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Conceptual Design of a Bioreactor for Tissue Engineering and Regenerative Medicine

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Abstract: The aim of the paper was to make an overview of current bioreactor systems in tissue engineering and introduces a conceptual design for a perfusion bioreactor tailored for vascular tissue culture. Perfusion bioreactors were selected for their ability to replicate the in vivo environment, particularly for blood vessels, by providing continuous media flow and applying shear stress, both critical for successful vascular tissue growth. Three bioreactor variants were designed, each incorporating specific modifications aimed at improving the tissue culture process. Variant A consists of a cylindrical chamber that allows for uniform external media flow. Variant B features an hourglass-shaped cultivation chamber, which increases pressure on the cultured tissue, thereby enhancing mechanical stimulation. Variant C, the most promising design, integrates both shear stress and longitudinal stress, aiming to produce vascular tissues with superior mechanical properties. Our results suggest that Variant C holds the greatest potential for achieving robust in vitro vascular tissue formation due to its combined mechanical stimulations. The design prioritizes scalability, ease of assembly, and sterilization, making it suitable for future applications in tissue engineering and regenerative medicine. However, further experimental testing is needed to assess flow dynamics, shear stress, and material performance to refine the bioreactor system and validate its effectiveness in laboratory conditions.

Keywords: bioreactor; stimulation; differentiation; tissue engineering; perfusion; tissue culture

1. Introduction

Tissue engineering bioreactors are specialized devices designed to regulate biological processes through mechanical, biochemical, and physical stimulation. The growing field of tissue engineering and regenerative medicine relies heavily on bioreactors to cultivate functional tissues in vitro that can be used to restore or replace damaged organs and tissues. Bioreactors not only provide controlled environments for cell differentiation and tissue growth but also ensure the adequate distribution of nutrients and gases to support three-dimensional cell cultures.

The aim of this paper is to expand the current knowledge and contribute to the advancement of tissue engineering and regenerative medicine by developing a conceptual design for a perfusion bioreactor, specifically tailored for the cultivation of vascular tissues. The ability of bioreactors to mimic in vivo conditions through continuous media flow and mechanical stimulation makes them an indispensable tool for tissue engineers. Additionally, the use of bioreactors can address limitations of traditional transplantation methods, such as the scarcity of donor tissues and the risk of immune rejection.

This study outlines the design and development of three perfusion bioreactor variants, each engineered to provide optimal conditions for vascular tissue growth. By incorporating mechanical forces such as shear stress and longitudinal stress, these designs aim to improve the mechanical strength and functionality of the cultivated tissues.

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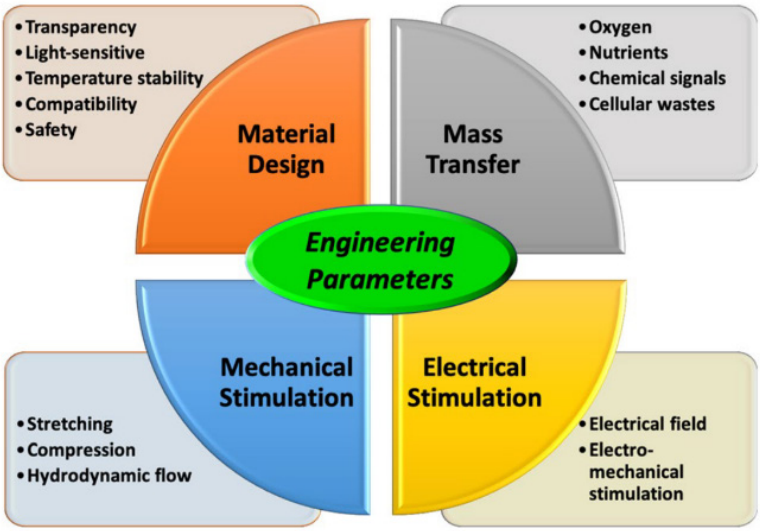

