Simulating Bioreactor Feature through CFD Tool for the Maturation of Fresh 3D Printed Human Organs

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Abstract
Bioprinting of tissues and organs can be defined as layer-by-layer additive robotic biofabrication of three-dimensional functional living macrotissues and organ constructs using tissue spheroids as building blocks. Closely placed tissue spheroids undergo tissue fusion, a process that represents a fundamental biological and biophysical principle of developmental biology-inspired directed tissue self-assembly. After the tissue spheroids structuring by rapid prototyping, the tissue/organ newly made is then carried out into a bioreactor which should play an important role of providing an adequate environment to the growth and maturation of the bioproduct. Bioreactors are used to accelerate tissue maturation through the control of their mechanical, biochemical and electrical conditions. Computer simulations are essential to the understanding of this context. So, this paper presents an initial study that reproduces part of the internal scenery of a bioreactor through simulations based on the finite element method run on Ansys CFX.

Keywords: 3D bioprinting, biofabrication, CFD, bioreactor, alginate.

1. Introduction
3D printing has figured as an emerging and hot technology in the last years. In this direction, the possibility to connect engineering with biological and medical concepts in order to get alternative ways for the tissue engineering is a great challenge and at the same time glad tidings for many people who are waiting for transplants and for solutions that can offer to them a new perspective of life. Bioprinting of human organs and tissues is on initial stage of development but some developments already achieved have shown that bioprinting is a potential technology.

2. 3D Organ Printing or Bioprinting
3D Organ Printing or Bioprinting gets feasible by association with additive manufacturing technology which can control the precise deposition of tissue spheroids digitally droplet by droplet shaping a volume according to a blueprint. Tissue spheroids
are spheres with some kind of hydrogel encapsulating live cells which will fuse with their neighborhood originating a premature organ which will need to be carried into a bioreactor to spend a time for maturation before being ready to its transplantation.

2.1. Bioprinting cycle
Bioprinting cycle (Figure 1) compounds four main phases: blueprint conception, bioprinting, maturation, and implantation. Just after bioprinting the new organ is not ready to be implanted. It needs a time to be maturated, which means to have the organ in physiological and mechanical stages that allow the organ to assume adequately its functions when already in vivo (after implantation).

![Figure 1. Flowchart of the 3D Bioprinting Cycle (CTI).](image)

2.2. Bioreactor for Maturation
Maturation of a biofabricated organ is a fundamental step. The new organ must be immersed into a bioreactor which is able to provide very specific conditions and therefore to simulate the real condition that organ will find after being implanted. Within the bioreactor the organ will accomplish its formation in terms of structure (shape) and functionality. A bioreactor may be defined as a fluidic system that simulates physiological or desired environment for the creation, maturation, physical conditioning, biomonitoring, and testing of viable tissue engineered constructs and/or isolated organs in vitro (Barron et al. 2003).

Dimensioning of a bioreactor for organ maturation is an arduous and extensive task. There are many variables to be considered and everything must be precisely controlled. It is expected that the bioreactor can supply, for instance, nutrients and oxygen, for the organ and, at the same time, take care of the outflow (excretion, for example). Further this, to control temperature, pH, pressure etc.

2.3. Irrigation dripping tripled perfusion bioreactor
It has been purposed a specific maturation system named as irrigation dripping tripled perfusion bioreactor (Figure 2a). In case of organ printing the function of perfusion bioreactor is to “buy time” necessary for post-printing tissue fusion, remodeling and maturation of bioprinted constructs. We introduce novel concept of irrigation dripping tripled perfusion bioreactor in order to allow bioprinted tissue construct including its vascular tree to maturate before initiating of biomimetic intravascular perfusion.
Three perfusion circuits in this novel type of perfusion bioreactor serve three purposes: one perfusion system provides wet environment around the printed constructs; second perfusion system is designed for intravascular perfusion of maturated build in vascular tree; and, finally, third perfusion circuits is designed for enabling the temporal interstitial flow through removable temporal porous minitubes (needles) (Figure 2b). These removable porous tubes also provide temporal support and serve as some sort of non-biodegradable but removable supporting structure or serve as an analog of scaffold in classic tissue engineering. The distance between these tubes as well as their porosity must be designed based on mathematical modeling and computer simulation.

Figure 2. Sketch of (a) the irrigation dripping tripled perfusion bioreactor concept (Mironov et al., 2009) and (b) a general needle.

