Tissue Engineering
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Lecture – 24
Bioreactors in Tissue Engineering

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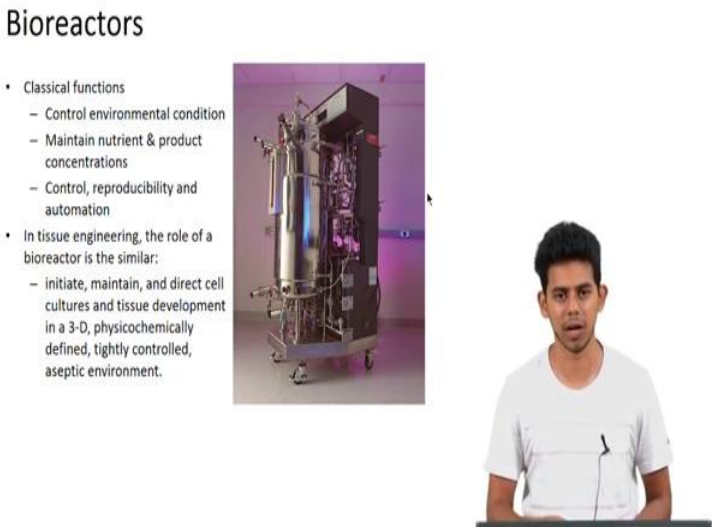

Hi everyone. Today we will see about bioreactors that are used in Tissue Engineering. So, what are bioreactors? And what is its function? Bioreactors are usually used to maintain a controlled environment, to ensure that the biological reaction can proceed. For that intent, you maintain the nutrient and product concentration. Also, you get a high degree of control and reproducibility of these products on using these bioreactors.

The role of bioreactors in tissue engineering too, is similar to what it is normally used for. It is to initiate, maintain, and direct cell cultures in a 3D environment by maintaining that environment and also the aseptic condition.

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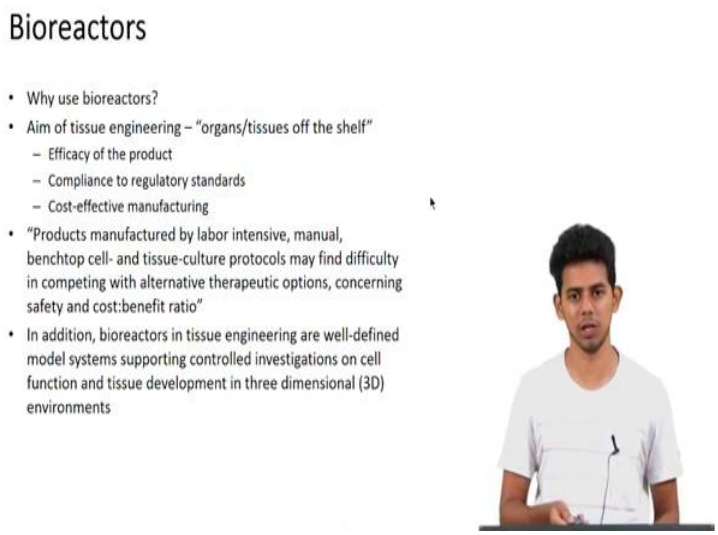

Why use bioreactors? The aim of tissue engineering has been to get organs or tissues off the shelf, wherein you are able to replace tissue or organ, which is damaged. The factors which play an important role in making this feasible are the efficacy of the product. So, the product has to do what the natural tissue or organ does. Then it has to comply with regulatory standards. Also, you should be able to produce these tissues or organs in a cost-effective manner.

This is where the bioreactors come in. Products that are manufactured on the tabletop in a lab. These might be useful to study how you can grow this organ in lab ex vivo. But to produce it on a large scale, you would need something like a bioreactor. In addition, such a controlled environment like a bioreactor ensures that we can study in detail on how tissue development happens by controlling all the parameters and changing whatever we require.

