TISSUE ENGINEERING BIOREACTORS: AN OVERVIEW ON POTENTIAL APPLICATION AND BENEFITS OF SCALE UP STRATEGY

ABSTRACT

Tissue engineering bioreactors have been used in order to achieve production of artificial tissue, increasing cell proliferation capacity and yield and/or in vitro tissue/disease modelling. Although it is still discussing how to obtain functional and vascular tissue with these bioreactors, preclinical and clinical studies are ongoing. Tissue engineering bioreactors have been used as lab-scale bioreactors until now.Crucial potential application areas can be created by increasing the production capacity and bioprocess efficiency of these bioreactors. In this review, recent technologies such as spinner flask bioreactors, rotating bed/wall bioreactors, hollow fiber membrane bioreactors, perfusion bioreactors and mechanical stimuli bioreactors are briefly presented in terms of their potential applications in medical field especially in the scope of scale-up approaches such as stirred tank reactor, bubble column, air-lift, membrane, packed bed and fluidized bed bioreactors.

Keywords: Bioreactors, tissue engineering, tailor made treatment, organ support systems, modelling in pharmaceutical/biological research.

INTRODUCTION

Cell culture has begun to use in medical sciencesas two dimensional-2D cell culture accompanying with many disadvantages such as not mimicking the *invivo* environment, mass transfer, gas exchange, waste management, inability to real time monitoring of the culture medium, harvesting the cells by enzymatic methods and eliminating the cell products from the medium during replacement [1,2]. The three-dimensional-3D culture systems have been developed by using bioreactors to eliminate the disadvantages of the static culture.Bioreactor is a device or system that supports a biologically active environment, which designed to grow cells or tissues in the context of cell culture. They are being developed for use in tissue or biochemical/bioprocess engineering.Bioreactors can be used forthe tailor made treatment, the organ support systems, increasing the number of cells before autologous cell implantation, *in vitro* tissue/disease modelling in pharmaceutical research and producing recombinant human proteins, vaccines, drugs and tissue grafts [3,4]. There are several types of bioreactors such as spinner flasks bioreactors, rotating bed/wall bioreactors, perfusion bioreactors, mechanical stimuli bioreactors and hollow fiber membrane bioreactors [5]. In this review, recent bioreactor technologies for tissue engineering briefly presented in terms of their potential applications in medical field especially in the scope of scale-up approaches.

TISSUE ENGINEERING BIOREACTORS

Five types of bioreactors, which can be used for tissue engineering are currently in use and commercially available. These are; a. spinner flask, b. rotating bed/wall bioreactors, c. hollow fiber membrane bioreactors, d. perfusion bioreactors and e. mechanical stimuli bioreactors [6]. Their mechanisms are briefly indicated as follows.

Spinner Flask Bioreactors

Spinner flasks are simple and frequently utilized bioreactor type. In this system; scaffolds are fixed the needles, magnetic bar stirs the medium. Along the seeding, suspended cells into the medium are transferred to the scaffold throughout by convection. In this way, cell seeding performance is increased by 3D seeding medium [7].

Rotating Bed/Wall Bioreactors

The first rotating wall vessel bioreactor has been originally projected by NASA in order to keep stable cell culture research in space. At the same time, this is revealing a potential for culturing cells on

Earth. Rotation rate of the wall allows the centrifugal force, hydrodynamic drag force and gravitational force [8-9].

Hollow Fiber Membrane Bioreactors

Hollow fiber membrane bioreactors are frequently utilized for culturing highly metabolic and sensitive cells which are needed high mass transfer [10]. Hollow fiber membrane bioreactorshave increased surface for cell attachment. Cells can be seeded inner or outer surface of the fibers. Moreover seeded into a gel in extra capillary space. Hollow fiber membrane bioreactors are utilized for several purposes; creation of engineered tissues and cell population expansion in the field of regenerative medicine, in vitro models for drug testing in pharmaceutical industry [11].

Perfusion Bioreactors

The perfusion bioreactors are based on a continuous flow of a fresh oxygenated medium through the seeded scaffold that is fixed compartment of the bioreactor. These features improve medium flow through the scaffold pores and provide a mechanical stimulus to the cells with optimized shear stress.In this way, cell viability and function are enhanced [12].

Mechanical Stimuli Bioreactors

There are several types of mechanical stimuli bioreactors which are utilize static, dynamic or combined effect. Compression bioreactors are utilized for the development of cartilaginous tissue that are required mechanical stimulus for proliferation. In strain bioreactors the force applied to the construct is a tensile force instead of a compressive one. These systems are utilized for tendons and ligaments engineering [13].