Figure 1: Schematic diagram of engineering parameters for bioreactor systems

2. Materials and methods

Tissue engineering is based on the collaboration of several disciplines, where biological sciences and technical principles are applied to the development of substitutes to restore and improve the function of a tissue or organ. Currently, tissues obtained from the patient himself (autologous transplants) or tissues from a human or animal donor (allogeneic and xenogeneic transplants) are commonly used. However, in both cases, several problems arise. In the case of autologous tissues, the limiting factor may be the lack of suitable tissue for transplantation, while tissues from donors may cause unwanted immune reactions that may even lead to de-healing (if they are perceived as foreign) of the tissue or the entire organ. [1] Methods in regenerative medicine use tissue-specific cells, biomaterials, and biologically active molecules to enhance regeneration and support the formation of new and functional tissues. [2–4]

Bioreactors are used in the development of new tissue in vitro by providing biochemical and physical regulatory signals to cells and inducing them to differentiate and/or produce extracellular matrix prior to implantation in vivo. [5] Mechanical stimulation in a bioreactor is used to transform stem cells into the desired cell phenotype. There is great potential for the use of mesenchymal stem cells and other multipotent cells to generate cells of a different tissue type. In bioreactors, these processes take place in a strictly monitored and controlled environment. They are sterile vessels with temperature regulators and gassing devices necessary to start the biochemical reaction. [6–9] In tissue engineering, bioreactors can be used in several areas. In the beginning, we need bioreactors for cell expansion. The cells are intended for direct transplantation. Second, we use bioreactors to grow 3D tissues before implantation, such as skin, cartilage, bone, blood vessels, and others. [10,11]

Bioreactor systems offer the opportunity to study cellular function, cell-to-cell interactions, and tissue development within controlled 3D models that are designed to provide cells with the conditions of their natural environment. In this regard, various culture systems have been developed to support tissue engineering constructs. [2]

During the design process, the specific use of the bioreactor must be kept in mind to ensure that all design constraints and conditions are met. If various parameters such as pH, nutrient concentration, or oxygen levels are to be monitored, these sensors should be built into the design. If a pump or motor is to be used, it must be small enough to fit in the incubator and usable in a humid environment. [11] The forces required for cell stimulation are very small, so it is important to ensure that the pump/motor has the sensitivity to accurately exert small forces. In any structure involving fluids, fluid sealing

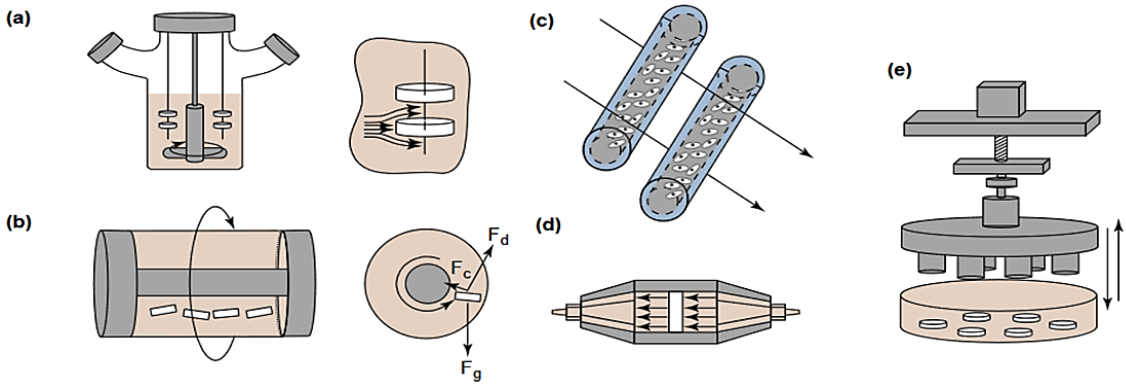

Figure 2: Types of dynamic bioreactor systems (a) rotating banks, (b) bioreactors with rotating walls, (c) bioreactors with hollow fibres, (d) direct perfusion bioreactors, (e) bioreactors with applied mechanical forces

problems may occur, and the need for seals should be minimized as far as possible. If a bioreactor prototype is being designed, it is worth thinking about innovative improvements from the very beginning. This may mean designing a device that can be scaled up relatively easily without changing its properties. [9–12]

The choice of materials is very important, as it must be ensured that the materials do not have negative effects and reactions with the cells during cultivation in the bioreactor. Any material that will be in direct contact with cells must be biocompatible and bioinert. [13]

