Stem Cell Bioreactors: Design, Structure, and Operation of Stem Cell Bioreactors

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Abstract

A bioreactor is a manufactured device or system that helps to produce biologically active substances such as yeast, bacteria, or mammalian cells under controlled environment. Bioreactor is basically a vessel constructed where chemical process is carried out in the presence of cells or organisms to produce large quantity of cells or their byproducts. The need for bioreactors arises when you need to produce large quantity of biological products, which is however not possible in the research laboratories. These bioreactors come with various sizes ranging from 2 L to 100s of L. These bioreactors are used to produce biopharmaceuticals, vaccines, or monoclonal antibodies, cell production and expansion, enzyme production, tissue engineering, algae production, protein synthesis, and also stem cell production. In this chapter, we will discuss significance, application, design, types, and operation of bioreactor especially focusing to mammalian or stem cell culture.

Keywords

Stem cell bioreactors · Design of bioreactors · Structure of bioreactors · Operation of stem cell bioreactors

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5.1 Introduction

A bioreactor is a manufactured device or system that helps to produce biologically active substances such as yeast, bacteria, or mammalian cells under controlled environment (Stephenson and Grayson 2018). Bioreactor is basically a vessel constructed where chemical process is carried out in the presence of cells or organisms to produce large quantity of cells or their byproducts (Stephenson and Grayson 2018). For large-scale production of biological active substances such as cells or tissues or organisms, the bioreactors are designed and constructed. These bioreactors come with various sizes ranging from 2 L to 100s of L. These bioreactors are used to produce biopharmaceuticals, vaccines, or antibodies and also used in converting raw materials into useful byproducts such as in the bioconversion of corn into ethanol.

5.2 Bioreactors and Its Application

Bioreactors are generally used to produce mammalian cells, stimulate cell differentiation and tissue formation under controlled environment supplied by nutrients. These bioreactors have been extensively applied to culture and expansion of mammalian cells, chimeric cells, induced pluripotent stem cells, and human mesenchymal stem cells, respectively. Furthermore, another advantage of bioreactors is that they allow delivery of biological and chemical ingredients on control manner to regulate the production of different types of cells with high purity and better functionality. The well-defined controlled environment in the bioreactors has many advantages such as it improves standardization and reproducibility of the products and also helps to produce in large quantities. It also produces clinically relevant cells with high purity and superior functionality. Over the past years as the field of regenerative medicine has grown up globally, so as the use of bioreactors also increased immensely. The different types and sizes of bioreactors have been designed and produced to meet the demands received from various biopharmaceutical and biotechnology companies to produce clinical grade stem cells for the cell therapy.

5.3 Types of Bioreactors

Bioreactors can be designed and produced based on the two major factors, one is that which types of cells need to be produced for example, bacterial cells, yeast and mammalian cells and second one how much volume (liters) of the cells need to be produced. Based on these factors, bioreactors are classified into six types such as fluidized bed bioreactors, and packed bed bioreactors, continuous stirred tank bioreactors, bubble column bioreactors, airlift bioreactors, and photo-bioreactors.

5.3.1 Continuous Stirred Tank Bioreactors

The bioreactor is also known as back-mix reactor or mixed flow reactor as this continuous-flow stirred tank reactor is used in chemical and environmental engineering. This bioreactor mainly used to assess the key unit operation variables to reach a specified output. This bioreactor can be used to in the forms of liquids and gases chemical compositions.

5.3.2 Bubble Column Bioreactors

The bubble column reactor is used wherein mixture of gases can be distributed in the liquid form by using a suitable distributor which travels upwards direction produced bubbles.

5.3.3 Airlift Bioreactors

Airlift bioreactors are used where the injection of a gas is made in the culture medium which can cause the broth to circulate between the riser and an interconnected down comer compartment of the bioreactor.

5.3.4 Fluidized Bed Bioreactors

In a fluidized bed bioreactor, mixture of culture medium is moved in upward direction through a packed bed of immobilized cells suspends them inducing a fluid-like behavior.