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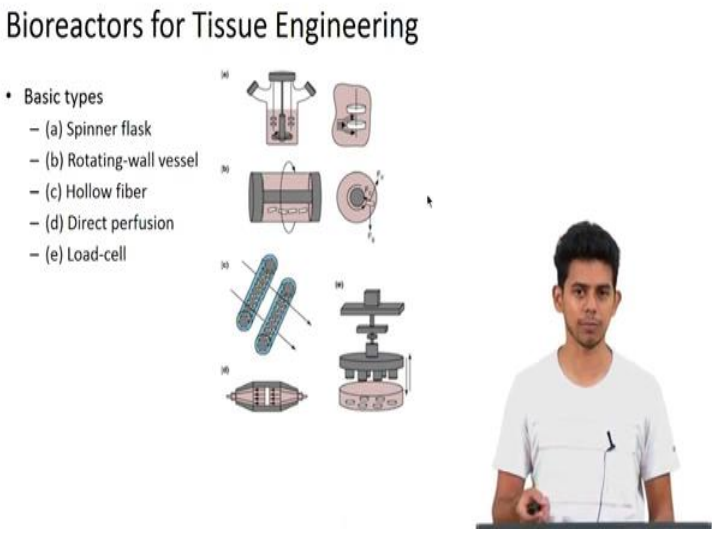

These are the basic types of bioreactors that are used in tissue engineering. The first one is a spinner flask wherein there is a low degree agitation, which is given to the flask. The second one is the rotating wall vessel wherein the cells remain in suspension, and the bioreactors keep rotating. So, this offers some slight degree of shear stress, which is required for cell lines like epithelial cells and all.

Then hollow fibers. So, these hollow fibers have capillaries that run through them carrying the media. And the media can diffuse outside, and on the outer surface of these capillary tubes, there are cells that are seeded on it. So, the spent media is then taken out and can be recycled and circulated again.

Direct perfusion. Here what happens is a cell and scaffold contract is placed in the center of the chamber, and the media is perfused through it. So, the spent media comes out on the other side, and you can re-circulate it or change the media. Then load cell bioreactors. So, this is when the tissue that you are growing require some sort of mechanical force to grow in the right way like tendons or bones. You can simultaneously stretch or compress the tissue as they are forming, thereby giving it the physiological conditions required for its development.

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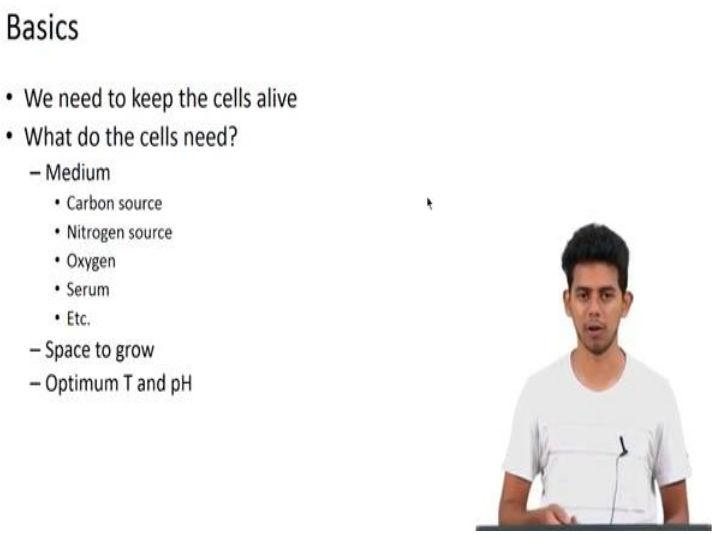

The basics that we look at is, what do we need to keep the cells alive. Obviously, we need media. This media needs a carbon source or nitrogen source, oxygen and serum, and other things like growth factors, etcetera. The cells need space to grow, and also you need an optimum temperature and pH.

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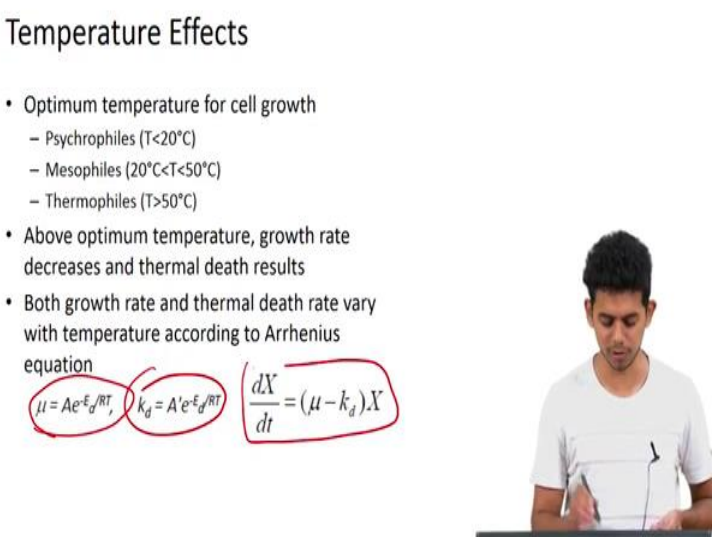