3.1. Perfusion bioreactor for the cultivation of blood vessels

Perfusion bioreactors have many advantages in vascular tissue engineering. [3] The chemical and mechanical stimuli necessary for proper development can be better achieved in a controllable manner than traditional static cell culture systems. Perfusion systems provide intra-luminal pulsatile flow providing the necessary shear stress for endothelial cells. Perfusion bioreactors for the cultivation of blood vessels are assembled from several main parts that form the basis of the bioreactor: one or more cultivation chambers, media container, pipe system and medium transfer pump (located outside the culture chamber). [3,14]

To culture blood vessels, it is necessary to have a carrier – scaffold. [3] Many natural and synthetic materials are used to make scaffolds, which can be permanent or biodegradable. Synthetic scaffolds can be made from a variety of non-biodegradable poly-

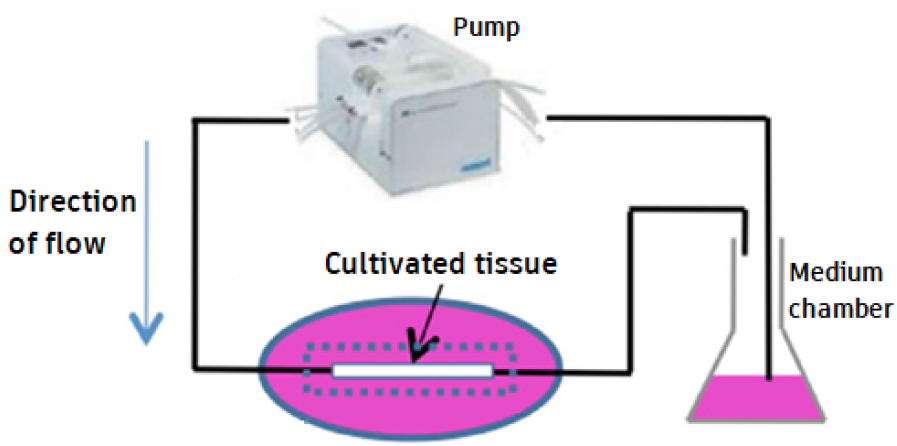

Figure 3: Main components of perfusion bioreactor system

mers. Non-biodegradable polymers can be coated with collagen to mimic the extracellular matrix and allow endothelial and smooth muscle cells to grow on its surface. Biodegradable polymers can also be used. Examples of materials used as natural scaffolds include collagen, fibrin and hyaluronan. Decellularized tissue can also be used as a carrier. They consist of the extracellular matrix (ECM) of the original source. They can come from sources in the body, such as human umbilical veins and the submucosa of the small intestine, or from xenografts, such as pig aorta, which can then be seeded with human cells. [1,9,10,15]

3.2. Design of different variants of bioreactor systems

In this part of the research paper, the design and operation of three variants of the perfusion bioreactor are described in detail. All parts for the perfusion bioreactor were designed in the SolidWorks program and the final models were modified in the Autodesk Fusion 360 program. The bioreactor was designed for the cultivation of vessels with a cylindrical cell carrier. Biodegradable polymer carriers are suggested for the scaffold. Collagen and elastin are also commonly used, as they are the two main components of the artery responsible for its characteristic mechanical properties. According to studies, the use of endothelial cells or smooth muscle cells has been the most proven. The culture medium in the inner and outer parts of the inoculated carrier is managed by two independent perfusion systems. Through a closed circulation system, the medium circulates continuously. The culture medium flows in a closed loop from the culture medium reservoir to the bioreactor and back to the reservoir. Since the intensity of the flow differs in the vessels and arteries, the behaviour of the cultured tissue and how it can withstand the loads will be observed. In the bioreactor, the fluid flow direction is parallel to the axis of the tube. The carrier is attached to two tubes (see Figure 4) which are inserted through opposite sides of the chamber walls to ensure medium perfusion through the lumen.

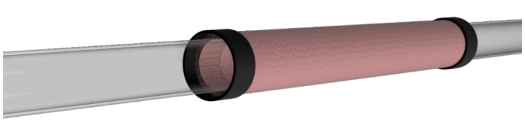

Figure 4: Scaffold fixing

Cultivation chambers (Variant A, B, C) are suggested to be constructed out of completely transparent biocompatible polycarbonate (PC). The size and shape of the culture chambers are designed based on the sample size and other requirements such as the volume of the media reservoir.