5.3.5 Packed Bed Bioreactors

Packed bed bioreactors are used in cell and tissue engineering applications. These bioreactors support the growth and expansion of different types of cell lines for long period of time under various culture conditions.

5.3.6 Photo-Bioreactors

In photo-bioreactor, light source is used to cultivate phototrophic microorganisms. These phototrophic microorganisms apply photosynthesis process to generate biomass by converting light energy into biomass using light and CO_2.

5.4 Design of Bioreactors

Bioreactor is basically a steel vessel which is covered by thermal jacket to prevent the loss of heat. The center of reactor is fitted with aerator panel to mix the cell culture which is equipped with motor where speed of rotation can be increased or decreased (Fig. 5.1).

These inlets allow the entry of air, media, and nutrients inside reactors which are monitored by the computerized system. In case outlet, which releases effluent outside. The temperature, pH, dissolved oxygen, and pressure of the reactor is monitored through sensors which are submerged in the culture media.

5.5 Function and Operation of Bioreactors

5.5.1 Culture Condition

The bioreactor is generally used to culture cells which are growing in the media and nutrients need to be regularly and steadily mixed all the time to be able to produce uniform and pure products. In case, the nutrients are not properly mixed in the bioreactor, the growing cells will not get sufficient amount of nutrients which may

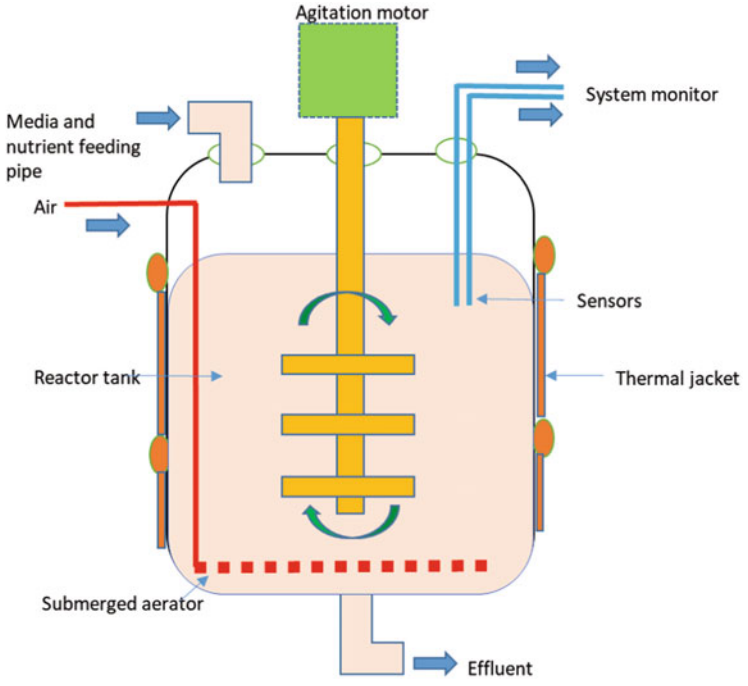

Fig. 5.1 Diagrammatic representation of the bioreactor for cell culture

Table 5.1 Rate of mixing in the bioreactor

Organism types	Rate of mixing
Bacteria, yeast, fungi	500–1500/min
Mammalian or plant cells	30–300/min

Table 5.2 Temperature range in different organisms

Type of organisms	Temperature range
Bacteria, yeast, fungi	+20 °C to +60 °C
Mammalian or plant cells	+25 °C to +37 °C

result in not getting the desirable products, and there will be deviation from one lot to another lot which is not acceptable as per the GMP guidelines. In some case, if the pH of the mixture is too high, which will have negative impact on both quality and quantity of the cell production and is not acceptable as per the GMP guidelines. Such deviations in the bioreactor not only reduce the efficiency of the bioprocess, but can also promote genetic modifications which may result in the financial loss to the company as such product is not approved for the consumption. In addition, temperature in the bioreactor is also very critical factor for the product development as any minute variation in the temperature may negatively impact the cell growth and culture. Hence, it's very important to regulate and maintain the bioreactor temperature uniformly and consistently. Another important issue is the stirring of the mixture in the bioreactor. It's very important to continuously mix the air and nutrients with growing cells, so that all growing cells get uniformly same amount of air and nutrients. The speed of stirrer is critical, as very slow and very high speed may not good for the cells and may cause damage to growing cells. It has been found that depending on the cell line, cell cultures may have a much more intense response to overly vigorous stirring which may lead to cell death. The mixing rate for different organisms is shown in Table 5.1.

