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Design and Fabrication of a Radial Flow Bioreactor to Decellularize Muscular Arteries

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Abstract. Tissue engineering seeks to obtain functional organs in laboratories due to the scarcity and difficulty of obtaining organs and tissues for donations. We must consider that there are patients who require organs and/or tissues for which there is currently no transplant protocol for them, for example, blood vessels. Currently, a scaffold biofabrication technique used in tissue engineering known as decellularization is used, with which we obtain a natural cellular scaffold in which we can seed cells to obtain a functional blood vessel. The aim of this work is to make the biochemical process of decellularization more efficient using a bioreactor that generates mechanical and hydraulic stimulations. A radial flow hydraulic circuit was designed and simulated in Solidworks by solving the Navier-Stokes equations to have a non-turbulent laminar behavior and to stimulate cell detachment without damaging the collagen matrix of the blood vessel. Finally, the bioreactor was printed using additive printing techniques using a photosensitive resin as shown in this work.

Keywords: Tissue engineering · Bioreactor · Flow simulation · Arteries · Additive print

1 Introduction

Although organ donations have increased exponentially, it is still not enough to meet the needs of people who are waiting for an organ or tissue, only the National Transplant Center (CENATRA), reports that at date there are 23,370 people on the waiting list. This without considering that there are patients who require organs and/or tissues for which there is currently no transplant protocol for them, for example, blood vessels [1]. Well, according to the press release of October 31, 2018, from the National Institute of Statistics and Geography (INEGI), it mentions cardiovascular diseases as the first cause of death in Mexico and it is also one of the main ones in the world [2].

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That is why, faced with this problem, there is a high demand in the search for surgical strategies for patients who require solutions to the lack of blood vessel substitutes, resorting to allotransplantation, (which consists of a transplant that comes from another human being), and in the particular case of arteries, to autograft (in which the patient is the recipient and donor) but to date it has not been shown to be a holistic solution [3].

Among the main cardiovascular diseases is atherosclerosis, (arterial occlusion that prevents blood flow), affecting any artery, like the arteries of the heart, arms, legs and other organs. Which can cause damage such as angina pectoris, or a heart attack, in addition to a stroke to the loss of kidney function. The placement of synthetic biological vascular grafts is the most recurrent clinical procedure to try to solve this type of problem [4].

In decellularization from tissues obtained from a biological model, a process is carried out by which cells are eliminated using different decellularization agents to promote the elimination of antigens that initiate the immune response in transplants, eliminating the need for immunosuppression and decreasing the rate of transplant rejection and even opening the possibility of xenotransplantation (transplantation between different species) maintaining a viable extracellular matrix that can serve as a cellular scaffold to be repopulated with the patient's cells and thus promote tissue regeneration [5].

Regarding biological grafts, the most used are autologous arteries or veins such as the saphenous; however, 40% of patients do not have vessels of adequate quality or length, and even with proper tissue, in vivo remodeling. Within the proposals of synthetic materials most used today we find expanded polytetrafluoroethylene and Dacron. However, these present problems for which they end up losing their function, such as their resistance to tension and high rigidity, which is why they are difficult to suture *in vivo*. [6].

Due to the above, currently seeking to achieve a scaffold of blood vessels that can be used to replace them in patients who require it and promote regeneration. This project seeks to propose a scaffold biofabrication technique used in tissue engineering known as decellularization, this methodology is since extracellular matrix proteins are highly conserved between different species. [7].

These underwent a decellularization protocol previously developed by our research group applied to bile ducts, which was effective in their decellularization. [8] Therefore, this protocol was applied in blood vessels to obtain a decellularized scaffold as well as the histological evaluation of the extracellular matrix and its potential use in vascular regenerative medicine by analyzing cell viability in the obtained scaffold. [4, 9].