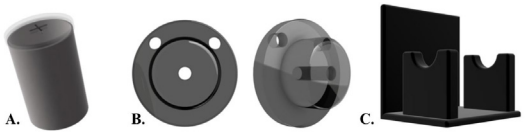

Figure 5: Bioreactor components: A. Gas exchange membrane, B. Closing lids, C. Bioreactor stand

The cultivation chamber is placed on a stand (Figure 5C) made of plastic using two oval lids for closing (Figure 5B), which ensure sealing of the chamber from both sides. They contain holes to ensure internal (and external) flow.

3.1.1. Input and Output of Gases

The perfusion bioreactor design ensures precise gas exchange to maintain optimal cell culture conditions. The cultivation chambers are equipped with silicone membranes that facilitate gas exchange, allowing for the continuous supply of a gas mixture (95% O2 / 5% CO2) directly to the medium reservoir. This setup maintains the necessary pH and oxygenation levels within the bioreactor. The air filter connected to the gas supply ensures sterility and prevents contamination.

3.1.2. Variant A

Variant A design uses a simple cylindrical chamber that ensures uniform external flow of the medium. The chamber itself does not contain a medium tank. The grafted scaffold will therefore be inserted through the opening on the chamber wall, at which time it will already be connected to the tube system.

A peristaltic pump would be used to ensure the constant flow of the medium. The pump is controlled by computer software that allows the speed and frequency of fluid flow to be adjusted, thus allowing detailed manipulation of the level of mechanical stimulation during tissue growth. To ensure two independent circuits, two peristaltic pumps are used, which are controlled separately. Pumps connected to the computer connect the culture vessel with the medium and the bioreactor itself through a system of tubes. The two perfusions are kept sepa-

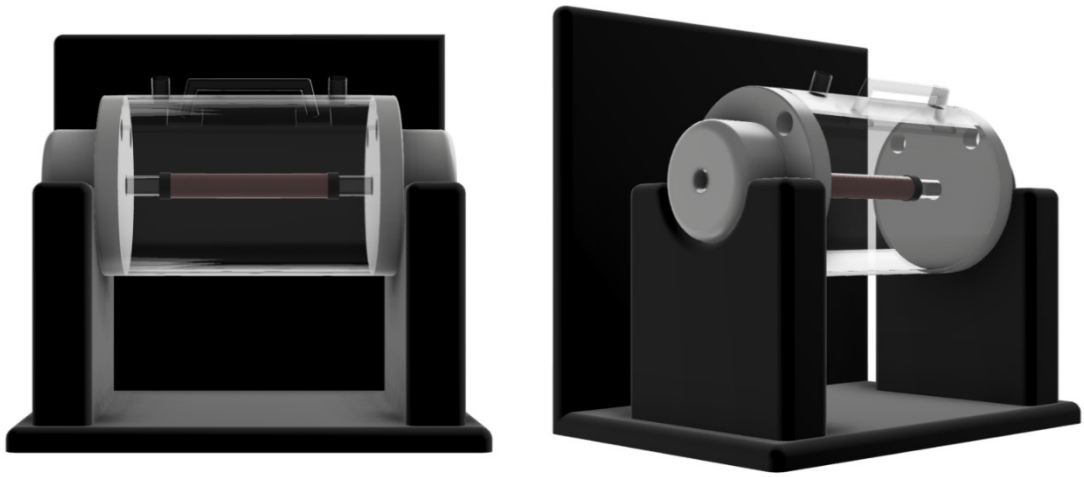

Figure 6: Concept design of a bioreactor for vascular cultivation (Variant A)

rate to avoid contamination between different cell types. The pumps provide adjustable flow that can be continuous or pulsatile with a frequency range of 50 to 200 beats per minute to cover the range from fetal to adult heart rates. The external medium is flushed at a slow and continuous rate to minimize shear stress on the outside of the artery. The perfusion of the internal medium is laminar and pulsatile to reproduce the physiological flow.

