

Design and fabrication of a bioreactor for the proliferation of mesenchymal stem cells

Diseño y fabricación de un biorreactor para la proliferación de células troncales mesenquimales

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ABSTRACT

Currently, the number of organ donors is low and there are different methods to deal with this situation, including cell cultures, decellularization and bioreactors, which play an important role in helping the required processes to be carried out in a faster and more optimized way. The objective of this work is to make the cell proliferation process efficient using a bioreactor as an alternative, in which flow simulations can be made to previously analyze how it works. The radial flow hydraulic circuit was designed and simulated in SOLIDWORKS solving Navier-Stokes equations to verify that we have a non-turbulent laminar flow and simulate cell detachment without damaging the cell matrices. The design was printed with additive printing techniques, using photosensitive resin. Bioreactors also play a part in tissue engineering, as they allow for changes that can be monitored and reproduced in particular environmental factors. This article shows the successful design of a bioreactor to proliferate mesenchymal stem cells printed and subjected to simulations, whose results lead to the conclusion that there is non-turbulent laminar flow in the behavior of the system without subjecting the cells to a possible stress that causes damage.

KEYWORDS: tissue engineering; mesenchymal stem cells; bioreactors; flow simulation.

RESUMEN

Actualmente, la cantidad de donadores de órganos es escasa y existen diferentes métodos para enfrentar esta situación, entre ellos los cultivos celulares, la descellularización y los biorreactores, que juegan un rol importante ayudando a que los procesos requeridos sean llevados a cabo de una manera más optimizada y rápida. El objetivo de este trabajo es hacer el proceso de proliferación celular eficiente usando un biorreactor como alternativa, en el cual se pueden hacer simulaciones de flujo para analizar previamente cómo trabaja. Se diseñó y se hicieron simulaciones del circuito hidráulico de flujo radial en SOLIDWORKS resolviendo ecuaciones de Navier-Stokes para comprobar que tenemos un flujo laminar no turbulento y simular el desprendimiento celular sin dañar las matrices celulares. El diseño fue impreso con técnicas de impresión aditiva, utilizando resina fotosensible. Los biorreactores también toman parte en ingeniería de tejidos, ya que permiten hacer cambios que pueden ser supervisados y reproducidos en factores del medio en particular. En este artículo se muestra el diseño exitoso de un biorreactor para proliferar células troncales mesenquimales impreso y sometido a simulaciones, cuyos resultados llevan a concluir que existe flujo laminar no turbulento en el comportamiento del sistema sin someter a las células a un posible estrés que cause daño.

PALABRAS CLAVE: ingeniería de tejido; células troncales mesenquimales; biorreactores; simulación de flujo.

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I. INTRODUCTION

More than three hundred clinical assays are registered in the research field to judge the potential of Mesenchymal Stem Cells (MSC)-based cell therapy worldwide. Anyway, the amount of cells that can be obtained from available donors isn't enough. A potential solution is to amplify the culture of these cells *in vitro* to generate a relevant number of cells for particular applications [1]. With the advancement of clinical trials, MSCs have successfully cured and treated many diseases, including neurological, cartilage, orthopedic, immune, and non-immune [2]. The MSCs can be easily isolated from the adipose tissue, bone marrow, umbilical cord, fetal liver, muscle, and lung. While the promising benefits of MSCs are now believed to be due to more complicated mechanisms, they can be found in almost all tissues, can be successfully cultured *in vitro*, and can be differentiated into cell types such as neurons, stem cells, adipocytes, osteoblasts, chondrocytes and myoblasts.

MSCs can be transported together on scaffolds designed from natural or synthetic biomaterials, depending on the implementation. Two differentiated or undifferentiated MSCs can be loaded onto scaffolds prior to implantation into sites with damaged tissue. MSC scaffolds have been used successfully to repair damaged cartilage and long bones, generating well-integrated and functional hard tissues [3].

The advantage of using a delivery system with tissue-engineered MSC recliners is the easy to control and manipulating the implanted cells and tissues, with acute lower side effects impacting other systems in the body. These advances between the scaffolds and MSCs will help develop a comprehensive medical application technology.

Microfluidics-based microscale cell culture provides a well-controlled system with physiologically real-life parameters, including cell-cell interactions under biological flow conditions, oxygen, nutrient and drug delivery, metabolite removal, shear stress, and other force effects in cell culture. It is known that cell culture microenvironments can be significantly influenced and potentially improved by flow [4]. The systematic difference in cells between the distance and angle, where the angle consists of the flow direction, flow velocity, Young's modulus, and the shape, allows to describe the actual influence on a cell [5].

