE, what will happen? The silicon in the area which is not protected by oxide will stay and the area which is protected by, not protected by oxide will get etched. The silicon in the area which is protected by oxide will stay and the area which is not protected by oxide will get etched right.

Now, I will remove silicon dioxide. How can I etch silicon dioxide? I can etch silicon dioxide by dipping the wafer in buffer hydrofluoric acid, on this I will pour PDMS, on this I will pour PDMS and then cure it, after putting PDMS we will cure it. After curing, we will strip it off, When I strip it off this is my PDMS right, if I strip this PDMS off the silicon after curing, I will have this PDMS. This PDMS, I will thick and I will just load it right over here right, you can if you want to have like this you can light over here correct. This is what? This is your PDMS. This makes your microfluidic chip; this makes your microfluidic chip you got it right. First step is a heater, next step is an insulator, next step is remove the contact from the remove the silicon dioxide from the contact area of the micro heater, next step would be deposit metal for interdigitated electrodes. Next step would be to pattern the inter digitated electrodes, On that we will create a silicon mould and we will pour PDMS, cure PDMS, strip off PDMS and then bond PDMS with glass which is having an interdigitated electrodes and a micro heater separated by insulator using oxygen plasma bonding, using oxygen plasma bonding.

So, to have a device which you can see right over here and this is a schematic represent that the cartridge is there in which, your device is placed within this cartridge. This cartridge consists of your antibiotic 1,2,3 waste reservoir blood media and wash buffer. There is a contact paid for impedance measurement, there are contact pads for applying voltage to the heater

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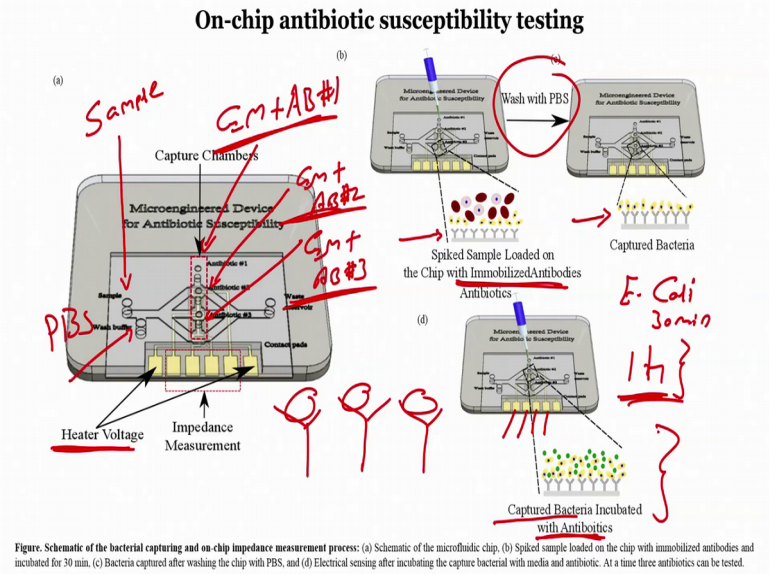

Figure. Schematic of the bacterial capturing and on-chip impedance measurement process: (a) Schematic of the microfluidic chip, (b) Spiked sample loaded on the chip with immobilized antibodies and incubated for 30 min, (c) Bacteria captured after washing the chip with PBS, and (d) Electrical sensing after incubating the capture bacterial with media and antibiotic. At a time three antibiotics can be tested.

So, how it will work? You will see here, there is a heater voltage you can see here in our device, because in the device that I have shown the heater pads and the impedance measurement pads are on the same side, so if there is a heater voltage that you can apply then, the it will be 37 degrees centigrade right.

These are capture chambers you can see 1,2 and 3, and this is the, these are electrodes for impedance measurement if you see in this particular case which is, your b case the first will load in each chamber 1,2 and 3. Each chamber will load antibodies, antibodies for E. coli, so it is anti E. coli antibodies for E. coli ok. In each chamber will load antibodies, after loading antibodies what we will do? We will load, we will load a sample at the inlet of the microfluidic chip; so, you I will show it to you here. It is easy to see here in this diagram right. You assume that the chamber has the immobilized antibodies right which, which looks like this gray color which looks like this y, like this alright.

Then we are loading a sample, it can be your spike blood, it can be urine in the channel and once the sample is loaded into the channel it is incubated at 37 centigrade for 30 minutes, 30

minutes ok. After that we will wash this, we will wash this channel with the help of PBS right you see here, wash with PBS. When you wash with PBS what will happen? That the bacteria present in the sample will get captured on the antibodies that is what you can see here. Bacteria that were present in the sample got captured on the antibodies right, after it gets captured on the antibody the next step that we will do is, we will load again here. In this, again means, in this chambers ok we load growth media plus antibiotic 1 right, growth media plus antibiotic 2, growth media plus antibiotic 3.