3.1.3. Variant B

As a second variant, a different shape of the cultivation chamber was proposed. The pressure that causes the flow to the outside of the cultured tissue is increased in the narrowed part of the chamber. Pressure monitoring sensors are built into the bioreactor, which are connected to a computer system.

The cultivation chamber for the second variant was designed in the shape of an hourglass (for variant B and C). The perimeter of the side parts of the chambers was kept from the first design, as well as the length of the chamber, so that the dimensions of the designed stand and lids did not have to be changed. Silicone membranes for gas exchange will be placed on both sides. With this type of chamber,

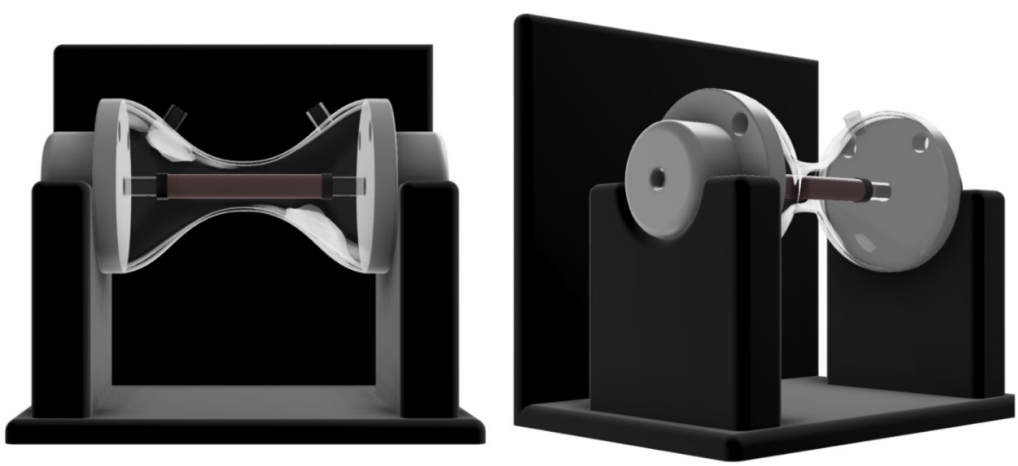

Figure 7: Concept design of a bioreactor for vascular cultivation (Variant B)

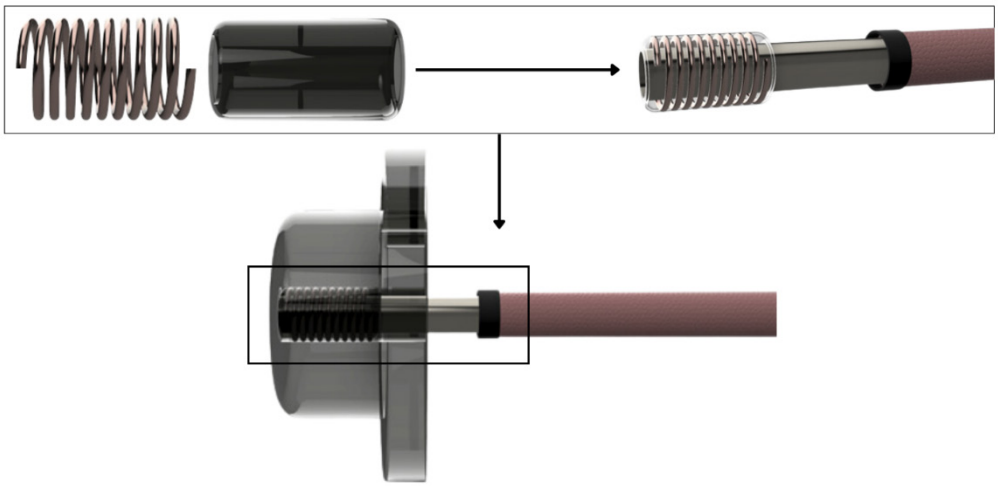

Figure 8: Copper coil with plastic insulation and the principle of incorporating the coil into the lid

the scaffold will be inserted from the side by first fixing it to one of the lids. It is then inserted from one side into the cultivation chamber and connected to the pipe system on the other side to close the circulation. Such embedding was designed to test different types of scaffolds embedding and evaluate which principle is more advantageous and takes less time.