The functional and efficient implementation of technologies linked to mesenchymal stem cells will require a specific potential to produce cell biomass with particular characteristics. Stem cell proliferation on bioreactors is a hopeful process for the future implementation of MSCs, depending on purpose and conditions. These culture systems provide the ability to manipulate physiological conditions such as pH, temperature, pressure, oxygen and CO₂ concentration levels, and nutrient supply. Bioreactors must be designed to produce proliferated stem cells with consistent properties that can help make reproducible differentiation into selected mature cell types of high purity [6].

The objective of this work is to increase the current and static cell proliferation technique with the alternative of using a bioreactor that shows flow simulations to verify that we will have a non-turbulent laminar flow behavior and will not have a shear stress which can change or damage the cells.

II. METHODOLOGY

SYSTEM DESCRIPTION

A bioreactor was designed with the SOLIDWORKS 3D CAD software to proliferate mesenchymal stem cells in a closed circuit. At the start, a 100 ml cylindrical chamber of MSC's media was added. On top, there is an input injector with holes for MSC's growth, besides three outputs with different sizes to keep the media moving.

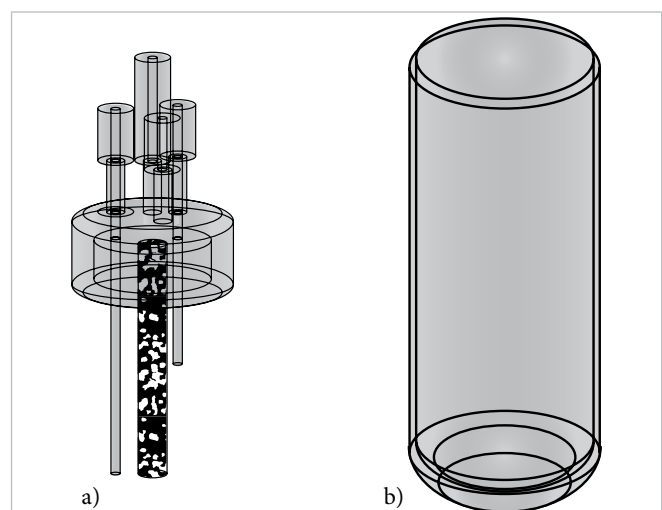


Figure 1. Unassembled parts of the bioreactor system: a) the injector (3.5 cm in length) with 3 outputs (2.8 cm in length) and b) the cylindrical chamber to contain the media (12 cm in length and 3.5 cm in radius).

3D DESIGN AND SIMULATION

The bioreactor system has a cylindrical chamber and an injector with holes and three exit tubes. Each one has a different length but the same diameter because it is intended to find out in which of the three the laminar flow couldn't damage the growth of the cells and have a homogeneous flow.

TABLE 1
DESCRIPTION OF THE INJECTOR TUBES SIZE

TUBE	LENGTH	DIAMETER
First	3.3 cm	0.4 cm
Second	5 cm	0.4 cm
Third	9 cm	0.4 cm

Flow Simulation in SOLIDWORKS provides tools for rotating regions selection and sliding mesh methods to accurately simulate system behavior based on the ge-

TABLE 2
SOLVER RESULTS OF THE BIOREACTOR MESHING

	TOTAL CELLS	FLUID CELLS	FLUID CELLS CONTACTING SOLIDS	CPU TIME PER LAST ITERATION	TRAVELS	ITERATIONS PER ONE TRAVEL
Meshing analysis	39,692	39,692	21,634	00:00:03	1.00069	1,770

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 eventual test with a higher resolution.

First, the pieces of the bioreactor were exported with .STL extension to add them to the Chitubox V2.2[®] 3D printing slicer software. It is designed to set up the models with just a few mouse clicks and to be able to set them up in horizontal or vertical positions to take advantage of more space and have a better printed model. In this case, the horizontal position was chosen for the two components. The resin parameters used were the following:

- Layer height 0.4 mm
- Lower/upper thickness 0.2 mm
- Print speed (mm/s) 6 sec
- Printing temperature 27 °C

The bottom of the object was cut 0.1 mm, overlay 0.15 mm and then 5 mm supports were added along the

ometry, angular velocity, and later displays the fluid response on the designed system.

For this flow simulation, the objective was to defeat the zero flow zones within the bioreactor to obtain a radial flow model that contributes the cell proliferation by optimizing the media volume to make three different comparisons using the three tubes to analyze at what height the cells can be multiplied without harming them.

The Meshing tool was also used to create finite elements that made the model and the corresponding analysis, as well as the solution of equations. A mesh with geometrical elements was created, taking into account the data of 39,692 fluid cells and 1,770 iterations. Previous of choosing the mesh, a primary mesh test was performed. A mesh with a double number of elements was created, being careful with the spatial distribution, and then the simulations were displayed.

length of the structure. Supports can be easily removed after printing.

After the components were configured, they were printed and then the parts were washed with isopropyl alcohol to remove any resin residue left on the components. Finally, the pieces rested for 20 minutes in water before being cured with the light of Anycubic Photon Mono X.

