



ADVANCED BIOREACTOR SYSTEM FOR THE IMPLANTABLE BIOMATERIALS TESTING AND TISSUE ENGINEERING APPLICATIONS

Mohd Ramdan¹ and Irza Sukmana²

¹MediTeg Research Group, Faculty of Biosciences and Biomedical Engineering, Universiti Teknologi Malaysia, Johor Bahru, Malaysia

²Department of Mechanical Engineering, Faculty of Engineering, University of Lampung, Bandar Lampung, Indonesia

E-Mail: irza.sukmana@eng.unila.ac.id

ABSTRACT

Tissue engineering scientists believe that the next generation of functional tissue and artificial organ replacements truly need the use of advanced bioreactor system. Bioreactor system, in which the culture conditions can be adjusted and studied, will support the development of tissues with optimal mechanical, chemical, and biological stimuli for a given application. Although there have been various types of bioreactors designed and tested for several implantable biomaterials and tissue engineering applications, the development of a complete artificial organ remains a dream. This review addresses recent advances and future challenges in designing and using advanced bioreactor system to support the mass production of vascularized engineering tissues and artificial organ. The potential application of micro-electromechanical system (MEMS) bioreactor technology for future advancement in tissue engineering is also highlighted.

Keywords: bioreactor, tissue vascularization, scaffold construct, micro-electromechanical system.

INTRODUCTION

Bioreactors have been used for many years in areas other than tissue engineering like fermentation, water treatment, food processing and pharmaceutical industry. In these bioreactors, biological and biochemical processes are taking place under a closely monitored and tightly controlled environment [1]. In tissue engineering research, bioreactors have been used for implantable biomaterial testing and to culture a larger cell population since 1980s [1]. Then, in 1990s the research on more advanced bioreactor for the combination of cells with biocompatible extracellular matrix (ECM) and scaffold for tissue replacement have begun [2].

Tissue engineering scientists acknowledged that bioreactor system may improve the cell culture stability and function of 3D engineered tissue construct [2, 3]. Furthermore, bioreactors may accelerate the development, evaluation, and delivery of engineered tissue products to patients or in clinical research [3]. Numerous types of bioreactors for tissue engineering applications that enable the production of tissue engineered either soft (e.g., skin, blood vessel, and cardiac) or hard tissues (e.g., bone, bladder, liver, and cartilage) as well as artificial organ within a specifically controlled biological and chemical environment [4, 5]. Each type of bioreactor has been designed differently depends on application involved. Further advancement in the field of tissue engineering is the using of Micro-Electro-Mechanical System (MEMS) became increasingly prevalent and found widespread use in producing mini and micro-scale reactor system [6, 7].

This paper will report status of the current research and development of bioreactor system as an advanced support system in tissue engineering. It is necessary to improve our understanding of the mechanisms behind bioreactor and its application. Additionally, other aspects including the challenges in

optimized and controlled the culture parameter using MEMS technology will also be highlighted.

BIOREACTOR DESIGN AND CONSIDERATION

Tissue engineering aims to combine the principles and methods of engineering and life science into the development of artificial organs or biological substitutes for the restoration, maintaining or improvement of human tissue and organ functions. One of the major challenges in designing tissue engineering bioreactor system is to translate the lab-bench-scale engineering tissues product with into large-scale production of functional tissues or artificial organs that are reproducible and economic [2,8]. A bioreactor is should also designed to provide the real cellular microenvironment mimicking in body environment in order to facilitate construct uniformity and overall viability [9, 10]. These requirements render the design and specification of tissue engineering bioreactor system [11].

In general, there are two main aspects in designing bioreactor for biomaterial and tissue engineering application; they are biological and mechanical aspects. Biological aspects are purposely control the growth of specific cell/tissues include accurate culture conditions (i.e., aseptic operation) and improved mass transfer (i.e., nutrient supply and waste elimination) [4, 8]. Mechanical aspects are purposely to control the environment of in vitro system included increased cell-seeding efficiency, appropriate physiological stimulation (e.g., mechanical, electrical, chemical and biological), design material, and monitoring cell/tissue growth techniques [12].