5.5.2 Temperature Monitoring During Culture

Temperature is critical factor for microorganisms and cell growth inside bioreactor and optimum temperature has been defined for the cell culture growth. The presence of enzyme in the nutrients and media is important for cell growth and action of enzyme may be changed with the change in the temperature. It's very important to maintain the reactor temperature uniformly and consistently during the cell culture growth and production. It has been found that mammalian cells are normally grown comfortably in the narrow range of 37 °C and any variation from 3 to 4 °C may cause damaging impact on the cell production and there is great possibility that growing cells might die due to high temperature. Platinum resistance sensors are used to check and monitor the bioreactor temperature. The typical temperature range in different organisms is shown in Table 5.2.

To avoid an overheating of the vessel, bioreactor is now active cooling system which quickly helps to reduce temperature and brings back to required temperature.

But none the less, the temperature must be constant throughout the cell production stage or cell cultivation. In some unique condition, where the products such as penicillin or recombinant proteins, changing in the temperature at the end of the growth phase may be useful to activate certain important genes for product formation. In addition, the temperature of the vessel is rarely dropped at the end of the bioprocess which allows finished product to remain stable for future use.

5.5.3 pH Monitoring During Culture

Like temperature, the measuring and controlling of pH of the growing cells inside bioreactor is critical for the bioprocesses, as any change in the pH may significantly alter growth conditions and may produce negative impact on cell growth and cell cultivation. It has been noted that culture media generally possess buffer substances that may cause the change in pH of the mixture, and any increase in the acidic pH may damage the cells and cells will eventually die. A typical pH range for different organisms is depicted in Table 5.3.

In the bioreactor, pH sensor is fitted to measure pH during the bioprocess and this pH sensor is also known as a combination electrode for pH. To properly control the pH of the mixture, the bioreactor has option for acid and an alkaline which is connected to the culture vessel via tubes and pumps.

5.5.4 Addition of Nutrients During Culture

Nutrients are the most constituent for the cell cultivation, and based on the different cell types, array of nutrients have been used and developed. The basic composition of a nutrient medium typically consists of water, glucose, carbon, nitrogen, and phosphorus, salts and trace elements. In certain specific conditions, there is also need for vitamins, essential amino acids to be added in the culture media. It's very important to maintain the quality of nutrients used in the cell cultivation and any variation in the quality of nutrients will have impact on the quality of cells product.

5.5.5 Providing Air During Culture

During the bioprocess of cell cultivation, it's very important to provide the growing cells with required amount of sterile oxygen into the culture medium. It has been recommended that constant stirring is required during the whole process of cell culture for successful and uniform gas distribution into cell culture media. While gas

Table 5.3 pH range for different organisms

Type of organisms	pH range
Bacteria, yeast, fungi	4.5–7.0
Mammalian cells	6.7–7.4

release into the mixture, which may lead to production of bubbles in the mixture which indicate that gas is mixing in the cell culture media. The bubble formation is important as it shows that microorganisms and cell cultures absorb the oxygen that has been dissolved in the nutrient mixture. The demand for oxygen may vary from cells to cells and level of oxygen can be monitored regularly.