When we do that, what we will see? We will see that see and then, we will incubate it for another 30 minutes. See, we can keep it for 1 hour as well depending on what kind of bacteria we have. Suppose we have E. coli. E. coli, the doubling time doubling time it gets doubled it is 30 minutes, 30 minutes ok. So, let us say we have incubated our device which is captured bacteria plus antibiotics plus growth media for about 1 hour and we measure the impedance, live impedance, whenever we from the when the point we have loaded the antibiotic right, to the point that 1 hour is over we continuously monitor the impedance.

So, the advantage of microfluidic would be at the same time you can see or understand the effect of 3 different antibiotics correct. At the same time, you can understand the effect of 3 different antibiotics. So, how the data looks like, how the data looks like.

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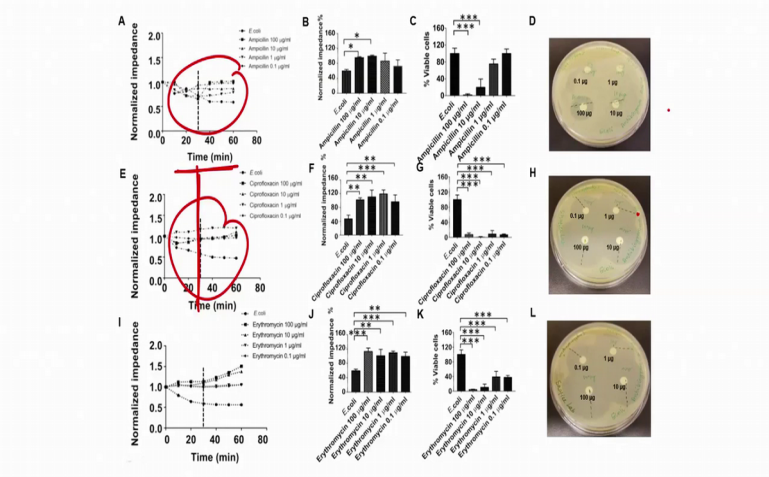

Figure. Electrical response of *E. coli* in the presence of Ampicillin, Ciprofloxacin, and Erythromycin over a period of 1 h. ~~*E.coli* in the presence of Ampicillin, Ciprofloxacin, and Erythromycin over a period of 1 h.~~ (A) Real time impedance measurement of *E.coli* in presence of ampicillin, (B) Statistical analysis of the effect of ampicillin on *E.coli* ($p= 0.0055$, n=3), (C) Viability test results of *E.coli* in the presence of ampicillin after an overnight incubation by measuring their optical density (OD) at 600 nm wavelength, ($p<0.0001$, n=3), (E) Real time impedance measurement of *E.coli* in presence of ciprofloxacin, (F) Statistical analysis of the effect of ciprofloxacin on *E.coli* ($p=0.1915$, ns, n=3), (G) Viability test results of *E.coli* in the presence of ciprofloxacin ($p=0.0850$, n=3), (I) Real time impedance measurement of *E.coli* in presence of erythromycin, (J) Statistical analysis of the effect of erythromycin on *E.coli* ($p= 0.0055$, n=3), (K) Viability test results of *E.coli* in the presence of erythromycin ($p=0.0850$, n=3), and (D, H, L) shows the antibiotic susceptibility of *E.coli* against ampicillin, ciprofloxacin, and erythromycin using disc diffusion method (24 h).

So, the data would look like, you can see here we have tested different antibiotics and you can see the first graph is for Ampicillin right. The second graph is for the Ciprofloxacin and

the third graph is for Erythromycin right, 3 graphs are here. If you see A,B and C, A B and C is tested for Ampicillin our first antibiotic, the next one which is our E F and G, this is tested for another antibiotic, and then the last one which is I J and K is tested for Erythromycin.

The D H and L these are our antibiogram right, you have seen this in the first slide. Antibiogram SA correct. So antibiogram SA you can see here for different concentrations right, 0.1 microgram, 200 microgram in all 3 cases and this is our gold standard, we are comparing the results with our gold standard. We also done the viable self-studies, and whatever impedance we get here, whatever the values we get here we have normalized it with here and this is a normalized impedance plotted for E. coli which is our control sample and then the E. coli captured with ampicillin of different concentration and we can see the effect of antibiotics by looking at the impedance change and we can also correlate it with viable cells here that the this is statistically different, the Ampicilin 100 microgram is statistically different, 10 microgram is also statistically different compared to the control. While, 0.1 microgram and 1 microgram is not different compared to the control right.

So, what we see is that the week from this study we can understand what is the minimum concentration of antibiotic that we should prescribe to a patient, also we can see that using this technique we can quickly correlate with the gold standard as well as we can rapidly measure the change in impedance as low as 1, 1 hour as low as or as fast as 1 hour.

So, this really rapid you can see here right. So, normalized impedance versus time about 1 hour we can see the change, that this is for 30 minutes 30 minutes is because, it is the doubling time of the bacteria right and if you see and read this figure, caption is a electrical response of E. coli in presence of ampicillin, ciprofloxacin and erythromycin over a period of 1 hour. In E. coli in presence of ampicillin again, this one for a period 1 hour real time impedance measurement, this is just a repeating just we should delete it. Do not worry about it.

