Overview Article: Bioreactors Designed for 3D Bioprinted Tissue and Process Parameters

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***Abstract*—In this review article, we would like to define the operation and parameterization of processes in devices called bioreactors. A bioreactor is a device that uses the setting of physical processes to influence biological processes. In tissue engineering and biomedical engineering, bioreactors can be used to aid in the development of new tissue in vitro. By providing and altering biochemical or physical regulatory signals, it is possible to induce a state in which the cells will differentiate or form an extracellular matrix prior to implantation.**

Next, we describe the physical procedures that must be maintained for the proper course of the cell development process in the field of bioreactors. For each cell that is inserted into the bioreactor, there is a predetermined set of parameters that must be observed. In this article, we will focus on the general parameters of bioreactors.

***Index Terms*—bioreactor, nutrient medium, biological processes, tissue, parameters**

I. Introduction

By standard definition, a bioreactor is a vessel in which a biological reaction or change takes place, by which we can also understand, for example, cell division [1]. The test structure for the bioreactor can be microorganisms, animal or plant cells or various tissue types. Before each process for which the bioreactor will be used, it is necessary to first know the behavior of the cell. There are a few parameters we need to know before using the bioreactor. The first parameter is the purpose of use, for example, fermentation, respiration or cell division [2]. We will be interested in cell division. In general, we divide bioreactors according to other basic parameters:

- by phase (liquid only, or also gas and particles),
- according to technical design (open, closed, with membrane),
- according to the method of mixing (mixed, unmixed, hydraulically mixed),
- according to the method of cultivation - supplementation of nutrients (for one filling, with gradual addition of nutrients) [3].

Other essential features include the method of culturing or sterilizing the bioreactor. The basic parameters we will discuss in Chapter III are the parameters of the bioreactor, which are often the common denominator of each process and metabolism. These include oxygen transfer, mixing, temperature stabilization, operational stability and reliability and, last but not least, not only the purchase costs but also the operation of the bioreactor [4]. The selected bioreactor should have an optimized operating mode with the most efficient and accurate parameter settings.

II. Background and Related Work

The development of tissue engineering provides rare outputs in the form of biological replacements for living organisms. One of them is the using of the Bioreactor as a tool for biological replacements. A bioreactor can be defined as a system in which biological reactions such as aerobic or anerobic cell culture are performed. The main function of bioreactors is the ability to create suitable conditions for the growth of the biological system in the bioreactor. The main function of bioreactors is the ability to create suitable conditions for the growth of the biological system in the bioreactor. This can be achieved by controlling the temperature, pH, rate of passage of the nutrient fluids, oxygen supply or CO_2 [5].

Tissue engineering studies that use the culture of living systems with bioreactors can be divided into stem cell culture research, cultured cell and tissue research for the analysis of physiological processes, or research on the effect of bacteria on cell and tissue culture [6]. The study of tissue engineering describes also new technology and applications for bioreactors. One of them is the study of a new bioreactor where they

investigated the increase in viability and function of cultured cells and tissues [7]. Three cell / tissue populations were examined: heparocellular carcinoma cells, human stem cells, and liver tissue. It has been shown that a new bioreactor has improved the structure and basic metabolic functions of heparocellular carcinoma cells. Hepatic tissue analysis showed better maintenance of cell viability.

Another study in the field of bioreactors is the cultivation of human mesenchynal stem cells in static cultures and suspension bioreactors [8]. The aim of the study was to elucidate the advantages of this technique in terms of productivity of cell formation and the ability to generate aggregates. It was found that the deposition of stem cells in suspension bioreactors created a more uniform population in smaller aggregates for 8 days. The suspension bioreactor is also used to differentiate human mesenchymal stem cells into smooth muscle cells [9].

Bioreactors are also used in the treatment of the heart, where study [10] describes the printing of pluripotent stem cells derived from cardiomyocytes. The authors of the study developed a new method of stem cell delivery using 3D bioprinted heart patches without biomaterials. The extruded patches in the bioreactor chamber showed action potential conduction as in the nervous system as well as uniform electrical conduction throughout the length of the patch. The subsequent implementation of these extruded patches into the native rat myocardium represents a significant advance in the treatment of stem cell heart failure.

