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Development of a Bioreactor by Computer Fluid Dynamics Simulations for the Maturation of 3D Printed Organs by Rapid Prototyping

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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. The microtissues and tissue spheroids are living materials with certain measurable, evolving and potentially controllable composition, material and biological properties. 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, 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. The creation of a representative environment inside the bioreactor is too complex since it can enclose a large range of variables. The simulation of this scenery is essential to the study and the success of tissues and organs bioprinting is straight linked to a set of an appropriate environment in the bioreactor that assure the feasibility, maturation, biomonitoring, tests, storing and transport of the involved elements on the generation of the new tissue such as the deposited cells and nutrients. Computational fluid dynamic (CFD) software packages have been a powerful tool to calculate flow fields, shear stresses and mass transport within and around 3D constructs, including a bioreactor environment. This work presents an initial study that reproduces the internal scenery of a bioreactor with some of the main variables through simulations based on the finite element method run on Ansys CFX package software.

1. The idea of printing human tissues and organs

The field of tissue engineering (regenerative medicine) aims to repair and regenerate damaged tissues by developing biological substitutes that mimic the natural extracellular matrix to help guide the growth of new functional tissue in vitro or in vivo to restore, maintain or improve tissue function (Langer and Vacanti, 1993). The ultimate goal of tissue engineering is the manufacture of living functional tissues and organs suitable for transplantation in reasonable time scales. Organ printing could be defined as computer-aided 3-D tissue engineering of living organs based on the simultaneous deposition of cells and hydrogels with the principles of self-assembly (Mironov et al., 2009). Bioreactors provide a fluidic environment for tissue engineered tissue and organs, and guarantee their viability, maturation, biomonitoring, testing, storage, and transportation. There are different types of bioreactors and they vary greatly in their size, complexity, and functional capabilities. Although progress in design and functional properties of perfusion bioreactors for tissue engineered blood vessels, heart valves, and myocardial patches is obvious, there are some challenges and insufficiently addressed issues, and room for bioreactor design improvement and performance optimization. These challenges include creating a triple perfusion bioreactor for vascularized

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tubular tissue engineered cardiac construct; designing and manufacturing fluidicsbased perfused minibioreactors; incorporation of systematic mathematical modeling and computer simulation based on computational fluid dynamics into the bioreactor designing process; and development of automatic systems of hydrodynamic regime control.

1.1 The 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).

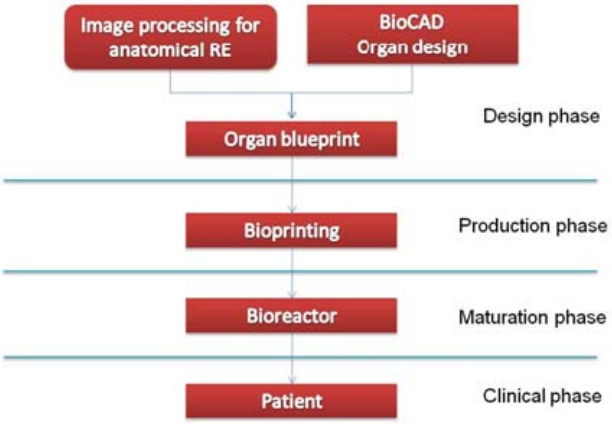

Figure 1: Flowchart of the 3D Bioprinting Cycle (CTI).

2. The bioreactor as organ maturator

A tissue engineering bioreactor can be defined as a device that uses mechanical means to influence biological processes (Darling and Athanasiou, 2003). Bioreactors can be used to aid in the *in vitro* development of new tissue by providing biochemical and physical regulatory signals to cells and encouraging them to undergo differentiation and/or to produce extracellular matrix prior to in vivo implantation. Bioreactors are devices in which biological or biochemical processes develop under a closely monitored and tightly controlled environment (Partap et al., 2010). First, they must maintain the viability of the engineered tissues. Second, they are often (but on always) used as a cell-seeding device. Often they can be used for accelerating tissue maturation using mechanical, biochemical, and electrical stimuli conditioning. They also can be used for testing and monitoring maturation processes in tissue-engineered constructs. Finally they can serve for storage, preservation, and transportation of tissue engineered constructs (Mironov et al., 2006). A bioreactor has to be able to operate over long periods of time under aseptic conditions since maturation of a functional tissue may take up to 3-4 months. Providing three-dimensional tissues with nutrients may rely on passive diffusion, or may be more actively delivered by direct perfusion. However, direct perfusion introduces a new level of complexity when scale-up is encountered, and the engineering challenges may be significant. Tissues that have been manufactured to date have relied on diffusion, although tissues envisioned for future products will require a more active delivery process (Korossis et al., 2005). 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. 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.