Temperature effects. The optimum temperature required by the cells can vary based on the cell type. They are divided into three classes based on their required temperature. Psychrophiles which required temperature is below 20 °C; Mesophiles, which require a

temperature range of 20 to 50 °C. And thermophiles, which require a high temperature greater than 50 °C.

Above this optimum temperature, growth rate decreases, and thermal death results. The effect of temperature can be shown by the Arrhenius equation. This shows the growth rate constant, and this shows the death rate constant. And using the death rate and the growth rate constant, we can calculate the rate of cell concentration with respect to time.

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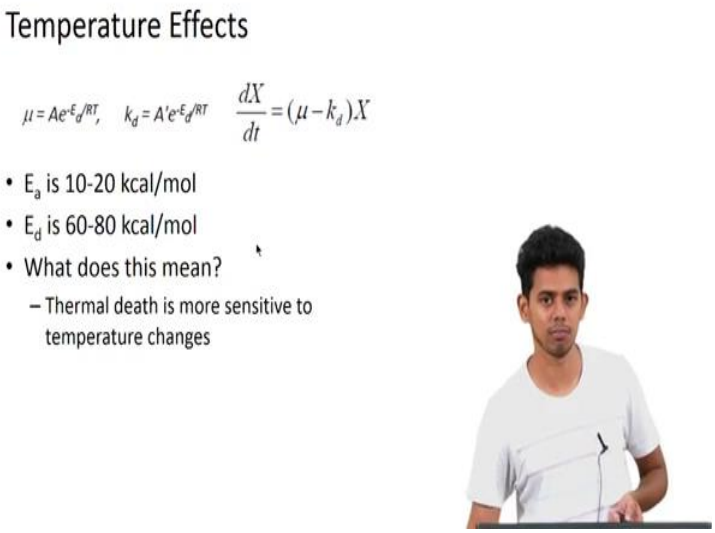

The activation energy for growth is usually around 10 to 20 kcal/mol and of death is around 60 to 80 kcal/mol. From this, we can see that if you can substitute it, that thermal death is more sensitive to temperature changes. So, having the right temperature range is quite important.

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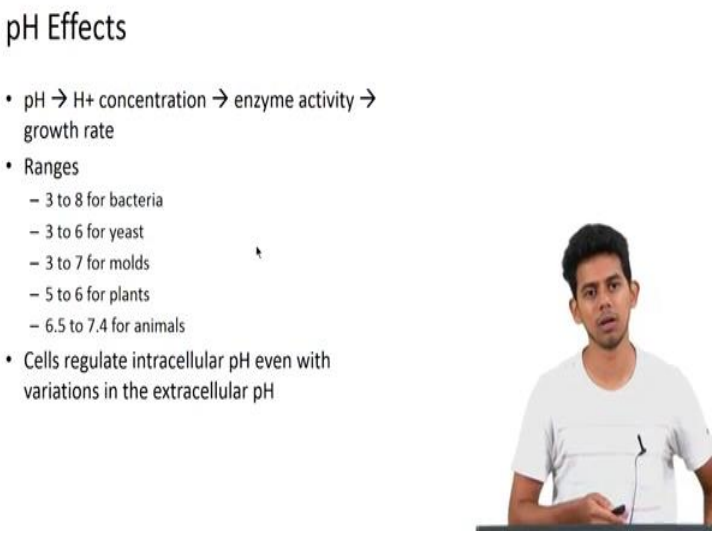

Then coming to the pH effect. pH is basically the H+ concentration, which in turn affects the enzyme activity and the growth rate. These are a few of the examples of different organisms and their preferable pH range. For mammalian tissues, its usually around 6.5 to 7.4 pH. The cells are capable of regulating their intracellular pH even when there is a variation in the external pH, so there is a buffering system usually for it.