Culture parameters such as temperature, pH, pressure, oxygen level and nutrients must be carefully controlled in vitro in accordance with the best conditions in the body [12, 13]. Some in vitro bioreactors have achieved to undertake these parameters, but facing problems on maintaining and optimizing culture



conditions [13]. Gas exchanger and heat exchanger are needed to be utilized in vitro for controlling pH, temperature and oxygen (O₂) concentration [10, 13]. Waste elimination also must be controlled and monitoring properly because it will effect on enzyme activities and defect the tissue growth [14]. Cell seeding method is also a crucial issue related to utilizing cells from limited human cell sources. Cells can be seeded either in a separate operation with bioreactor (static seeding) or directly seed onto 3D scaffold in the bioreactor (dynamic seeding) [14, 15]. Dynamic seeding provides more advantages technique compares to conventional static method; include higher attachment efficiencies and more homogeneous cell distribution [11, 16].

Related to mechanical aspect of the reactor, some studies found that mechanical stimulation (e.g., mechanical compression, hydrodynamic pressure, fluid flow) may affect cellular morphology and function, as well as improve mechanical properties of engineered tissue construct [16]. Mechanical stimulation is also play a role on extracellular matrix (ECM), such as increases the mechanical stiffness of ECM in short culture time [17]. Furthermore, electrical stimulation, such as pulsatile electrical current may also affect cells behavior and phenotype differentiation [18].

TYPES AND APPLICATION OF BIOREACTORS

There are numerous types of bioreactor systems that are designed and used in various tissue engineering applications. Each type of bioreactors is developed based on the specific requirements of tissue that be constructed. Spinner flasks and rotating wall are two types of first generation of bioreactor system that widely utilized in biomaterial and tissue engineering field [19]. Spinner flask or stirred flask bioreactors have been used for seeding of cells into 3D scaffolds by fluid convection and are effective at creating a homogenous media solution on the exterior of the scaffold [19, 20].

The advantages of this bioreactor are improve the efficiency of cell seeding and viability and reduce the external mass transfer limitation [21]. However, the turbulent flow and higher shear stress produce by spinner flask and rotation wall reactor may cause cell damage and defect of tissue construct then resulting an irregular tissue surface [21].

Therefore, Wavy-wall bioreactor (WWB) recently has been design as the modification of spinner flask bioreactor to enhance axial mixing at lower shear stress rate and introduce smooth waves that mimic baffles. The low-shear hydrodynamic environment produce by WWB will enhance ECM development and cell proliferation compared with spinner flask [22].

Rotating wall vessel (RWV) bioreactors are another simple system used to enhance media mixing and been used for generate 3D growth of cartilage [20] and bone [23]. This culture system consists of two concentric cylinders with culture media and scaffolds filling the space between. RWV creates microgravity environment for cells grown by adjusting the vessel rotation speed at the rate that balance the scaffold in the medium [23]. The dynamic

flow generated by rotating fluid will expose scaffolds and cells in low shear stress environment and high mass transfer rate [24]. RWV designed by the National Aeronautics and Space Administration (NASA) and there are three derivative systems including the rotating wall perfused vessel (RWPV), slow lateral turning vessel (SLTV) and high aspect ratio vessel (HARV) as described in different literatures [24,25]. Most studies stated that both of spinner flask and rotating wall bioreactors do not effectively perfuse media into a scaffold [20, 26]. This limitation can be solved by using perfusion system which is use a pump system to perfuse media continuously through the porous scaffold [26].

The basic design of the perfusion bioreactors consists of a media reservoir, a pump, a tubing circuit and a culture chamber. The main characteristic of perfusion system is the medium flow continuously through a bio-chamber consists of a cell-biomaterial construct to provide microcirculation. The microcirculation produce by perfusion system beneficial in long term culture period in order to achieve tissue mass and can minimized the risk of contamination since the medium changed automatically. Most literatures stated that perfusion system be able to simulate in vivo conditions with continuously deliver nutrients as well as waste removal and expose the cells to physiological stimuli [17,27]. Perfusion system also have been shown can be used to enhance nutrient diffusion while mechanically stimulating cells to increase matrix production [28].

Dynamic culture using a flow perfusion bioreactor improved core cell activity and density compared to static culture [28]. There are various perfusion bioreactors have been designed for various tissue engineering applications including the radial flow, parallel plate, direct perfusion, microfluidic, rotating shaft, and pulsatile flow bioreactors [28-31]. Each kind of these bioreactors have their own design, either different on entire part or only some part (e.g., culture chamber and type of scaffold), but the basic concept is same for all bioreactors (i.e., providing efficient and uniform distribution of media flow and mass transfer) [30, 31].

Most of these bioreactors perfuse the media through the porous scaffolds excluding the hollow fibre and microfluidic bioreactors which have be designed with different cell proliferation and differentiation support [31,32]. Microfluidic bioreactors are the novel and advances technology in fabrication of micro- and nano-system in order to culture cells in tissue engineering application [33-35].