The external and internal flow of the medium is handled in the same way as in Variant A. In this case, however, attention must be paid to the intensity of both flows. As previously mentioned, laminar flow creates shear stress inside the scaffold. Due to the change in the shape of the culture chamber (see Figure 8), care must be taken that due to the narrowed part in the centre of the chamber, the external flow of the medium will create pressure on the outer wall of the cellular carrier. Therefore, it was decided that the external medium will be circulated very slowly and continuously.

3.1.4. Variant C

Longitudinal stress acts on arteries and veins in the human body. Therefore, for the variant C, in addition to shear stress (which arises because of the intensity of the internal flow), it was decided to apply longitudinal stress based on the principle of electromagnetism. The application of a defined longitudinal stress can be used to cultivate a vessel that is mechanically strong. For this reason, the construction materials of some parts had to be changed, as well as the con-

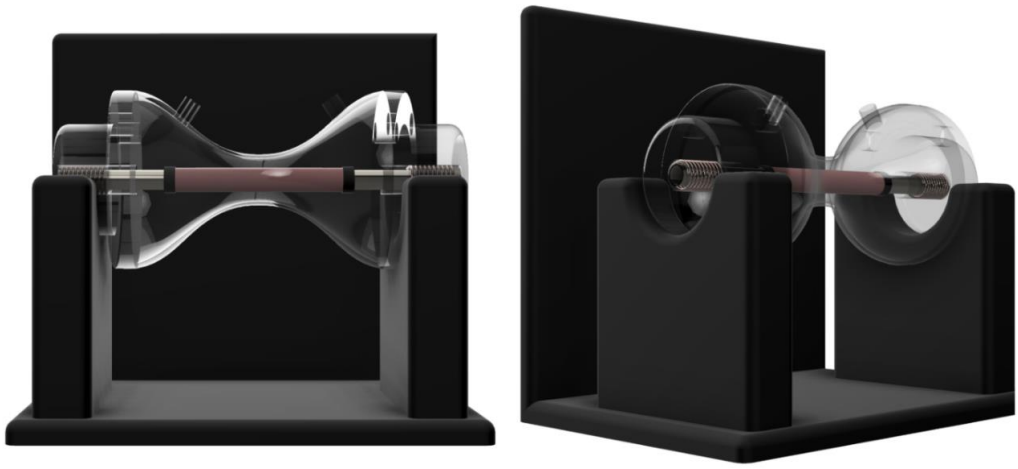

Figure 9: Concept design of a bioreactor for vascular cultivation (Variant C)

struction of the lids. They will have a built-in gap for inserting a coil with isolation (plastic).
The size (diameter and length) of the coil was designed according to the diameter of the nickel rod. The two holes on opposite sides of the insulation were designed to closely surround the nickel rod and ensure insulation.

It is suggested that the coil would be made of copper, because it has excellent electrical conductivity properties. The individual turns of the coil must be isolated from each other. Conductive contacts will be connected to each end of the coil for the supply of electricity. When an electric current flows through the coil, an electromagnetic field is created around it. The tubes to which the scaffold will be attached must be made of magneto-strictive material - nickel. Nickel was chosen as a suitable material because it has great resistance to temperature and is chemically stable. If we insert a nickel rod into the cavity of the coil and connect a direct current to the coil, the coil will create a magnetic field in its cavity. The rod placed in the cavity is magnetized. However, the magnetization of the nickel rod will cause its length to change. A gap will be created between the insulation and the gap in the lid - freedom for longitudinal movement. Due to the movement of the nickel rod, longitudinal stress will be applied to the scaffold. Since the application of longitudinal stress is important for the third design, good insulation of the medium from the electric current must be taken care of.

The mechanical forces provided by these bioreactor designs are essential for stimulating stem cells to differentiate into desired cell types and for enhancing the mechanical properties of the engineered tissue. This dynamic environment supports the formation of extracellular matrix and improves overall tissue strength and functionality

3. Results and discussion

The best reported results in the field of vascular tissue engineering are achieved by combining classical grafting or needling techniques with a dynamic culture system. Several perfusion devices and bioreactors have already been developed for these purposes. This principle is also proposed within this paper. Several criteria were considered when designing the bioreactor concept: media flow control, continuous media exchange, durable, easy to manufacture, easy to use and secured gas exchange.