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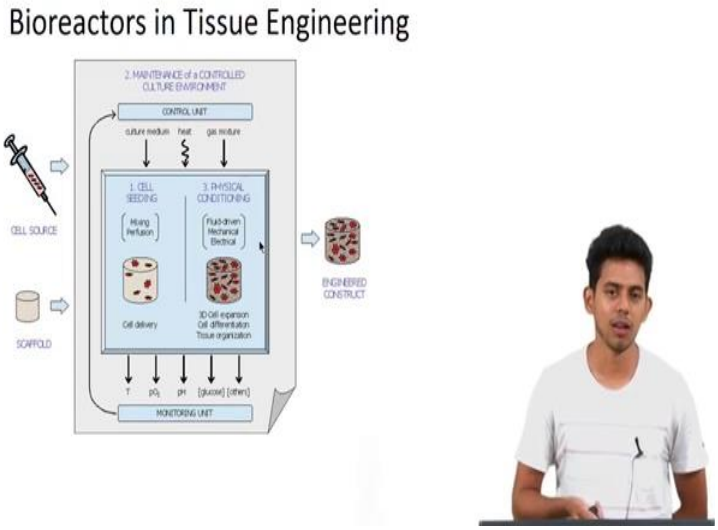

Now, looking at the main functions of a bioreactor in tissue engineering. We have a cell source and a scaffold. The cells initially need to be seeded on to the scaffold, and then

we should be capable of maintaining the right environment for cell proliferation and growth. There should be some level of physical conditioning, which is required. So, it varies depending on the tissue that you are trying to grow. It can be fluid-driven, or mechanical, or electrical conditioning. Also, all these parameters are continuously monitored and using sensors; they can be controlled so that the preferable environment is maintained.

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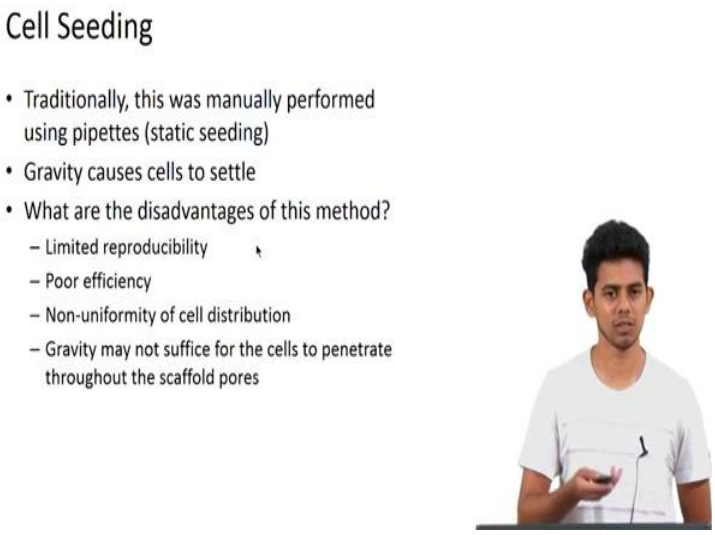

First, let us look at the cells seeding part. Traditionally, cell seeding was done by dropping the cells onto a scaffold using a pipette. Here, you rely on gravity to take the cells into the scaffold. But the disadvantage of this is; there would be a uniform distribution of the cells into the scaffold. There may not be complete penetration of the cells throughout the scaffold, and its poor efficiency and reproducibility are also affected.

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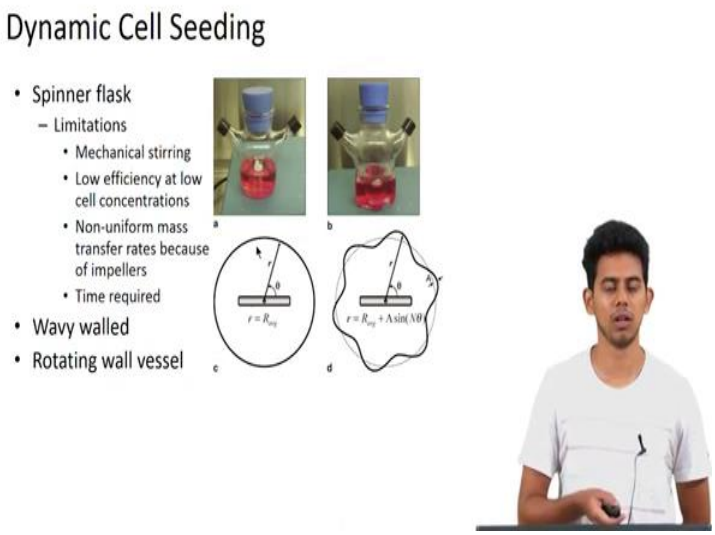