Unlike to microorganisms, cell cultures are not only needed oxygen in the mixture but also get influenced by pure nitrogen and pure oxygen present in the mixture. The precise composition of the various gases in the mixture generally depends on type of the cell culture. The gases conditions in the vessel can be monitored through computerized control system. It has been suggested that during the starting time of the bioprocess, the amount of oxygen required is minimum but its requirement increased when cell cultivation progresses. In addition to ensuring a constant supply of the desired oxygen, the bioreactor should be also delivered the right amount of gas at the right time. It has been suggested that the gases amount will be varied during the bioprocess and it's directly related to amount of cells which are grown in the vessel. During the mixing of different gases, the air bubbles will form which will uniformly be distributed in the vessel which is very critical for obtaining the optimum cell products. The efficiency of cell production is also depended on the mixing of oxygen during culture, which means, if oxygen is not properly distributed in the vessel, it will produce a negative impact on the quantity and quality of the cell production.

5.5.6 Monitoring Pressure During Culture

The vessel pressure is critical factor for cell cultivation. It has been found that higher the pressure in the vessel will cause the more oxygen to dissolve in the mixture. The cell culture vessels which are made of glass can only take pressure up to 0.5 bar, which is not sufficient for the cell cultivation and if you increase the pressure, there is great possibility that glass bioreactor will get burst. Unlike glass culture vessels, stainless steel bioreactors are designed for higher pressures. Hence, it's highly recommended that all bioreactors should be made of steel material. Even, in the steel made bioreactor, outlet should be provided so that access gas can be released from the bioreactor. The standard steel vessel can take pressure up to 2 bar.

5.6 Application of Bioreactors in Stem Cells Production

The bioreactors have been extensively used in the production of clinical grade stem cells for the regenerative medicine, tissue reconstruction, and cell transplantation application. As large number of stem cells or differentiated cells are required for the regenerative medicine, it can only be produced by using bioreactors. The cells can be produced for both allografts and allogeneic transplantation. As per WHO, FDA, all the clinical grade stem cells or differentiated cells must be produced as per GMP compliance and guidelines. GMP compliance manufacturing facility needs a huge

investment, and biotechnology companies and hospitals need to create separate GMP facilities. The advantage of GMP facility allows you to produce millions of cells in the bioreactor which are needed for the cell transplantation. The bioreactors are capable of supporting industrial-scale, ultra-high-density cell suspension cultures with controlled microenvironments, standardization, and uniformity of culture conditions in order to generate homogenous populations of stem or lineage-specific cells. There are different types of bioreactors such as Rocking bed (wave motion) (Shekaran et al. 2016) which has size (1–500 L), has advantages—versatile single-use bags, and has limitations—limited scale-up potential for hMSCs. Stirred tank (Surrao et al. 2016; Gasperini et al. 2014; Lawson et al. 2017; Markert and Joeris 2017) has size (100 mL–1000 L), can produce large volumes with limitation of cell death due to force and may be useful for hMSCs, hASCs, hiPSCs, and murine ovary cell production. In the case of rotating wall vessel (Varley et al. 2017), which has size (100 mL–10 L) with advantages of low turbulence and can simulate microgravity but has produced less than 10 L of cells with hMSCs. In case of perfusion bioreactor (Nguyen et al. 2016a, b; Ball et al. 2016) has size (100 mL–5 L) and has advantage of limited turbulence and can be automated with limitations that may affect cells due to large force and useful for hMSCs production. In case of isolation/expansion automated systems (Mock et al. 2016; Priesner et al. 2016) have size (100 mL), and it has many advantages such as versatile single-use bags, automated cell isolation, manipulation, and expansion, GMP-compliant. But it can only produce 100 mL of human lymphocytes.

In addition to non-anchoring or attached cells, bioreactor can also be used to culture adherence or anchored cells. There are different types of cells which can only be grown in adhesion form, not in the suspension form. It's challenging to grow the anchored cells in the bioreactors and it's now possible to grow anchored cells in the bioreactors by using hollow fibers in perfusion systems, encapsulation, or microspheres. The attachment of anchored cells with this methods increase surface area of suspension bioreactor. In addition, packed bed bioreactors can also be used to grow mesenchymal stem cells. Moreover, there are few studies which have shown that adherent cells such as bone marrow derived mesenchymal stem cells can be successfully cultured on protein coated microspheres (Stephenson and Grayson 2018). It has been reported that cells which are grown on these microspheres retain their morphological and functional properties. By using this approach, it's possible to produce the cells in large volume (1000–2000 L) of cells by using Mobius (EMD Millipore) stirred tank bioreactor. This bioreactor is commercially available in sizes ranging from 50 to 2000 L. There is an issue with this method, as high speed is required for such bioreactor which may cause the stem cells differentiation into different cell types and this problem can be tackled by encapsulating them into microspheres. This type of encapsulation method is commercially expensive method (Stephenson and Grayson 2018).