There are also studies where the bioreactor plays a role as a tool for further analysis as in study [11] where a new bioreactor for combined magnetic resonance spectroscopy and optical representation of metabolism in 3D cell cultures is described. A similar use of the bioreactor in diagnostics is described in a study of the active metabolism of the protein-protein interaction by nuclear magnetic resonance (NMR) [12]. In this case, the bioreactor was used to keep the cells in an active metabolic status. The bioreactor maintained adenosine triphosphate (ATP) levels above 95% for up to 24 hours, allowing further analysis of metabolic processes. Another area of tissue engineering research is the study of the bacterial community for bioreactor tank culture. Study [13] describes this area of tissue engineering research where they investigated the biodegradation of nonylphenol and the effects of microbial populations at low temperatures in a bioreactor. By analyzing the fluorescence hybridization of the microbial population in the biofilm, they found that all the bacteria belonged to the Proteobacteria strain. The proportion of bacteria bound to the measuring probe was 60%, 43% and 24% at $15°C$, $10°C$ and $5.5°C$.

An important tool in the evaluation of bacterial culture in bioreactors is the analysis of 16srDNA. This analysis was also used in a study [14] examining the decolorization of Direct Red 28 with a mixed culture of bacteria in a countercurrent bioreactor. In this case, the bioreactor operated at 2 aeration rate settings, at $0.4\ mmol/min$ and $0.6\ mmol/min$. The flow rate of the bioreactor was set at 60, 90 and 120 ml/h. The study concluded the results that a higher aeration rate of $0.6\ mmol/min$ at a flow rate of 60 ml/h is more suitable for decolorizing Direct Red 28 in a bioreactor.

III. Bioreactor Process and Parameters

In this chapter we will describe a standard bioreactor designed to work with cells. In the specific example in Figure 1, we can see the basic structure of the wave bioreactor.

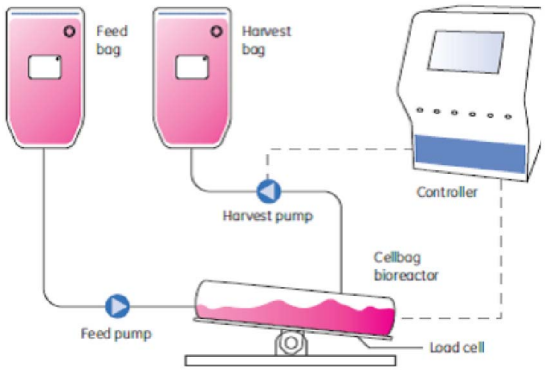

Fig. 1. Wave bioreactor with one filling bag and one collection bag. The bioreactor is checked by a computer, which also collects data from the entire culture. [15]

Depending on the type of cells, it is necessary to set their process of staying in the bioreactor. The basic structure elements as on the wave reactor are repeated for all common types of bioreactors. The bioreactor in Figure 1 is not a closed loop of nutrient medium. A closed case of nutrient medium cycling is often used in human or animal cell research. In the case that it is not a closed loop, the contents of the feed bag are pumped into the harvest bag during the process. The nutrient medium contains an environment suitable for the targeted process. During cultivation, excipients, nutrients, media stabilizers or antibiotics may be added to the feedbag [16]. During the process, it is necessary to observe stable required quantities of a particular bioreactor:

- nutrient medium flow,
- temperature of nutrient medium and chamber,
- carbogen content and gaseous ratio,
- acidity of the solution,
- if movement of the chamber is necessary,
- control of substances in the nutrient medium.

In most cases, the flow of the medium is ensured by peristaltic pumps, which are self-priming and do not come into contact with the medium. They do not contaminate the transfer of nutrition in the system and do not change the structure of the transfer medium. Often the bioreactor system contains multiple peristaltic pumps and peristaltic hoses are used to communicate with the chamber [17]. In closed-loop, continuous-flow reactors, peristaltic pumps include a control that is responsible for the amount of medium that flows through the cell chamber.