3. Scenery of Simulations
Aiming at having a well dimensioned bioreactor its engineering should start by the most simplified analysis. Perfusion of the new organ will be done by needles-like structures that will help to support the organ and at the same time supply it and remove its excretions. An important starting step is to project the suitable needles. Parameters such as the number of pores at needle surface, the distance between these pores and the amount of parallel lines of pores are some of the geometry aspects to be known and primarily explored. Regarding these variables and that the inner of a bioreactor beholds a flow of fluids and dynamic behaviors, computational fluid dynamics software were adopted for the creation of similar sceneries and simulation of phenomena involved. Computational Fluid Dynamic software Ansys CFX® 12.0 has been used.

The needle (Figure 2b and Table 1) is filled with a fluid with water-like viscosity in a inflow velocity of 1mm/s. The needle is inserted in a volume with gel (alginate-like viscosity) (Figure 3). In this work, it was chosen an intermediary alginate concentration of 3%, where k = 6 Pa.s and n = 0.84 with density equals to 1.4 g/cm³ and a shear rate range from 0.01 to 100 s⁻¹ and an average viscosity in the range of 2.8 to 12.5 Pa.s (Rezende et al. 2009). The objective is to check how the water-like fluid spread throughout the needle penetrating the region with alginate. As much regular the distribution as better the flow diffusion and as more appropriate is the design of needle.

Figure 3. Needle with water within an alginate volume.
Table 1 – The needle geometry parameters adopted.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-newtonian system</td>
<td></td>
</tr>
<tr>
<td>Needle length</td>
<td>1500</td>
</tr>
<tr>
<td>Uniform pore diameter</td>
<td>40</td>
</tr>
<tr>
<td>External needle diameter</td>
<td>470</td>
</tr>
<tr>
<td>Internal needle diameter</td>
<td>420</td>
</tr>
<tr>
<td>All the units in μm</td>
<td></td>
</tr>
</tbody>
</table>

The instantaneous equations (Eqs. 1 to 3) of continuity and momentum of the fluid dynamic phenomenon can be written as follows:

The Continuity Equation:
\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{U}) = 0
\]  

(1)

The Momentum Equation:
\[
\frac{\partial (\rho \mathbf{U})}{\partial t} + \nabla \cdot (\rho \mathbf{U} \otimes \mathbf{U}) = -\nabla p + \nabla \cdot \tau + S_{M}
\]  

(2)

where the stress tensor, \( \tau \), is related to the strain rate by:
\[
\tau = \mu (\nabla \mathbf{U} + (\nabla \mathbf{U})^T) - \frac{2}{3} \delta \nabla \cdot \mathbf{U}
\]  

(3)

4. Results and Discussion

Water-like material was inside needle and alginate-like outside. Water-like flow was set to force its exit from needle penetrating externally the alginate volume. Simulations results show the spread interface between alginate and water through the volume fraction. Two cases were analyzed: (1) needle without internal microchannels and (2) needle with internal microchannels.

In Case 1, with no internal microchannels, the simulation showed the tendency the flow has to leave the needle through the first rings of pores. In Figure 4a, the number of pores per ring is constant and in Figure 4b is variable. This variability got a little bit more spreading inside the needle, however, for the time considered in the simulation, the flow kept the tendency of leaving the needle by the first rings.

Figure 4. Needles of 14 rings: 8 pores per ring (a) and variable number of pores (b).

In Case 2, microchannels were incorporated internally in the needle. Here, two different angles for the microchannels orientation were taken: 60° and 90°. By Figure 5a-b, it can be observed that the spread outside the needle is much more uniform for both cases than that showed in Case 1, reaching a larger range in the alginate volume and also in out-
front of the needle. Figure 6 shows the design and the mesh for 60° angle scenery. Only as detail, Figures 4 and 5 are mirrored to each other with no impact.

![Figure 5. Microchannels oriented by angles of 60° (a) and 90° (b).](image)

![Figure 6. Illustration of design (a) and mesh (b) of microchannels oriented in 60° angle.](image)

5. Conclusions

Bioreactor designing is too complex but is fundamental count on computer simulations which aid to direct the way and to get shorter the time spent on the developments. Microchannels have demonstrated to be potential to this application. Primarily, the study has been carried in order to know the behavior of spread of two different viscous materials within each other through a microscopic needle. This flow understanding is part of the global concept of the irrigation dripping tripled perfusion bioreactor. One of the next steps after this current work is to integrate many needles in parallel and analyze the bioreactor internal flow.

6. Acknowledgements

Our thanks by the financial support of The São Paulo Research Foundation (FAPESP), The Brazilian Institute of Biofabrication (INCT-Biofabris), and The National Council for Scientific and Technological Development (CNPq) through PIBIC program.

References