Which was also effective in blood vessels, since a decellularization of 95.65% ± 4.65% was obtained, in addition to the fact that no damage was observed in the extracellular matrix and the elastic fibers were maintained. On the other hand, in the cell viability assay it is shown that a viability of around 87% was obtained [10, 11]. This indicates that good results are obtained when using the same protocol as in the bile ducts, to have blood vessels, mostly decellularized and without apparent damage to the extracellular matrix and gives rise to the possibility that this tissue in regenerative medicine. [12, 13].

The objective of this work is to make the biochemical process of decellularization more efficient using a bioreactor that generates mechanical and hydraulic stimulations.

A radial flow hydraulic circuit was designed a simulated to corroborate the optimization of the biochemical process between the static and the dynamic process [6].

2 Methodology

2.1 System Description

The proposed system (spiral support for arteries and their irrigation) were designed with Solidworks® (CAD software). The media reservoir and the system were designed to use with 100 ml media, mimic the internal blood circuit like a spiral radial flow geometry, decellularization media was delivered with a peristaltic pump driven the spiral irrigation system. The spiral system was placed in the center of the bioreactor to obtain a radial flow along the support to allow a laminar, non-turbulent and constant flow of media along the arteries to decellularize. The bioreactor dimensions are 35 mm height × 18 mm radius. Connectors were designed with 6 mm radius to plug in the peristaltic pump. To close the circuit, we add an output pipe that absorbs the processed media and return to the spiral through a peristaltic pump as shown in Fig. 1. The decellularization methodology used in this project is based on a process previously developed in the Biomaterials Laboratory of the UNAM Materials Research Institute, which involved physical-chemical-enzymatic decellularization agents [4].

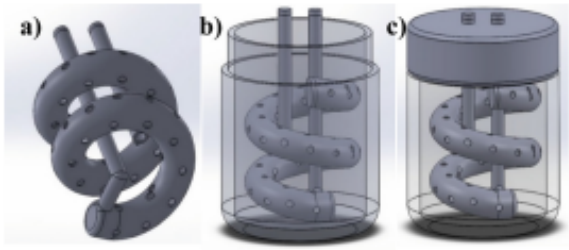

Fig. 1. Spiral-shaped bioreactor components a) Spiral-shaped tissue support and the output pipe, b) Cylindrical media chamber, c) Bioreactor assembly.

2.2 Model Design and Flow Simulation

The flow simulation goals were, suppressed the zero flow zones on the media chamber in the bioreactor to obtain a radial flow model that support the artery to decellularize, optimizing the media volume for different tissue lengths. The assembly was exported to CosmosFloworks (fluid simulation software) to solve the Navier-Stokes equations to simulate and calculate the speed fields and stress against the tissue [6]. Our values and simulation conditions were:

- Outlet conditions Pressure: 101,325 Pa (1 atm)
- Inlet conditions Flow rate: 0.83×10^{-05} m^3/s
- Fluid temperature: 310.15 K (37 °C)
- Density: 1000 kg/m^3.

A mesh consisting predominantly of hexahedral and tetrahedral elements was generated, with a total of 3,470,820 elements and 1,774,332 nodes (Fig. 2). Before choosing the final mesh, a preliminary mesh test was performed with the default values. A mesh with twice the number of default elements was created, keeping the same spatial distribution and nodes, and then the simulations were performed. The maximum velocities values obtained by both meshes were observed, and a difference of less than 3% was found in the results.

Fig. 2. Meshing for flow simulation.

2.3 3D Printing

The bioreactor parts were printed on a resin photolithography additive printer (Creality LR-002) using a photosensitive hard resin to have the complex figures in one piece to generate the finally test. To achieve this, the Chitubox V2.2® slicing software was used to print the resine parts with the follow parameters used for resin printing:

- Layer height 0.4 mm
- Thickness 0.2 mm
- Print speed (mm/s) 6 s
- Printing temperature 27 °C
- Enable fan
- Cut the bottom of the object 0.0 mm
- Overlay 0.15 mm
- Supports along the structure 5 mm.

Finally, the slices software images shown in Fig. 3.