That is why dynamic cell seeding is used mainly in bioreactors. Here, what is done is, it ensures that the cells end up penetrating throughout the scaffold, and they are distributed in a more even fashion. These are some other techniques normally used for seeding the cell within the bioreactor. A spinner flask uses normal mechanical steering to ensure that the cells are seeded completely into the scaffold.

But the spinner flask has some limitations, which are mentioned here. So, we use wavy walled, which has a sinusoidal pattern. Waves can be created because it has a patterned outer layer. So, this structure allows for enhanced perfusion of the cells into the scaffold. Then the next one is a rotating wall vessel that we saw before.

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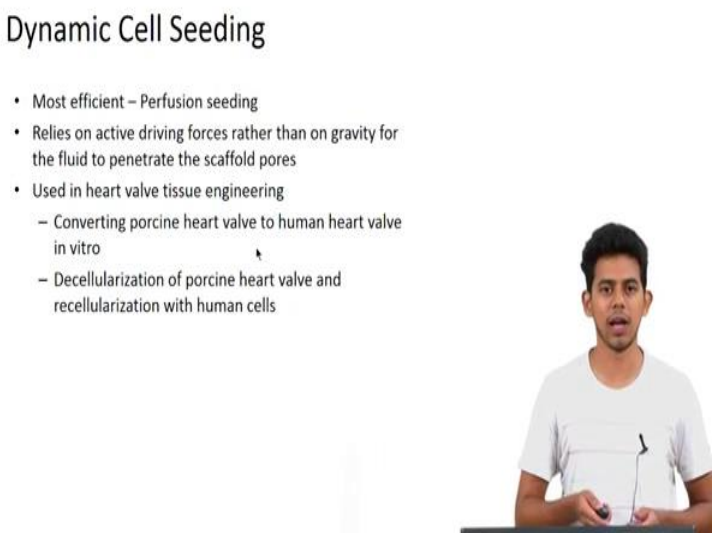

Amongst the dynamic cells seeding techniques, the most efficient one is perfusion seeding. It relies on active perfusion rather than using gravity for seeding the cells onto the scaffold. So, it can penetrate throughout the scaffold, and in a uniform fashion, the cells can be seeded. It has been used in converting a porcine heart valve by decellularizing it and recellularizing it with human cells by perfusion.

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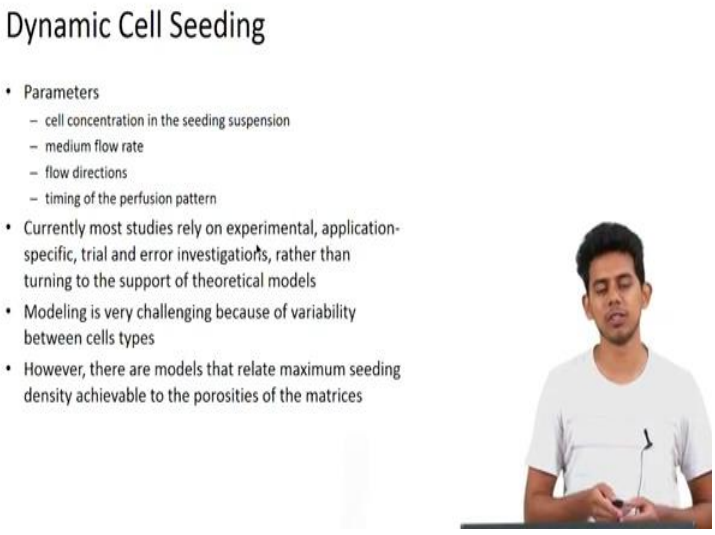

The main parameters which determine how the cells are seeded are the cell concentration that you use for your seeding. The flow rate of the medium, the flow direction, and

timing of the perfusion pattern. Currently, moreover what is used is a trial and error approach in determining what is the best parameters that need to be used to achieve the best seeding. Modeling is quite challenging because each cell type can have a lot of variability. Still, there are models that can help us achieve maximum seeding density based on the porosity of the matrices.

