Advances in Additive Manufacturing of Materials: Current status and emerging opportunities

Prof. Bikramjit Basu
Materials Research Center, Indian Institute of Science, Bangalore

Lecture 17

In this scientific case study, we will be discussing the process as well as experimental results of one of the published study from our group related to the inkjet cell printing. Essentially, it will be binder jet 3D printing but using biological cells, live cells. The challenge is to maintain the cell viability and cell functionality of inkjet printed cell volume when they will be essentially printed on some substrate. If you look at this particular slide, this is from one of the published studies where people use the different velocities from 150 to 300 mm/m. And they have used different cells like you know different type of cells and they check what is the cell viability when this 3D binder jet printed cells were incubated up to 24 hours. You can see that when the cells are live cells they can be stained and in this particular case you can see in the inset they have shown this live and dead cells as well.

How this cell printing takes place? You can use it as a scaffold free conditions that means the cells are directly being printed without surrounding scaffolds and then how this essentially this particular process works. You have a piezoelectric inkjet nozzle. Let us say the size is 80 micron. Typically biological cells , their size varies in the range of 15 to 20 micron.

If the inject nozzle size is 80 micron, so at a time you can imagine that 3 to 4 cells can be injected. And when these cells are being printed on a substrate it is in a XY stage movement and then essentially you can print the cells on this particular substrate and what is shown that you know how the live cells can be printed using that inkjet printing conditions. You have a piezoelectric inkjet nozzle, you have ink reservoir. This ink reservoir actually contains the live cells with the cell culture medium. During the process of the cell printing at different time step like 167 microsecond to up to 1000 microsecond, this is that images are being captured just to show how these droplets is formed.

Now, droplet is submerged, cells are sinked and cell dish contact and cell adhesion taken place with the different time step. This is the sinking displacement with time up to 12 to 14 s, and how this cell printing took place. Some of the things are very important in the context of the cell printing because we like to see that cells when they are being printed

their life and also these cells when you are contained in a tissue engineering scaffold, These cells they experience normal biological function or natural biological function and they should also cause angiogenesis or tissue regeneration purpose. angiogenesis like blood vessel formation. some of the tissue engineered scaffolds which are being implanted into an animal body and then you can see that how blood vessel formation is taken place in different areas of the scaffolds.

Let us move on to that experiments or collaborations that we had with University of Manchester. We means our research group from Indian Institute of Science had with University of Manchester. This is a 3T3 cell culture using standard culture protocols confluent cells. Then you trypsinize with 0.25% trypsin and 1mM EDTA Na.

Then fresh media, it is centrifuged. Then pellet is ready. That once the pellet is ready, then you resuspend in PBS (phosphate buffer saline). Then, you tailor the cell density like you have to see that whether 10^3 or 10^4 number of cells can be printed and then inkjet printing of 3T3 cells using different voltages takes place. Why we have to adjust the voltage? You want the cells to survive this kind of printing conditions.

Even the printing parameters are very aggressive, then the cell viability can be compromised. And post printing, what are the cell functionalities that you can measure? It is that what is the cell viability, proliferation and cell membrane damage. Because cell membrane damage is likely to take place because of the printing conditions and if the printing conditions are aggressive then the membrane can be damaged. Piezoelectric inkjet bioprinting safety on mammalian cells and dynamic cell membrane healing was the major objective of this particular study. Continuing with our discussion on the cell printing.

what we have done as part of this work we have also measured some of the physical properties of this cell laden ink before doing that binder jet cell printing. what are the properties that we have measured? We have measured the viscosity, both the static viscosity and dynamic viscosity. static viscosity we have measured as a function of shear rate and dynamic viscosity we have measured as a function of angular frequency. in both the cases as you see that dynamic viscosity somehow attain the steady state value of around 20 mPa.s from 40 frequency whereas static viscosity that reaches a kind of a steady state around 1.

5 mPa.s from around 20 second inverse shear rate. this particular cell laden ink, PBS ink, that phosphate buffer saline has an ohnersorge number is 0.2 and this ohnersorge number is quite good. This is the 3T3 murine fibroblasts. This is the cell printing setup that were used.

you have the bio ink reservoir here. And this is the print head through which these printed cells are being collected in the cell culture tube the high speed camera is focused on the inkjet stream and then when high speed camera is focused, essentially you will get this ink droplet diameter and then you can get several shadow graphs and from shadow graphs you can see the camera controller you can see that how this cell laden ink is being printed. just to start with we have done with the fibroblast cells because mouse fibroblast cells is very widely used in many of the cell compatibility or cytocompatibility evaluation of the scaffolds or materials. Now as I said that we use the different voltages during the cell printing and these voltages varies over 80 to 100 V and we have compared the percentage live cells with that of the unprinted cells that means cells which did not experience the printing induced stresses. the major idea is that how printing will induce stresses on the cells and then how printing induced stresses essentially compromise the cell viability.