The average temperature value in systems containing human or animal cells is the temperature of the organism, i.e. $36 - 37°C$. The heating of the nutrient medium is solved

differently in the bioreactors. The most common solution is a heated pad under the chamber. It is necessary to maintain the reliability of such a system, because if the heating of the chamber fails, the whole experimental process may fail. Many bioreactors use incandescent light to heat the chamber, which ensures a continuous temperature. As with peristaltic pumps, the medium and the heating element do not come into contact. The control system (Controller on Figure 1) takes care of a closed control loop for heating the medium in the bioreactor. The temperature sensors are already in direct contact with the nutrient medium.

To maintain proper nutritional values, it is necessary to measure and control the gas content. The reactor is equipped with probes for measurement of dissolved oxygen and pH. Regulation of both quantities is ensured by the addition of carbogen (95% O_2 and 5% CO_2 [18]) to change the content of gases consumed during the reaction. And by adding a solution that changes the pH in the nutrient medium itself.

As mentioned in Chapter I, a number of bioreactors, mainly for the production of drugs, are mixed. In the case of bioreactors intended for cell culture, the chamber is tilted. The movement of the bioreactor chamber should be applied according to the tissue to be regenerated. For example, external physical stimuli (tension and pressure) with different exposure times improve chondrocyte differentiation and proliferation. However, for blood vessels and nerve cells certain physical stimuli (solution stimulation) should be applied at a constant speed and direction [19].

The last factor we will address is the control, nutrients [20] and substances needed for successful cell culture or proliferation. During processes where the ability of the bioreactor to maintain a friendly environment for cells is used, it is necessary to supplement substances that prevent infections and undesirable conditions. For all controlled processes in the bioreactor, the sensor part must contain such sensors that are calibrated and of high quality throughout the processes. If any of the sensors fails during use, as well as peristaltic pumps or said heating, the whole experiment may be unsuccessful.

IV. Cell Cultivation

Cell culture is directly conditioned by the ability of a cell to multiply and survive. To ensure these two properties, cell adhesion within the cultured cell mass is very important. Each cell must be able to ensure its viability and the subsequent viability of cells which, by their interconnections, form the basis for the subsequent construction of tissue mass, either alone or within a scaffold which gives the cultured product a precise shape. [21] To ensure this process, it is necessary not only to supply nutrients but especially to form and distribute extracellular and intracellular matrix proteins.

The extracellular matrix (ECM) is a non-cellular component that provides differentiation, morphogenesis and homeostasis of tissues. [22] These biomechanical and biochemical stimuli are necessary for building the basic structure of the cellular component.

Spontaneous cell adhesion to the surface of the artificial material under in vitro conditions is also produced by extracellular matrix proteins. Excallular matrix molecules synthesized by the cells themselves are deposited on the surface of the material. The cells use the following basic ECM Molecules to attach to the material: fibronectin, collagen, vitronectin, elastin, osteoponin, tenascin, thrombospodine, and certain types of laminate. [23]

During various physiological processes such as migration, proliferation, differentiation, metabolic activity or viability, we can determine how individual cells will behave under the action of adhesion molecules. [21] Chemical bonds such as Van der waals forces, electrostatic interactions or hydrogen bonds also help us to bind ECM proteins to the surface of the material.

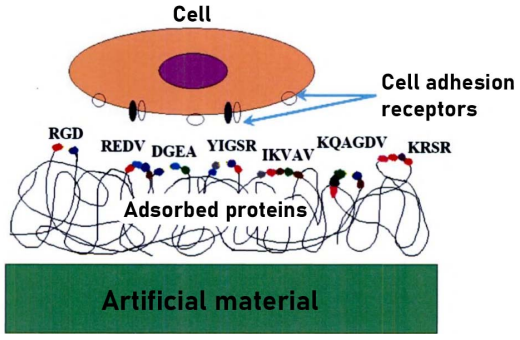

Fig. 2. Cell adhesion to the artificial material via the amino acid sequence of the extracellular matrix [23]

Using adhesion receptors, cells recognize specific amino acid sequences that are important for cell adhesion to EMC molecules that are bound to the scaffold or tissue. Certain amino acid sequences are typical of certain cells.

• The amino acid sequences of Agr-Gly-Asp (RGD), which is found on fibronectin, and Asp-Gly-Glu-Ala (DGEA), which is present on collagen, are not specific for cells of a particular type. [24]

• For endothelial cells, the specific amino acid sequence is Arg-Glu-Asp-Val (REDV), which is also present on fibronectin. [25]

• For smooth muscle and vascular cells, the specific sequences are Lys-Gln-Ala-Gly-Asp-Val ,(KQAGDV) which is present on vitronectin and Ala-Pro-Gly (VAPG) which is present on elastin.