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Maintenance of control culture environment. A convective media flow around the construct and also perfusion of this media through the pores can aid in overcoming the diffusional transport limitations. Perfusion can enhance the formation and deposition of ECM, as shown in these examples. And also improve cell proliferation in blood vessels, etcetera.

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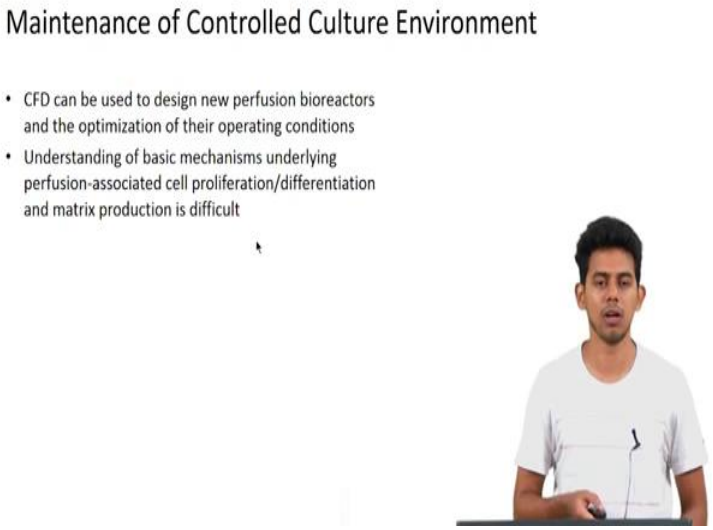

Computational fluid dynamics can be used to design new perfusion bioreactors and also to optimize their operating conditions. But what is required for this mainly is to get to know the underlying mechanisms associated with perfusion. So, what is important for this to happen is to get a good grasp of the underline mechanisms.

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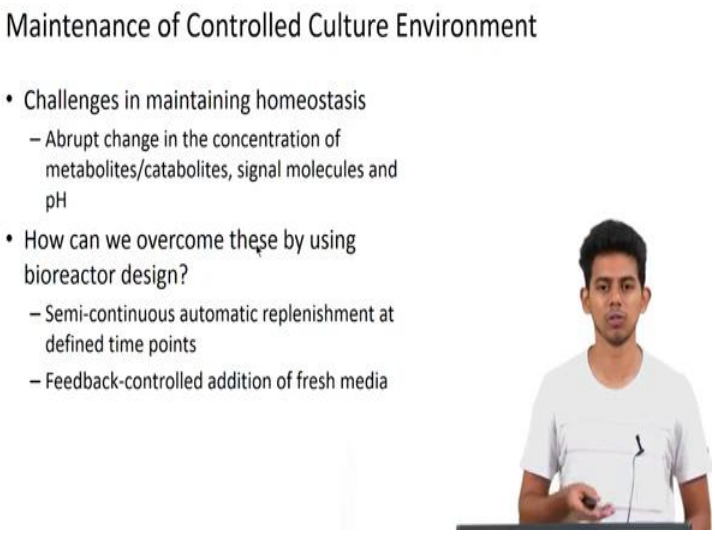

The challenge in maintaining homeostasis is an abrupt change in the concentration of metabolites and catabolites. This happens, especially in the lab culture environment. The challenge in maintaining homeostasis is the abrupt change in concentration of

metabolites and catabolites. This can be overcome in a bioreactor by using a semi-continuous or automatic replenishment; thereby there is not a huge and sudden change in the metabolites or the catabolites during the reaction. Also, we can use a feedback-controlled addition of fresh media.

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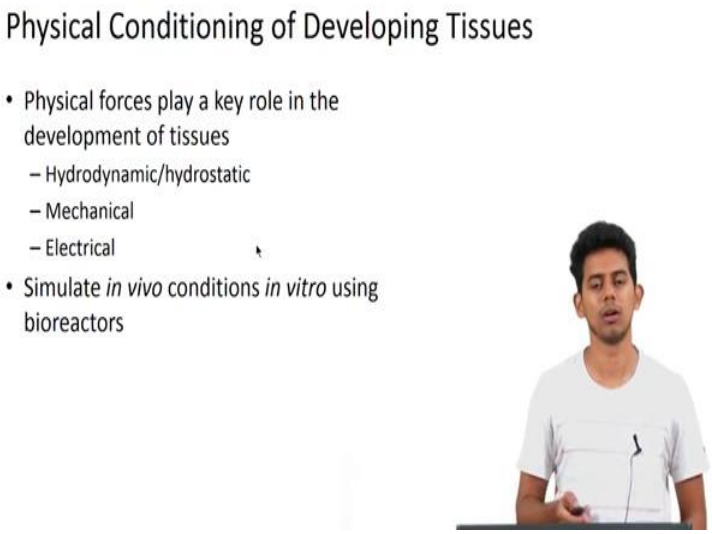