Now what you see in the fluorescence images here, any green stained region, this is the green stained cells, these are like live cells. Any red stained cells like this which are being arrowed, these are like dead cells. This is dead cells. what you see from this fluorescence image of the 3T3 cells printed using 80 V for 2 hours post printing cell viability and you can see very clearly that most of the cells are still alive. Now, if you look at this typical optical or SEM microscope images of the unprinted cells, you can see when they are grown for 120 hours or 72 hours like 3 days or 5 days, the cells retain their very healthy morphology.

And when cells were printed at 90 Volt printing conditions, You can see at the 90 volt and 100 volt and 80 volt. compared to these unprinted conditions, this healthy morphology is very much comparable, although there may be some differences in terms of the cell numbers that you can see. But when you grow for 5 days in culture, this is like a 5 days culture conditions, same mouse fibroblast cells, so this is unprinted cells. If you compare with 80 volt to 90 volt cells, printing conditions to 100 volt and you can see that cell number wise, cell morphology wise, there is no differences. This Alamar blue assay is commonly used for quantitative analysis of the number of cells which are being grown from one day, day 1 to day 5 that how that growth that is taking place for the cells which are being printed.

According to the fundamental of cell biology, if cells retain their healthy status then cells must be able to proliferate over time. What it means that if you grow the cells for multiple days in culture then you should be able to see the cell numbers will increase with time. And that is very clear if you look at when you have used that 80 volt as a printing parameters, initial growth process is little bit sluggish. But when it goes to 4 days and 5

days, this is very, very significant increase in the cell growth. The same thing you can see that initial cell growth is very minimal, but from 3 days onwards up to 5 days this is very, very significant.

this is shown irrespective of the printing parameters like 80V, 90V and100V And this growth behaviour is fairly comparable because this you can consider as the reference behaviour like the cells which are not printed like as received cells and they are being grown on the culture plate up to 5 days and it shows the kind of growth pattern which was very much shown in scientific language you can mention similar cell growth pattern. And if the similar cell growth pattern is being recorded over time up to 5 days, then you can say this kind of printing parameters are the optimal cell printing parameters for 3T3 mouse fibroblast cells these are the things that I thought that I will be mentioning to you so that you remember, so what are the things that you need to really look for. Now as I said that when the cell laden ink is being injected through the inkjet printhead and the piezo electric inkjet printhead at different voltage conditions, it is quite likely the cells will experience significant stresses and those stresses can cause the membrane damage. and then one membrane damage takes place, the membrane poration evolves, then how this membrane, the pores that will evolve in the cell membrane region, how they will be dynamically healed when they will be grown in culture in the post printing conditions. if you compare the standard material printing and with the cell printing, in the case of standard material printing what you have learnt from the previous few lectures is that you do 3D binder jet printing of metals or ceramics and in specifically what you learnt for Ti6Al4V alloys or zirconia based bioceramic materials.

Then after the printing is over, you do heat treatment or chemical conversion techniques. Here what you see that after the cell culture, you need to grow the cells under the normal culture conditions to see that whether printing induced stresses are healed or as printed cells are able to proliferate in the same manner, identical manner like unprinted cells. these two aspects are very important and third point is that if some damage has taken place on the as printed cell surfaces membranes then whether those damage can be healed dynamically over few days in culture. this is what microfab inkjet head and that is what the cell printing you can see that how layers of cells are being printed very design manner and sequentially in this is meant for biofabrication. this is like acoustic porated cells in the printhead this is like porations you can see and when the PI staining is done so you can essentially see how texas red conjugated dextran molecules can enter through the pores in the cell membrane.

we have used different type of driving voltages during this inkjet printing of cells 80, 90, 100 and fluorescently tagged molecular probes like you know Texas-rate conjugated dextran molecules like 3000- 70000 Dalton and these are molecular probes were used and

probing time points like after printing like 0.25 to 2 hours post printing conditions. Now if you look at this numbers what you see here this is number of permeable cells and number of permeable cells what you see here this when printing voltage is 80 volt this is the PI. This PI you can see that you know that how this kind of number of permeable cells decrease. If you go to the 100 volt then again printing voltage it is reduced and then PI tagged cells also consequently reduced.

When you consider that percentage of PI in unprinted cells there is no evidence of higher probes then you can see that it also decreases. what I am trying to show you here that percentage PI in the unprinted cells is much much less compared to the percentage PI number of permeable cells with respect to PI in case of the printing cell printing at different voltages like 80, 90 or 100 volt. this part of the work was published in ACS Biomaterials Science and Engineering and this is again another collaboration between Indian Institute of Science and University of Manchester, Brian Derby's group and there Srimanta actually visited this university and did some of the experiments and then we have done lot of analysis of the particularly cell biology related and then we published together. what all these scientific case studies essentially provides you a flavor of scientific studies that one can do in case of the binder jet printing for metals, for ceramics, and also biological cells. major highlights of this particular lecture are essentially to show you that binder jet cell printing under the optimal printing conditions do not compromise the cell viability and do not compromise the cell proliferation or cell growth kinetics.

In fact, cell growth behaviour follows the similar temporal trend like time dependent trend like you know reference sample that is unprinted cells. And also even if the membrane poration takes place they were able to be dynamically healed during the post printing culture conditions. these are some of the aspects one needs to take care when they will do this binder jet 3D printing of cells. Thank you.