The main difference between the types of perfusion bioreactors is the number of flow circuits, which are generally either one or two. For a wider range, three variants of the perfusion bioreactor were designed, all with two closed circuits. Variant C should, according to the theory, create the mechanically strongest vessels due to the application of shear stress and longitudinal stress. A suitable material for the scaffold that allows cell adhesion, differentiation and proliferation also must be selected. It should also provide a surface to support the growing tissue until the cells create their own extracellular matrix – the scaffold will be made of biodegradable material. The aim was also to design the bioreactor in such a way that easy sterilization and possible recycling of individual parts were possible.

Mechanical stimulation within the bioreactor is achieved through controlled media flow and shear stress, so to sum up, the bioreactor variants include:

– *Variant A: This design uses a simple cylindrical chamber that ensures uniform external flow of the medium, allowing for detailed manipulation of fluid dynamics to simulate different mechanical stimuli during tissue growth. The system includes two independent circuits that allows for pulsatile flow to mimic physiological conditions*

– *Variant B and C: These designs incorporate hourglass-shaped cultivation chambers, which modify flow intensity and increase pressure at specific points. This configuration enhances the mechanical stress applied to the tissue, promoting better cell differentiation and tissue cohesion. Pressure sensors are integrated for real-time monitoring and adjustments*

– *Variant C: Additionally, this variant applies both shear stress and longitudinal stress to create mechanically robust vascular tissues. This is achieved through advanced flow control mechanisms that simulate the natural environment of blood vessels*

The mentioned bioreactor designs unite the goal of this paper, which was to propose a concept for a perfusion bioreactor for the cultivation of blood vessels. As for the comparison of the individual proposals presented in this work, it is only possible from a theoretical point of view. Only practical measurements will show the real advantages, respectively disadvantages and financial difficulty. In a laboratory with a built prototype, it will be possible to evaluate the following parameters: viscosity of the medium, flow rate, magnitude of shear stress, magnitude of pressure, maintaining a constant temperature in the bioreactor, sterile environment, ensuring impermeability, biocompatibility of materials, chemical and mechanical resistance of plastic, correct selection of scaffold for vascular cultivation.

4. Conclusions

Cardiovascular tissue engineering is a rapidly growing field in biomedicine. Further successful development in this area will require optimization of existing perfusion bioreactors and the development of a new generation tailored to the specific needs of tissue engineering constructs. Designing high-performance perfusion bioreactors will necessitate a multidisciplinary approach, involving close collaboration between tissue engineers, cardiovascular biologists, physiologists, cardiologists, biomechanical engineers, and software specialists. [16]

In this study, we focused primarily on the design aspects of perfusion bioreactors rather than the detailed input parameters and requirements. Three conceptual designs for perfusion bioreactors were proposed, focusing on the mechanical constraints critical for cell differentiation, tissue cohesion, and strength. The combination of induced media flow and shear stress, particularly with the additional longitudinal stress in Variant C, shows potential for beneficial vascular tissue formation in vitro.

It is important to note that these designs are currently conceptual. Future research will involve rigorous experimental validation to refine these designs and evaluate their practical applications. By continuing this research, we aim to contribute significantly to the development of viable tissue-engineered blood vessels for future clinical trials and therapeutic interventions.

Acknowledgments

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References

[1.] Lim, D., Renteria, E. S., Sime, D. S., Ju, Y. M., Kim, J. H., Criswell, T., Shupe, T. D., Atala, A., Marini, F. C., Gurcan, M. N., Soker, S., Hunsberger, J., & Yoo, J. J. (2022). Bioreactor design and validation for manufacturing strategies in tissue engineering. Bio-design and manufacturing, 5(1), 43–63. https://doi.org/10.1007/s42242-021-00154-3

[2.] Saltzman W., (2004), Oxford University Press; 1st edition, Yale University, Tissue Engineering: Engineering Principles for the Design of Replacement Organs and Tissues, ISBN-13: 978-0195141306