MEMS TECHNOLOGY IN BIOREACTOR

Micro-electro-mechanical system (MEMS) is a new technology that is utilized in almost all research fields' applications as well as in biological and biomedical applications. "Bio-MEMS" or biological micro-electromechanical systems play an important role in tissue engineering applications, as they provide new methods as well as produce powerful components to reduce the complex tissue engineering issues [36]. Tissue engineered noticed that this advanced technique offers unique



advantages than conventional methods including improved biological function, better quality cell-based data, decrease reagents consumption, simplicity, large scale integration and lower cost [36, 37].

Microfluidic devices or micro-bioreactor has applied for tissue engineered and this technique for cell culturing to study the biological behavior of cells (from single cells to multi-cells levels) [38]. This technique has been utilized to control of the accurate cell environment (i.e., chemical and physical environment) [33, 37], formation of perfuse able networks and study of minimally functional modules of complex tissue (e.g., liver, kidney and lung) [39]. Due to similar scale with the dimensions of most cells, microfluidic device has the potential to improve the creation of the in vivo microenvironment for cells culture and tissues growth [40]. Micro-system has the necessary tools to recreate in vivo like micro-environment and provide advanced approaches in controlling the factors

that affect the cells behaviors such as mechanical forces (e.g., shear stress, bubbles and cell deformation) and transport phenomena (e.g., type of flow, diffusion, surface area to volume ratio and effective culture volume) [38, 41].

The design of this micro-system needs more expertise to fulfil the requirements of the cells culturing system. Although the basic design is simple and easy to build, however, without an appropriate technique it will impact on the viability and performance of this micro-system. Similar to macro-systems, the design of micro-system needs to consider a few aspects include material selection, physiological factors (mechanical and chemical), fluidic, sensing and control element without requiring a cell culture incubators [41, 42]. Complete resume of the bioreactor systems and their applications in biomaterial and tissue engineering research is presented in Table-1.

Table-1. Advanced bioreactor system and their application in tissue engineering.

No.	Types of bioreactors	Applications	Novel findings	References
1.	Spinner Flask	Improve cell seeding into 3D scaffolds by fluid convection-Bone and cartilage generator.	Wavy-wall with modification of spinner flask-produce lower shear stress rate-enhance axial mixing and ECM development-produce more robust cartilage construct	[21,21]
2.	Rotating Wall	Creates microgravity environment and enhance media mixing -Generation of cartilage, bone and liver tissues.	Derivative systems- rotating wall perfused vessel (RWPV), slow lateral turning system (SLTV) and high aspect ratio vessel (HARV)	[23,25]
3.	Radial flow	Perfused the medium radially through the vertical porous scaffold.	cell seeding and growth with a proportional cell distribution throughout the entire scaffold.	[16,28]
4.	Direct perfusion	Directly perfused the medium through the pores of the scaffold.	Effects the proliferation and differentiation osteoblast cells	[26,27]
5.	Microfluidic	Diffusion of the nutrients and waste products across micron distances.	Long term of culture of hepatocytes, hematopoietic cells and osteoblast - advanced technique to produce microenvironment for cell culturing	[29, 32, 33, 42]
6.	Rotating shaft	Perfused type system for growing cartilage tissue by rotates the shaft.	Efficient oxygen and nutrient transfer and lower shear stress that is exerted the construct	[30]
7.	Hollow fiber	Hollow membrane-based growth systems (i.e., membrane hollow fibers and membrane capillary tubes.	For 3D high cell density culture - bone tissue growth - transplantation of human bone marrow stromal cells (BMSCs).	[31,34]
8.	Pulsatile flow	Produce desire pulsatile pressure similarly physiological pulse rate	Tissue engineering of cardiovascular such as heart valve and blood vessel	[13,35]

CONCLUSIONS

Basic bioreactor system used to facilitate testing for implantable biomaterials and engineering tissue construct in vitro. Advanced tissue engineering bioreactor system aims to mimic and reproduce physiological conditions in order to maintain and support higher cell

number for tissue generation. The ultimate advantage of bioreactor systems is their ability to produce reproducible culture conditions that enhance cell proliferation and differentiation with minimum handling of scaffold and human support. Some techniques and approaches of tissue engineering bioreactors have been designed and tested in



order to develop functionalized tissue construct and artificial organs. A new development technology is needed to support more precise and better scalable reactor when compare to the previous design. Biomedical engineers, natural science experts as well as medical doctors are challenged to have more collaborative effort in designing advanced bioreactor system for the production of complex engineering tissues and artificial organs.