As per the published data, it has been found that maximum 3 L of mesenchymal stem cells and adipose-derived stem cells can be produced in the bioreactors (Stephenson and Grayson 2018). In another report, it has been found that more than 50 L of stem cells can be produced in the bioreactor with some modifications.

Another advantage of this method is that stem cells don't lose their stem cell-like characteristics as these cells retained pluripotency and showing the stem phenotypic markers (CD44 and CD90), when compared with cells cultured under traditional conditions. But, this method has some disadvantage as this method requires certain growth factors and animal serum which is clinically not accepted as per the FDA guidelines.

Another strategy for better production of clinical grade stem cells is to produce them in the self-assembled aggregate forms. It has been suggested that cellular aggregates showed better and improved survivability and tissue forming capabilities (Stephenson and Grayson 2018). The impact of bioreactors on the mesenchymal stem cell aggregation and cell size has been examined by using commercially available WAVE Bioreactors™, and this bioreactor provides better stirring capabilities.

5.6.1 Induced Pluripotent Stem Cell Expansion

Over past few years, different types of bioreactors have been designed and constructed for producing embryonic and induced pluripotent stem cells such as in rotating flasks bioreactors, rotating wall bioreactors, stirred tank bioreactors, and WAVE Bioreactors™ (Wang et al. 2014; Kropp et al. 2017). It has been found that cell method with aggregation approach is considered to be the best method to generate natural pluripotent stem cells with natural characteristics. While producing pluripotent stem cells, care should be taken to avoid differentiation of pluripotent stem cells into different terminally differentiated cells. The differentiation of pluripotent stem cells can be avoided by providing them with specialized media and growth factors with proper monitoring and supervision. The stem cell aggregate size can be maintained to avoid differentiation. It has been found that stem cell aggregate sizes are regulated by chemically using Rho-kinase inhibitors and mechanically by using physical disruption techniques. Furthermore, the dissolved oxygen concentrations and dilution rate in the cell mixture also effect the stem cell (Abecasis et al. 2017).

5.7 Future Prospects in the Advances of Bioreactors

One of the limitations in the currently used cell culture is 2-dimensional form of cell production, which is not closely related to natural process of stem cells development. In fact all the body cells and tissues are grown in 3-dimensional form and cells grown in 3-dimensional form are physiologically and functionally better than 2-dimensional form. Hence, recently efforts are made to design bioreactors in such way that stem cells can be grown 3-dimensionally. Three-dimensional bioreactors support the production of different cells with applications. The 3-dimensional approach can be used to produce large number of cells and tissues. One of the challenges of the 2-dimensional approach, is that due to large size, it's difficult to

provide the nutrients and growth factors to all parts of the cells, whereas in case of 3-dimensional approach cells receive nutrients and growth factors and required amount of oxygen as all the sides are exposed to them. Efforts are also being made to design bioreactors to enhance the functionality of the cells by incorporating them with biomimetic physiological stimuli and sensors in the cell construct. These sensors help to monitor the health, growth, and viability of the cells on time-scale. The use of computer and artificial intelligence based approach also helps engineers to design automatic bioreactors with better efficiency and productivity of functional cells and tissues (Stoppel et al. 2016; Luciani et al. 2016; Dikina et al. 2017; Guo et al. 2016; Mellor et al. 2017). The application of computational modeling found to be effective in improving the predictability of the clinical grade cell product (Shakeel et al. 2013; Guyot et al. 2016; Nguyen et al. 2016a, b).

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