[3.] PaezMayorga, J., HernándezVargas, G., RuizEsparza, G. U., Iqbal, H. M. N., Wang, X., Zhang, Y. S., ParraSaldivar, R., & Khademhosseini, A. (2018, May 8). Bioreactors for Cardiac Tissue Engineering. Advanced Healthcare Materials, 8(7). https://doi.org/10.1002/adhm.201701504

[4.] Roseti, L., Parisi, V., Petretta, M., Cavallo, C., Desando, G., Bartolotti, I., & Grigolo, B. (2017, September). Scaffolds for Bone Tissue Engineering: State of the art and new perspectives. Materials Science and Engineering: C, 78, 1246–1262. https://doi.org/10.1016/j.msec.2017.05.017

[5.] Freed, L. E., Guilak, F., Guo, X. E., Gray, M. L., Tranquillo, R., Holmes, J. W., Radisic, M., Sefton, M. V., Kaplan, D., & Vunjak-Novakovic, G. (2006, December). Advanced Tools for Tissue Engineering: Scaffolds, Bioreactors, and Signaling. Tissue Engineering, 12(12), 3285–3305. https://doi.org/10.1089/ten.2006.12.3285

[6.] Ikada, Y. (2006, April 18). Challenges in tissue engineering. Journal of the Royal Society Interface, 3(10), 589–601. https://doi.org/10.1098/rsif.2006.0124

[7.] Zhao, J., Griffin, M., Cai, J., Li, S., Bulter, P. E., & Kalaskar, D. M. (2016, May). Bioreactors for tissue engineering: An update. Biochemical Engineering Journal, 109, 268–281. https://doi.org/10.1016/j.bej.2016.01.018

[8.] Partap, S., A., N., & J., F. (2010). Bioreactors in Tissue Engineering. InTech. DOI: 10.5772/8579

[9.] Burdick, J. A., & Mauck, R. L. (Eds.). (2011). Biomaterials for Tissue Engineering Applications. https://doi.org/10.1007/978-3-7091-0385-2

[10.] Chabanon, M., Duval, H., Grenier, J., Beauchesne, C., Goyeau, B., & David, B. (2019, March 21). Histological Method to Study the Effect of Shear Stress on Cell Proliferation and Tissue Morphology in a Bioreactor. Tissue Engineering and Regenerative Medicine, 16(3), 225–235. https://doi.org/10.1007/s13770-019-00181-3

[11.] Ahmed, S., Chauhan, V. M., Ghaemmaghami, A. M., & Aylott, J. W. (2018, October 27). New generation of bioreactors that advance extracellular matrix modelling and tissue engineering. Biotechnology Letters, 41(1), 1–25. https://doi.org/10.1007/s10529-018-2611-7

[12.] Saini, G., Segaran, N., Mayer, J., Saini, A., Albadawi, H., & Oklu, R. (2021, October 26). Applications of 3D Bioprinting in Tissue Engineering and Regenerative Medicine. Journal of Clinical Medicine, 10(21), 4966. https://doi.org/10.3390/jcm10214966

[13.] Revete, A., Aparicio, A., Cisterna, B. A., Revete, J., Luis, L., Ibarra, E., Segura González, E. A., Molino, J., & Reginensi, D. (2022, November 7). Advancements in the Use of Hydrogels for Regenerative Medicine: Properties and Biomedical Applications. International Journal of Biomaterials, 2022, 1–16. https://doi.org/10.1155/2022/3606765

[14.] Blose, K. J., Krawiec, J. T., Weinbaum, J. S., & Vorp, D. A. (2014).

Bioreactors for Tissue Engineering Purposes. Regenerative Medicine Applications in Organ Transplantation, 177–185. https://doi.org/10.1016/b978-0-12-398523-1.00013-6

[15.] Gensler, M., Leikeim, A., Möllmann, M., Komma, M., Heid, S., Müller, C., Boccaccini, A. R., Salehi, S., Groeber-Becker, F., & Hansmann, J. (2020, November 30). 3D printing of bioreactors in tissue engineering: A generalised approach. PLOS ONE, 15(11), e0242615. https://doi.org/10.1371/journal.pone.0242615

[16.] Stephenson, M., & Grayson, W. (2018, April 30). Recent advances in bioreactors for cell-based therapies. F1000Research, 7, 517. https://doi.org/10.12688/f1000research.12533.1