ACKNOWLEDGEMENTS

Financial support from The Minister of Research, Technology and Higher Education (Kemenristekdikti) of Indonesia under Hibah Penelitian Produk Terapan (PPT) Y2017 for author (Irza Sukmana) is acknowledged. Mohd Randan is supported by UTM Tier-1 (vote# 03H12), MOHE-FRGS (vote# 4F128) and UTM RU (vote# 00G670).

REFERENCES

- [1] Freshney, R.I., *et al.*, Chapter Twelve - Principles of tissue culture and bioreactor design, in Principles of Tissue Engineering (Third Edition), L. Robert, *et al.*, Editors. 2007, Academic Press: Burlington. p. 155-183.
- [2] Martin, I., D. Wendt and M. Heberer, The role of bioreactors in tissue engineering. Trends in Biotechnology, 2004. 22(2): p. 80-86.
- [3] Wendt, D., *et al.*, Bioreactors in tissue engineering: scientific challenges and clinical perspectives. Advances in Biochemical Engineering/Biotechnology, 2009. 112: p. 1-27.
- [4] Freed, L.E., *et al.*, Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. Tissue Engineering, 2006. 12(12): p. 3285-3305.
- [5] Chen, H.-C. and Y.-C. Hu, Bioreactors for tissue engineering. Biotechnology Letters, 2006. 28(18): p. 1415-1423.
- [6] Figallo, E., *et al.*, Micro-bioreactor array for controlling cellular microenvironments. Lab on a Chip, 2007. 7(6): p. 710-719.
- [7] Bashir, R., BioMEMS: state-of-the-art in detection, opportunities and prospects. Advanced Drug Delivery Reviews, 2004. 56(11): p. 1565-1586.
- [8] Freed, L.E., *et al.*, Advanced Material Strategies for Tissue Engineering Scaffolds. Advanced Materials, 2009. 21(32-33): p. 3410-3418.
- [9] Couet, F. and D. Mantovani, A New Bioreactor Adapts to Materials State and Builds a Growth Model for Vascular Tissue Engineering. Artificial Organs, 2012. 36(4): p. 438-445.
- [10] Zhang, Z.-X., *et al.*, Design of a novel bioreactor and application in vascular tissue engineering. Applied Surface Science, 2008. 255(2): p. 541-544.
- [11] Hutmacher, D.W. and H. Singh, Computational fluid dynamics for improved bioreactor design and 3D culture. Trends in Biotechnology, 2008. 26(4): p. 166-172.
- [12] Martin, Y. and P. Vermette, Bioreactors for tissue mass culture: Design, characterization, and recent advances. Biomaterials, 2005. 26(35): p. 7481-7503.
- [13] Chouinard, J.A., *et al.*, Design and validation of a pulsatile perfusion bioreactor for 3D high cell density cultures. Biotechnology and Bioengineering, 2009. 104(6): p. 1215-1223.
- [14] Hsu, S.-h., *et al.*, The effect of dynamic culture conditions on endothelial cell seeding and retention on small diameter polyurethane vascular grafts. Medical Engineering & Physics, 2005. 27(3): p. 267-27.
- [15] Sukmana, I., Microvascular guidance: a challenge to support the development of vascularised tissue engineering construct. The Scientific World Journal, 2012. 2012: p. 201352-201352.
- [16] Shkilnyy, A., *et al.*, Bioreactor controlled by PI algorithm and operated with a perfusion chamber to support endothelial cell survival and proliferation. Biotechnology and Bioengineering, 2012. 109(5): p. 1305-1313.
- [17] Mathes, S.H., *et al.*, A bioreactor test system to mimic the biological and mechanical environment of oral soft tissues and to evaluate substitutes for connective tissue grafts. Biotechnology and Bioengineering, 2010. 107(6): p. 1029-1039.
- [18] Wiesmann, H.-P., *et al.*, Electrical stimulation influences mineral formation of osteoblast-like cells in vitro. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 2001. 1538(1): p. 28-37.
- [19] Ratcliffe, A. and L.E. Niklason, Bioreactors and Bioprocessing for Tissue Engineering. Annals of the



