

Tissue Engineering
Prof. Vignesh Muthuvijayan
Department of Biotechnology
Indian Institute of Technology, Madras

Lecture – 24
Bioreactors in Tissue Engineering

(Refer Slide Time: 00:27)

Bioreactors

- Classical functions
 - Control environmental condition
 - Maintain nutrient & product concentrations
 - Control, reproducibility and automation
- In tissue engineering, the role of a bioreactor is the similar:
 - initiate, maintain, and direct cell cultures and tissue development in a 3-D, physicochemically defined, tightly controlled, aseptic environment.



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.

(Refer Slide Time: 01:12)

Bioreactors

- Why use bioreactors?
- Aim of tissue engineering – “organs/tissues off the shelf”
 - Efficacy of the product
 - Compliance to regulatory standards
 - Cost-effective manufacturing
- “Products manufactured by labor intensive, manual, benchtop cell- and tissue-culture protocols may find difficulty in competing with alternative therapeutic options, concerning safety and cost:benefit ratio”
- In addition, bioreactors in tissue engineering are well-defined model systems supporting controlled investigations on cell function and tissue development in three dimensional (3D) environments



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.

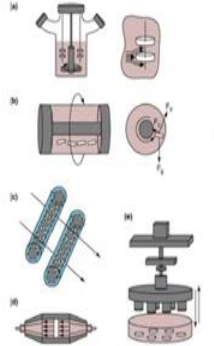
This is where the bioreactors come in. Products that are manufactured on the tabletop in a lab. These might be useful to study how you can grow this organ in lab ex vivo. But to produce it on a large scale, you would need something like a bioreactor. In addition, such a controlled environment like a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

(Refer Slide Time: 02:21)

Bioreactors for Tissue Engineering

- Basic types

- (a) Spinner flask
- (b) Rotating-wall vessel
- (c) Hollow fiber
- (d) Direct perfusion
- (e) Load-cell



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 construct 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.

(Refer Slide Time: 03:52)

Basics

- We need to keep the cells alive
- What do the cells need?
 - Medium
 - Carbon source
 - Nitrogen source
 - Oxygen
 - Serum
 - Etc.
 - Space to grow
 - Optimum T and pH



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.

(Refer Slide Time: 04:15)

Temperature Effects

- Optimum temperature for cell growth
 - Psychrophiles ($T < 20^{\circ}\text{C}$)
 - Mesophiles ($20^{\circ}\text{C} < T < 50^{\circ}\text{C}$)
 - Thermophiles ($T > 50^{\circ}\text{C}$)
- Above optimum temperature, growth rate decreases and thermal death results
- Both growth rate and thermal death rate vary with temperature according to Arrhenius equation

$$\mu = A e^{-E_d/RT}, \quad k_d = A' e^{-E_d/RT}, \quad \frac{dX}{dt} = (\mu - k_d)X$$



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.

(Refer Slide Time: 05:04)

Temperature Effects

$$\mu = A e^{-E_a/RT}, \quad k_d = A' e^{-E_d/RT} \quad \frac{dX}{dt} = (\mu - k_d)X$$

- E_a is 10-20 kcal/mol
- E_d is 60-80 kcal/mol
- What does this mean?
 - Thermal death is more sensitive to temperature changes



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.

(Refer Slide Time: 05:25)

pH Effects

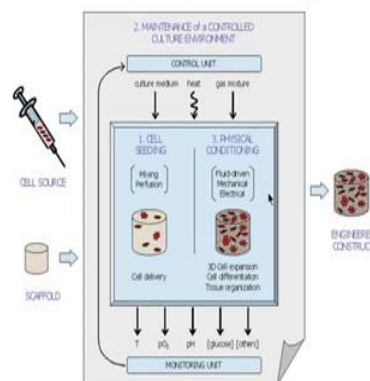
- pH \rightarrow H⁺ concentration \rightarrow enzyme activity \rightarrow growth rate
- Ranges
 - 3 to 8 for bacteria
 - 3 to 6 for yeast
 - 3 to 7 for molds
 - 5 to 6 for plants
 - 6.5 to 7.4 for animals
- Cells regulate intracellular pH even with variations in the extracellular pH



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.

(Refer Slide Time: 05:57)

Bioreactors in Tissue Engineering



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.