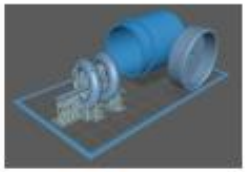

Fig. 3. Preparation slices for additive printing.

3 Results

3.1 Flow Analysis

Functional simulation results are shown in Fig. 4 with three different views, in a) the media show a laminar vortex in the chamber, and non-static zones were observed. In b) the simulations showed that the radial flow through the support and the artery is constant along the spiral and the holes with velocities of 0.013 m/s in the media, 0,014 m/s across the input and output. C) shows the laminar and uniform flow that reduce shear stress by selecting and appropriate flow, geometry, and position.

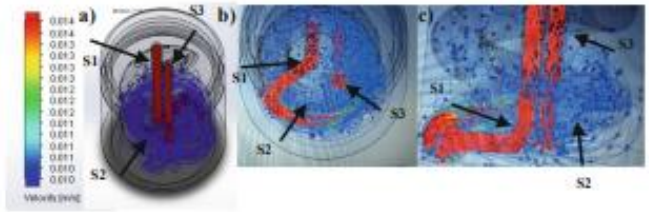

Fig. 4. Flow simulation components a) Cylindrical media chamber flow fields. b) Spiral-shaped radial flow vectors, c) Velocity fields across the spiral-shaped support.

An isometric view of the fluid simulation system is shown in Fig. 4. In this figure were shown 3 important sections, S1, S2 and S3, those positions and geometries generate the change of the fluid's velocity and the interaction whit the arteries' location. The inlet and spiral section (S1), has the peak velocity values determined in the fluid simulation, with the highest speed of 0.014 m/s produced by the peristaltic pump inlet, where the fluid travels through the support and the spiral. Then, the fluid travels through S1 and combines with the media reservoir (S2) in a radial form. The support got holes along their geometry; this section shows a reduce the velocity values that goes from 0.014 m/s to 0.011 m/s. The flow stabilizes while flow across the bioreactor along S2, where the values of the media velocity decrease and shows a laminar flow characterization with values between 0.011 m/s and 0.010 m/s.

Finally, S3 shows the outlet section, and it has another peak velocity values calculated in the fluid simulation, with the maximum speed of 0.014 m/s created by the peristaltic pump suction.

3.2 Printed Model

The design manufacturing depends on the 3D printer precision and specifications of the stereolithography system. The dimensional tolerance of 0.2 mm was contemplated for a non-permeable and correct mechanical assembly. The spiral support is the main structure, all the components were designed following this geometry; the structure purpose is not only to offer a decellularized system, however we also pretend to use it to seed cells within the obtained collagenic structures. To realize the purpose, we need to integrate the pieces to redesign the bioreactor system shown in Fig. 5, to be a fluid system that allows Recellularized the scaffolds.

Fig. 5. Printed spiral-shaped bioreactor model and the whole fluid system.

4 Conclusions

A radial flow bioreactor was designed and fabricated following the simulations results considering the geometry, flow characteristics and the radial circulation of the decellularized media. Artery tissue could be decellularized more efficiently in our dynamic system against the static protocol used in the past. The velocity curves described a radial flow model, and the velocity fields and vectors may be used to predict optimal media volume for different tissue lengths.

The resulting performance of the final design is useful to decellularized arteries while handling and performing flow simulation, because the technique was probed in static form. Moreover, by using the radial flow in the bioreactor we infer that the experimental results will be enhanced to decellularize arteries tissues.

The materials for stereolithography, it was convenient to generate translucent pieces for easy visualization of the bioreactor function. Considering the fluid flow simulation along the spiral support irrigation shows low velocity values which permit a laminar and non-turbulent fluid flow and then the decellularized media, disseminated along the reservoir system. Then, the peaks in the velocity values at inlet and outlet does not represent a danger for the vascular tissue since the flow runs to stabilize along its route.

Finally, we conclude, that the bioreactor system will offer a constant laminar and non-turbulent flow for the decellularization tissue experiments to reduce the time and efficiency of the technique.

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