So, the A is a real time impedance measurement of E. coil in presence of ampicillin. B is the statistical analysis of the effect of ampicillin and then C would be the viability test, same things tends sells true for E F and G, I J and K. The idea is that now we can rapidly diagnose we can rapidly diagnose the, or we can rapidly capture the bacteria and we can understand whether the bacteria is resistant to antibiotic or not.

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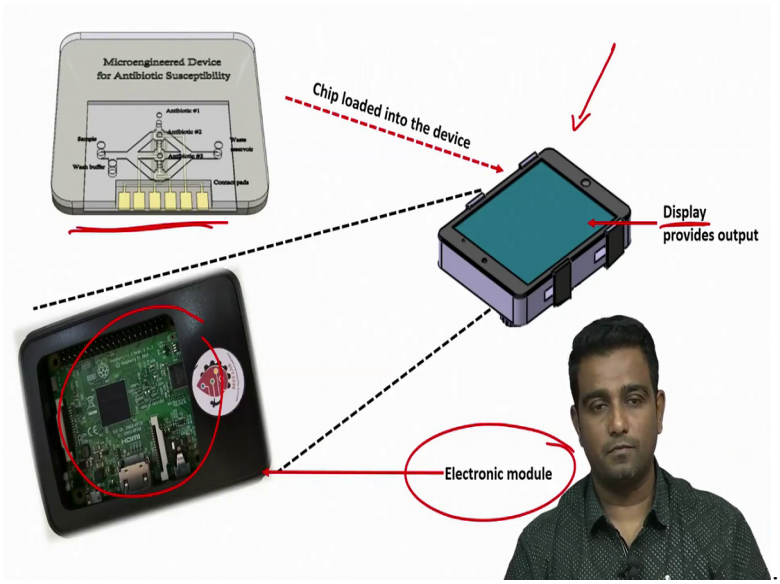

Also see here, very interesting; we can also form a electronic module and this electronic module will be indicated with a display and then there is a port to load this chip inside this whole electronic module right, this is the module. This is a final device, in which there is a display and then there is a port to load the chip inside this device and then we have the electronics within this device, so that whenever we understand whenever we measure the impedance we can convert this impedance to a display format whether the antibiotic is effective or not. So, if you see this slide if you see this slide you can see that, if the antibiotic is effective you will see a significant difference between the normal and the between the control and the actual sample and the example that is studied right guys. So, this is how we can quickly rapidly measure the effect of antibiotic. We can capture the capture the bacteria in a in a effective way and the reason of your reason of happening this is because, we are using microfluidics.

So, micro channels if you use you had to kept, the bacteria, amount of bacteria that doubles we can immediately see the change using the electrical sensing. So, can we use this technology, can we use this device for other applications like capturing other bacteria in case of sepsis? This is what we had talked about E. coli and E. coli is related to UTI. So, for UTI this can be a potential device that can measure the or capture the bacteria from urine sample in less than 1 hour and we can measure the effect of antibiotics on those bacterias and prescribe the patient a particular antibiotics rather than prescribing 4 to 5 pet antibiotics.

There is a, there is a concept ok, this is a concept. These are only initial or preliminary results.

This field is open wide. You guys can perform this experiment in a controlled environment along with a person who knows how to culture bacteria. See, bacteria is extremely harmful. Do not; do not mess up with bacteria. In any case, you had to go through a proper training right. Follow the protocol, get the approval, whether it is a biosafety approval required or whether is a ethical committee approval required. Get all the approvals in place then and then only test your sample, it is very important to understand. Do not just start fabricating a device and start loading the sample that it does not work in this particular way, because bacteria and cells are enemy. If you have seen an incubator and if you add bacteria in the incubator when the cells are there, cells will die.

So, if the if we get infected with bacteria it will affect our body, it will start killing the cells so it is harmful. Always have a proper precaution; always go through a laboratory protocol before you perform any of this experiment, not only this one. Any of the lectures that we have seen, you have to undergo a proper training and then and then only operate or work in this area of research right, that the idea is that can you design a device that can be used to understand the antibiotic susceptibility of a bacteria or you can say a bacterial resistance to a particular antibiotic and at the same time can you capture it and the same time is it rapid and at the same time does it require a small volume, because now we are talking about mega fluidics right.

So, we have to use only 10 to 20 micro liter of sample rather than taking 2 to 5 ml of blood right. So, so think about this. Learn this. Read the lectures for once again and I will see you in the experiment class where you will be shown different equipment a different equipment that are used for clinical applications whether it is a incubator or it is a peristaltic pump or there are microscopes or it is a micro manipulator or it is an impedance analyzer it is a biosafety hood right, it is an oven or we can have a small vacuum pump where we can perform the we can perform soft lithography by pouring PDMS on a mould and then stripping it off. So, these things will be shown in the laboratory that we have here at electronic systems engineering, Indians of science. So, till then you take care and I will see you later. Bye.