SCALE-UP STRATEGY AND POTENTIAL APPLICATION FIELDS

Tissue engineering bioreactors are lab-scale bioreactors based on tissue production or modeling. In addition to the works carried out to achieve the goal of tissue production, other outcomes of these systems were also benefited. Scale-up approaches and techniques are coming with problems to overcome. These problems are also parameters that need to be optimized such as operating time, production efficiency and capacity, temperature, pH, oxygenation, continuous monitoring, mass transfer, gas exchange, obtaining products and control of secondary processes.If repeatable and reproducible systems are obtained by optimizing scale-up conditions, potential applications of bioreactors in the medical field can be better succeded [5-13].Potential applications of bioreactors in the medical field can be listed as; i. taylor made treatment, ii. in vitro tissue/disease modelling in pharmaceutical/biological research, iii. producing recombinant human proteins, vaccines, drugs and tissue grafts [4,14-17].

Table 1. Application fields, types and examples of bioreactors in medical fields [14-40].

Applications Fields of Bioreactors	**Types of Application**	**Application Examples**
The tailor made treatment	Organ support systems	Bioartificial kidney system [4] Bioartificial liver support system[18]
	Increasing the number of cells before autologous cell implantation	Chondrocyte [19] Hepatocyte [20] Stem cell [21] Platelet rich plasma [22]
Modelling in pharmaceutical/biological researches	*In vitro* tissue modelling	Bone tissue[23] Cornealtissue [24] Skeletal muscle [25] Vascular smooth muscle tissue[26]
	Disease modelling	Modelling fibrosis[27] Modelling colon cancer [28] Modelling acute liver failure [29] Modelling chronic obstructive pulmonary disease [30]
Human medicinal products	Vaccines	Viral vaccine production (H1N1) [17]

bioprocess		Monoclonal antibodies [31]
	Recombinant human proteins	Recombinant human serum albumin [32] Recombinant human insulin[33]
	Drugs	Antibiotics (phenoxymethylpenicillin)[34] Citric acid[35] Pyruvic acid[36] α-Cyclodextrin[37]
	Tissue grafts	Vascular tissue graft [38] Osteochondral graft [39] Bone graft [40]

The Tailor Made Treatment

Conventional treatment methods include generalized protocols based on common indications. On the other hand in some clinical scenarios, patients' individual feature and medical charts of patients may vary from patient to patient. The tailor made treatment with bioreactors can be achieved as application of organ support systems and increasing the number of cells before autologous cell implantation. Some organs have synthesis, filtration, metabolization and detoxification function such as kidney and liver. In this point, the extracorporeal organ supporting systems is beneficial, especially on the cell based therapy for example; stem cell, platelet rich plasma, autologous cell implantation, etc., cell proliferation capacity and harvested cell number differ with patient to patient by cell origin, age and gender. Because of these individual changes, the tailor made treatment has been gained importance [14,15].

Modelling in Pharmaceutical Research

Animal studies and their outcomes are naturally piece of development of therapeutic systems. However, there are some ethical concern come from 3R approach. In this point *in vitro* tissue, disease and physiological system modelling are preferable because of saving animals and also avoid consuming time, budget and working power. Scientists have been still working on tissue modelling such as cardiac, liver, breast and bone tissue modelling in order to work targetted organ. On the other way, there are some studies as to disease modelling such as bone fracture, damaged tissue, cancer tissue in order to work disease based therapeutical agents [16]. Multicellular spheroid, hollow fiber and multicellular layer are utilized for modelling pharmaceutical research such as understanding cytotoxicity, drug metabolism and pharmacokinetics [4].

Producing Human Medicinal Products

The batch processing is conventionally utilized in order to gather human medicinal products such as vaccine, drug and recombinant proteins. In this point, there are some concerns about Good Manufacturing Practices (GMP) requirements, yield performance, process management requirements, monitoring, which also must be evaluated in a standardized manner to ensure quality control. Some critical parameters such as surface marker analysis, proteomics, functional assays, and sterility testing can be used toensure the quality control [15,41,42]. Although the mentioned concerns, the bioreactors seem to be a good a solution with acoustic settlers, hollow fiber bioreactors and hollow fiber based perfusion systems including tangential flow filtration or alternating tangential flow technologies [17].

CONCLUSION

The critical advances concerning the generation of bioengineered tissues and complex organs reported in the earlier studies highlight the multifaceted role of bioreactors technologies. In a future perspective, the biomimetic and tightly controlled microenvironment provided by these systems, appears to be essential for the creation of bioengineered grafts with optimal morphology and function. In addition, the development of bioscaffolds generated through perfusion decellularization techniques provides a potential solution towards the bioengineering of whole parenchymal organs with a highly organized vascular network. Several concerns still remain for clinical approaches. Particularly for protocol standardization, the choice of appropriate cell lines for clinical use, the set-up of the whole procedure according to GMP and the establishment of optimal recipient's selection criteria are important issues [42,43]. Additionally, scale-up approaches and techniques are coming with problems to overcome. These problems are also parameters that need to be optimized such as operating time, production efficiency and capacity, temperature, pH, oxygenation, continuous monitoring, mass transfer, gas exchange, obtaining products and control of secondary processes. If repeatable and reproducible systems are obtained by optimizing scale-up conditions, potential applications of

bioreactors in the medical field can be better succeded. From the perspective of the future, it is anticipated by the related studies that while the developments in bioreactor systems continue, there will be significant developments regarding the use of plants as bioreactors in drug development [44-45].

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