Physical conditioning of developing tissue, this would be the third aspect that bioreactors need to look at. Physical forces, as mentioned in the previous talk, do play an important role. Different types of physical forces play a role like hydrodynamic or hydrostatic forces, mechanical or electrical forces. The aim of the bioreactor here would be to simulate these in vivo conditions inside the bioreactor too.

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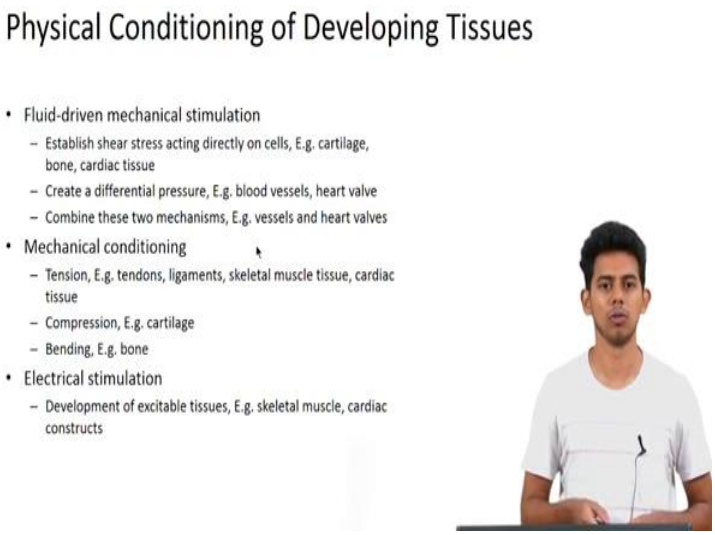

Some of the basic physical conditions in vivo are a fluid-driven mechanical simulation, wherein shear stress can be induced on the cells by the fluid, especially in the heart valve, etcetera. Also, create a differential pressure; we can even combine these two mechanisms, especially in making vessels and heart valves. In the mechanical condition, wherein you give a tension or compression like in bone, cartilage, etcetera. And electrical stimuli, which is used in cardiac, skeletal and muscle constructs.

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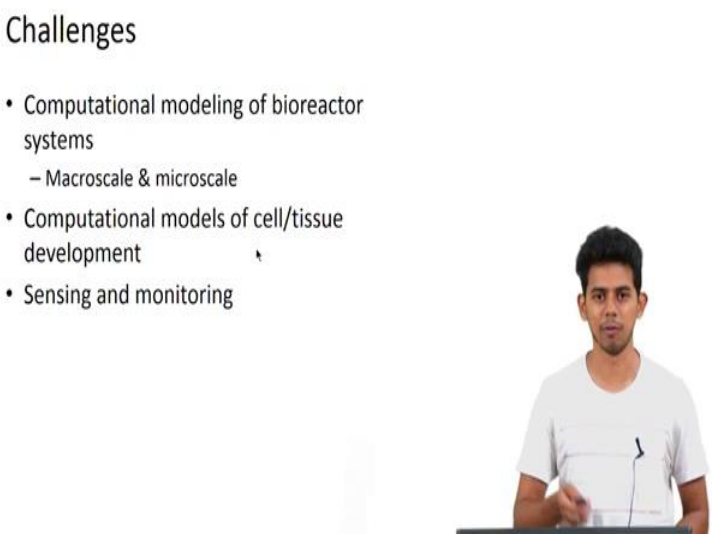

The challenges in developing a bioreactor would be that we need a very thorough understanding of what is happening in the in vivo condition. If we can make a computational model of the cell and tissue development in the bioreactors setting. The sensing and monitoring techniques could be improved wherein we can get real-time information on how the construct is being developed and what its parameters are like its morphology etcetera.

Thank you.