(Refer Slide Time: 06:44)

Cell Seeding

- Traditionally, this was manually performed using pipettes (static seeding)
- Gravity causes cells to settle
- What are the disadvantages of this method?
 - Limited reproducibility
 - Poor efficiency
 - Non-uniformity of cell distribution
 - Gravity may not suffice for the cells to penetrate throughout the scaffold pores

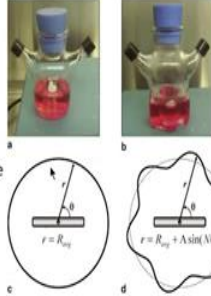


First, let us look at the cells seeding part. Traditionally, cell seeding was done by dropping the cells onto a scaffold using a pipette. Here, you rely on gravity to take the cells into the scaffold. But the disadvantage of this is; there would be a uniform distribution of the cells into the scaffold.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

(Refer Slide Time: 07:16)

Dynamic Cell Seeding

- Spinner flask
 - Limitations
 - Mechanical stirring
 - Low efficiency at low cell concentrations
 - Non-uniform mass transfer rates because of impellers
 - Time required
- Wavy walled
- Rotating wall vessel



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.

(Refer Slide Time: 08:14)

Dynamic Cell Seeding

- Most efficient – Perfusion seeding
- Relies on active driving forces rather than on gravity for the fluid to penetrate the scaffold pores
- Used in heart valve tissue engineering
 - Converting porcine heart valve to human heart valve in vitro
 - Decellularization of porcine heart valve and recellularization with human cells



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.

(Refer Slide Time: 08:42)

Dynamic Cell Seeding

- Parameters
 - cell concentration in the seeding suspension
 - medium flow rate
 - flow directions
 - timing of the perfusion pattern
- Currently most studies rely on experimental, application-specific, trial and error investigations, rather than turning to the support of theoretical models
- Modeling is very challenging because of variability between cells types
- However, there are models that relate maximum seeding density achievable to the porosities of the matrices



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.

(Refer Slide Time: 09:19)

Maintenance of Controlled Culture Environment

- Convective media flow around the construct and direct medium perfusion through its pores can aid in overcoming diffusional transport limitations (specifically via oxygen and metabolite supply and waste product removal)
- Perfusion enhances
 - calcified matrix deposition by marrow-derived osteoblasts
 - Viability, proliferative capacities and expression of cardiac-specific markers of cardiomyocytes
 - Cell proliferation in engineered blood vessels
 - Extra-cellular matrix deposition, accumulation and uniform distribution by chondrocytes



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.

(Refer Slide Time: 09:45)

Maintenance of Controlled Culture Environment

- CFD can be used to design new perfusion bioreactors and the optimization of their operating conditions
- Understanding of basic mechanisms underlying perfusion-associated cell proliferation/differentiation and matrix production is difficult



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.

(Refer Slide Time: 10:17)

Maintenance of Controlled Culture Environment

- Challenges in maintaining homeostasis
 - Abrupt change in the concentration of metabolites/catabolites, signal molecules and pH
- How can we overcome these by using bioreactor design?
 - Semi-continuous automatic replenishment at defined time points
 - Feedback-controlled addition of fresh media



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.

(Refer Slide Time: 10:56)

Physical Conditioning of Developing Tissues

- Physical forces play a key role in the development of tissues
 - Hydrodynamic/hydrostatic
 - Mechanical
 - Electrical
- Simulate *in vivo* conditions *in vitro* using bioreactors



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.

(Refer Slide Time: 11:24)

Physical Conditioning of Developing Tissues

- Fluid-driven mechanical stimulation
 - Establish shear stress acting directly on cells, E.g. cartilage, bone, cardiac tissue
 - Create a differential pressure, E.g. blood vessels, heart valve
 - Combine these two mechanisms, E.g. vessels and heart valves
- Mechanical conditioning
 - Tension, E.g. tendons, ligaments, skeletal muscle tissue, cardiac tissue
 - Compression, E.g. cartilage
 - Bending, E.g. bone
- Electrical stimulation
 - Development of excitable tissues, E.g. skeletal muscle, cardiac constructs



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.

(Refer Slide Time: 12:03)

Challenges

- Computational modeling of bioreactor systems
 - Macroscale & microscale
- Computational models of cell/tissue development
- Sensing and monitoring



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.