- New York Academy of Sciences, 2002. 961(1): p. 210-215.
- [20] Yeatts, A.B. and J.P. Fisher, Bone tissue engineering bioreactors: Dynamic culture and the influence of shear stress. *Bone*, 2011. 48(2): p. 171-181.
- [21] Bueno, E.M., *et al.*, Increased rate of chondrocyte aggregation in a wavy-walled bioreactor. *Biotechnology and Bioengineering*, 2004. 88(6): p. 767-777.
- [22] Bilgen, B., *et al.*, Flow characterization of a wavy-walled bioreactor for cartilage tissue engineering. *Biotechnology and Bioengineering*, 2006. 95(6): p. 1009-1022.
- [23] Takebe, T., *et al.*, Human Elastic Cartilage Engineering from Cartilage Progenitor Cells Using Rotating Wall Vessel Bioreactor. *Transplantation proceedings*, 2012. 44(4): p. 1158-1161.
- [24] Plunkett, N. and F.J. O'Brien, Bioreactors in tissue engineering. *Technology and Health Care*, 2011. 19(1): p. 55-69.
- [25] Klement, B.J., *et al.*, Skeletal tissue growth, differentiation and mineralization in the NASA Rotating Wall Vessel. *Bone*, 2004. 34(3): p. 487-498.
- [26] Jaasma, M.J., N.A. Plunkett, and F.J. O'Brien, Design and validation of a dynamic flow perfusion bioreactor for use with compliant tissue engineering scaffolds. *Journal of Biotechnology*, 2008. 133(4): p. 490-496.
- [27] Sailon, A.M., *et al.*, A Novel Flow-Perfusion Bioreactor Supports 3D Dynamic Cell Culture. *Journal of Biomedicine and Biotechnology*, 2009. 2009.
- [28] Kitagawa, T., *et al.*, Three-dimensional cell seeding and growth in radial-flow perfusion bioreactor for in vitro tissue reconstruction. *Biotechnology and Bioengineering*, 2006. 93(5): p. 947-954.
- [29] Ferrell, N., *et al.*, Albumin handling by renal tubular epithelial cells in a microfluidic bioreactor. *Biotechnology and Bioengineering*, 2012. 109(3): p. 797-803.
- [30] Chen, H.-C., *et al.*, A Novel Rotating-Shaft Bioreactor for Two-Phase Cultivation of Tissue-Engineered Cartilage. *Biotechnology Progress*, 2004. 20(6): p. 1802-1809.
- [31] Das, D.B., Multiscale simulation of nutrient transport in hollow fibre membrane bioreactor for growing bone tissue: Sub-cellular scale and beyond. *Chemical Engineering Science*, 2007. 62(13): p. 3627-3639.
- [32] Mehta, K., *et al.*, Quantitative inference of cellular parameters from microfluidic cell culture systems. *Biotechnology and Bioengineering*, 2009. 103(5): p. 966-974.
- [33] Velte-Casquillas, G., *et al.*, Microfluidic tools for cell biological research. *Nano Today*, 2010. 5(1): p. 28-47.
- [34] De Napoli, I.E. and G. Catapano, Perfusion enhances solute transfer into the shell of hollow fiber membrane bioreactors for bone tissue engineering. *The International Journal of Artificial Organs*, 2010. 33(6): p. 381-391.
- [35] Hildebrand, D.K., *et al.*, Design and hydrodynamic evaluation of a novel pulsatile bioreactor for biologically active heart valves. *Annals of biomedical engineering*, 2004. 32(8): p. 1039-1049.
- [36] Puleo, C.M., H.-C. Yeh, and T.-H. Wang, Applications of MEMS technologies in tissue engineering. *Tissue Engineering*, 2007. 13(12): p. 2839-2854.
- [37] Gao, D., *et al.*, Recent developments in microfluidic devices for in vitro cell culture for cell-biology research. *TrAC Trends in Analytical Chemistry*, 2012. 35(0): p. 150-164.
- [38] Sodian, R., *et al.*, Tissue-engineering bioreactors: a new combined cell-seeding and perfusion system for vascular tissue engineering. *Tissue Engineering*, 2002. 8(5): p. 863-870.
- [39] Inamdar, N.K. and J.T. Borenstein, Microfluidic cell culture models for tissue engineering. *Current Opinion in Biotechnology*, 2011. 22(5): p. 681-689.
- [40] Ziolkowska, K., R. Kwapiszewski, and Z. Brzozka, Microfluidic devices as tools for mimicking the in vivo environment. *New Journal of Chemistry*, 2011. 35(5): p. 979-990.
- [41] Walker, G.M., H.C. Zeringue, and D.J. Beebe, Microenvironment design considerations for cellular scale studies. *Lab on a Chip*, 2004. 4(2): p. 91-97.
- [42] Pasirayi, G., *et al.*, Microfluidic bioreactors for cell culturing: A review. *Micro and Nanosystems*, 2011. 3(2): p. 137-160.