• Neuronal cells are specific sequences Tyr-Ile-Gly-Ser-Arg (YIGSR) and Ile-Lys-Val-Ala-Val which are typical for laminin. [26]

By choosing a correct material to make a scaffold, We can ensure that the cell mass of the selected, cultured tissue adheres properly to the cellular carrier. Subsequently, after the natural degradation of the scaffold, we are left with cell mass or tissue which can then be used for medical purposes. The choice of scaffold for the tissue construct will also impact on mass transfer. The thickness of some "artificial" substrates hinders mass transfer, and pore sizes may not reflect in vivo

tissue organisation. Decellularised tissues may offer a better scaffold environment [27].

V. Conclusion

In this work we present the current state of bioreactor development in the world. We consider the topic of human and animal cell culture to be the future of tissue research and development. Bioreactors will play an indispensable role in this process. The problematic part nowadays is the application of 3D printed body parts. Immediate tissue transplantation is not possible after successful printing. The cells of the extrudate must undergo successful proliferation. After a successful process of cell proliferation transplantation becomes real.

We plan to design and implement a real bioreactor with 3D printed parts in the near future. At present, in a state where the world is threatened by a pandemic, the question of the development and research of bio-printing and subsequent transplantation of artificial organs is an acute topic. Therefore, this overview should serve as a springboard for gaining knowledge in the field of bioreactor operation.

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References

[1] Jian-Jiang Zhong. Recent advances in bioreactor engineering. *Korean Journal of Chemical Engineering*, 27(4):1035–1041, 2010.

[2] Georgia Antonopoulou, Maria Alexandropoulou, Chris Lytras, and Gerasimos Lyberatos. Modeling of anaerobic digestion of food industry wastes in different bioreactor types. *Waste and Biomass Valorization*, 6(3):335–341, 2015.

[3] Klaas Van't Riet and Johannes Tramper. *Basic bioreactor design*. CRC press, 1991.

[4] CH Benson, Morton A Barlaz, DT Lane, and JM Rawe. Practice review of five bioreactor/recirculation landfills. *Waste Management*, 27(1):13–29, 2007.

[5] Zulfiqar Ali Raza, Muhammad Rizwan Tariq, Muhammad Irfan Majeed, and Ibrahim M Banat. Recent developments in bioreactor scale production of bacterial polyhydroxyalkanoates. *Bioprocess and biosystems engineering*, 42(6):901–919, 2019.

[6] Robert M Nerem. Cellular engineering. *Annals of biomedical engineering*, 19(5):529–545, 1991.

[7] HW Hoyle, LA Smith, RJ Williams, and SA Przyborski. Applications of novel bioreactor technology to enhance the viability and function of cultured cells and tissues. *Interface Focus*, 10(2):20190090, 2020.

[8] Leah M Allen, John Matyas, Mark Ungrin, David A Hart, and Arindom Sen. Serum-free culture of human mesenchymal stem cell aggregates in suspension bioreactors for tissue engineering applications. *Stem cells international*, 2019, 2019.

[9] Chris Slavin. Stirred suspension bioreactor differentiation of human mesenchymal stem cells into smooth muscle cells. *Stem cells international*, 2020.

[10] Chin Siang Ong, Takuma Fukunishi, Huaitao Zhang, Chen Yu Huang, Andrew Nashed, Adriana Blazeski, Deborah DiSilvestre, Luca Vricella, John Conte, Leslie Tung, et al. Biomaterial-free three-dimensional bioprinting of cardiac tissue using human induced pluripotent stem cell derived cardiomyocytes. *Scientific reports*, 7(1):1–11, 2017.

[11] Benjamin L Cox, Sarah Erickson-Bhatt, Joseph M Szulczewski, Jayne M Squirrell, Kai D Ludwig, Erin B Macdonald, Robert Swader, Suzanne M Ponik, Kevin W Eliceiri, and Sean B Fain. A novel bioreactor for combined magnetic resonance spectroscopy and optical imaging of metabolism in 3d cell cultures. *Magnetic resonance in medicine*, 81(5):3379–3391, 2019.