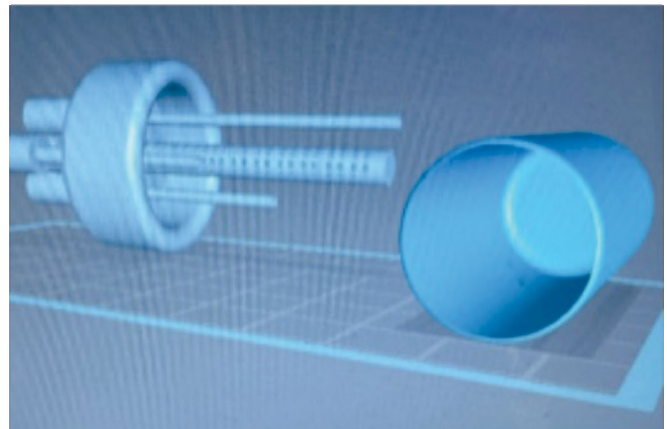


Figure 2. The pieces of the bioreactor were configured in horizontal position on the Chitubox software, ready to be printed.

III. RESULTS AND DISCUSSION

FLOW ANALYSIS

Flow simulation made it possible to quickly and easily simulate liquid flows through and around the bioreactor design to calculate the system performance and capabilities. For the initial simulation, the water density of 996 kg/m^3 was used. Functional simulation results are shown in Figure 3 with four different views.

In a), the media show a laminar vortex in the chamber with velocities of 0.021 m/s and 0.150 m/s in the three tubes, and no static zones are observed.

The first individual simulation b) was performed by the tube's length of 3.3 cm , and revealed the radial flow through the cylindrical chamber constantly showing a spiral shape at the top of the bioreactor with a speed of 0.129 m/s in the bioreactor's chamber and 0.064 m/s on this tube.

The simulation in c) showed a radial flow inside the second tube of 5 cm , with chamber's speed of 0.150 m/s and 0.70 m/s in the tube.

The last simulation d) corresponds to the tube's length of 9 cm , with a flow path concentrated at the bottom of the bioreactor with a speed of 0.171 m/s in the chamber and 0.107 m/s in the tube. The speed through our injector varies depending on the simulation in the tube. For the third tube, the speed is better at 0.214 m/s .

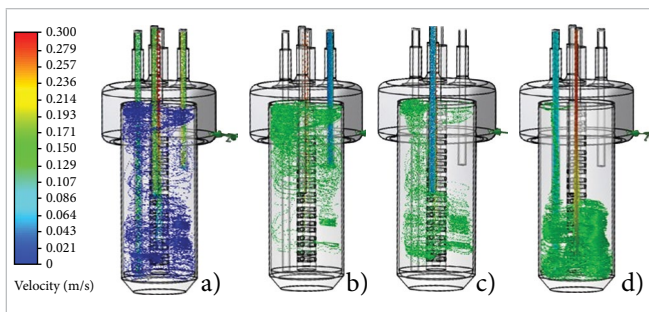


Figure 3. Flow Simulation components: a) showing all the flow trajectories, b) 3.3 cm tube flow trajectory, c) 5 cm tube flow trajectory, and d) 9 cm tube flow trajectory.

BIOREACTOR DESIGN PRINTED

Figure 4 shows the photo-sensitive resin 3D printed model and the cured model with ultraviolet light inside

the Anycubic Photon Mono X to generate translucent pieces for an easy view of the bioreactor function.



Figure 4. Bioreactor design for mesenchymal stem cells printed: a) curing the model inside the Anycubic printer with UV light and b) the printed model.

ADVANTAGES OVER THE MONOCULTURE SCAFFOLD

The bioreactor is advantageous to the proliferation of the MSCs because it can enhance its development and the cells could pass and circulate through the biomaterials covering the injector during the cell proliferation process, using the suitable biomaterials like sponges, culture forms a well-organized tissue like architecture that resembles organ histology. The cell layers of this architecture adhere to the fibers building a matrix able to form spheroids-shape colonies more efficiently than in a monoculture scaffold [7].

IV. CONCLUSION

The design of a bioreactor for the proliferation of mesenchymal stem cells has achieved the stated objective. The bioreactor, printed using a photosensitive hard resin following the flow simulations, allows us to conclude that the flow is non-turbulent laminar and without shear stress that can affect the cells, because if the liquid flow causes shear stress, there would be potential forces that would crash the cell morphology and performance in some way, such as cells reacting to shear stress by changes in gene transcription and translation, ion channel activation, and the restructuring of the whole cell layer.

As seen in the flow simulation results figures, the green and blue colors validate a correct laminar behavior in the system. Therefore, this bioreactor represents a proposal that could be used as a cell culture alternative, helping to succeed in methodical technology for medical and engineering applications.

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