[12] Leonard Breindel, David S Burz, and Alexander Shekhtman. Active metabolism unmasks functional protein–protein interactions in real time in-cell nmr. *Communications biology*, 3(1):1–9, 2020.

[13] Ana Soares, Marika Murto, Benoit Guieysse, and Bo Mattiasson. Biodegradation of nonylphenol in a continuous bioreactor at low temperatures and effects on the microbial population. *Applied microbiology and biotechnology*, 69(5):597–606, 2006.

[14] Bella Devassy Tony, Dinesh Goyal, and Sunil Khanna. Decolorization of direct red 28 by mixed bacterial culture in an up-flow immobilized bioreactor. *Journal of industrial microbiology & biotechnology*, 36(7):955–960, 2009.

[15] Christian Kaisermayer and Jianjun Yang. Highly efficient inoculum propagation in perfusion culture using wave bioreactor™ systems. In *BMC proceedings*, number S6 in 7, page P7. Springer, 2013.

[16] Paul Schoeberl, Mounir Brik, Marina Bertoni, Rudolf Braun, and Werner Fuchs. Optimization of operational parameters for a submerged membrane bioreactor treating dyehouse wastewater. *Separation and Purification Technology*, 44(1):61–68, 2005.

[17] John C Vellinger, Kenneth W Barton, Mark S Deuser, and Mark E Wells. Bioreactor apparatus and cell culturing system, April 3 2007. US Patent 7,198,940.

[18] N Jane Taylor, Hiram Baddeley, Kate A Goodchild, Melanie EB Powell, Michelle Thoumine, Linda A Culver, J James Stirling, Michele I Saunders, Peter J Hoskin, Heather Phillips, et al. Bold mri of human tumor oxygenation during carbogen breathing. *Journal of Magnetic Resonance Imaging: An Official Journal of the International Society for Magnetic Resonance in Medicine*, 14(2):156–163, 2001.

[19] Hun-Jin Jeong, So-Jung Gwak, Nae-Un Kang, Myoung Wha Hong, Young Yul Kim, Young-Sam Cho, and Seung-Jae Lee. Bioreactor mimicking knee-joint movement for the regeneration of tissue-engineered cartilage. *Journal of Mechanical Science and Technology*, 33(4):1841–1850, 2019.

[20] Pragyansri Pathi, Teng Ma, and Bruce R Locke. Role of nutrient supply on cell growth in bioreactor design for tissue engineering of hematopoietic cells. *Biotechnology and bioengineering*, 89(7):743–758, 2005.

[21] L Bačáková, E Filova, F Rypáček, V Švorčík, and V Starỳ. Cell adhesion on artificial materials for tissue engineering. *Physiol Res*, 53(Suppl 1):S35–S45, 2004.

[22] Christian Frantz, Kathleen M Stewart, and Valerie M Weaver. The extracellular matrix at a glance. *Journal of cell science*, 123(24):4195–4200, 2010.

[23] L Bacakova and V Svorcik. Cell colonization control by physical and chemical modification of materials. *Cell Growth Processes: New Research*, pages 5–56, 2008.

[24] Erkki Ruoslahti and Michael D Pierschbacher. New perspectives in cell adhesion: Rgd and integrins. *Science*, 238(4826):491–497, 1987.

[25] Martin J Humphries, Steven K Akiyama, Akira Komoriya, Kenneth Olden, and Kenneth M Yamada. Identification of an alternatively spliced site in human plasma fibronectin that mediates cell type-specific adhesion. *The Journal of cell biology*, 103(6 Pt 2):2637–2647, 1986.

[26] John P Ranieri, Ravi Bellamkonda, Evan J Bekos, Joseph A Gardella Jr, Hans J Mathieu, Laurence Ruiz, and Patrick Aebischer. Spatial control of neuronal cell attachment and differentiation on covalently patterned laminin oligopeptide substrates. *International Journal of Developmental Neuroscience*, 12(8):725–735, 1994.

[27] Clare Selden and Barry Fuller. Role of bioreactor technology in tissue engineering for clinical use and therapeutic target design. *Bioengineering*, 5(2):32, 2018.