2.1 Irrigation dripping tripled perfusion bioreactor

It has been purposed a specific maturation system named as irrigation dripping tripled perfusion bioreactor (Figure 2). In case of organ printing the function of perfusion bioreactor is to “buy time” necessary for post-

printing tissue fusion, remodelling 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). 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.

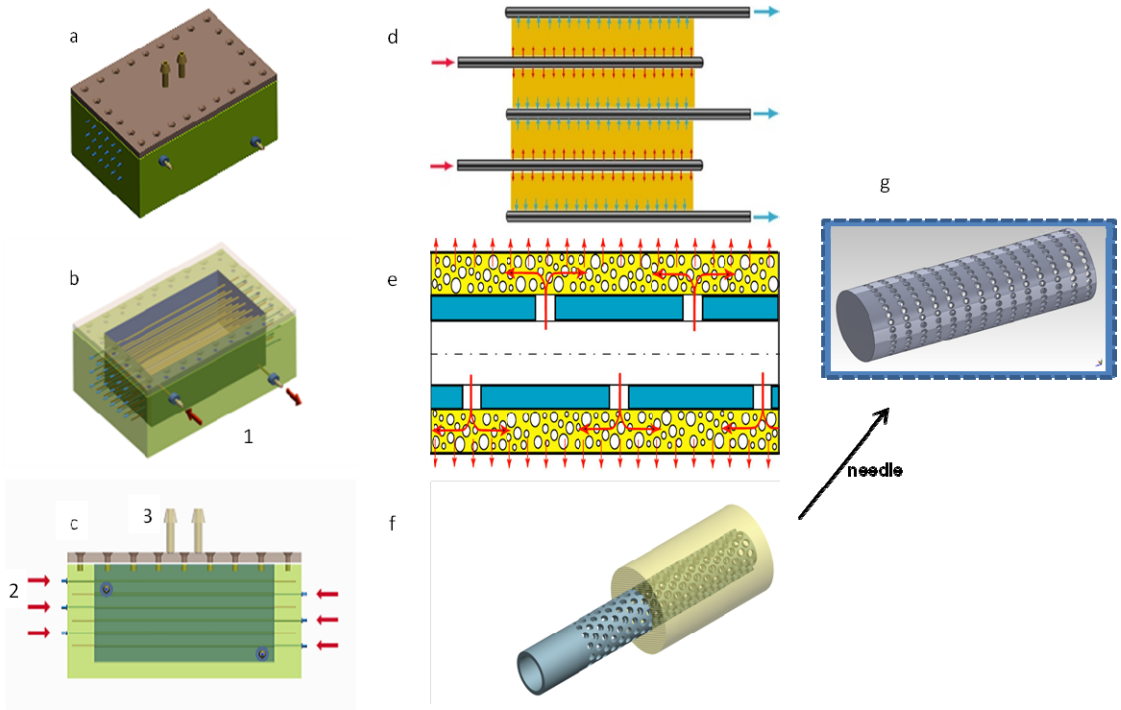

Figure 2. Design of an irrigating triple perfusion bioreactor.

The design of the irrigating triple perfusion bioreactor is basically comprised by: (a) Design of the triple perfusion bioreactor chamber; (b) Design for the placement of temporally removable porous tubes into the triple perfusion bioreactor chamber. The inlet and outlet for the extra-construct perfusion circuit (1); (c) Design of the second circuit (2) based on the use of temporally removable porous tubes for interstitial intra-construct perfusion, and the inlet and outlet of third circuit (3) for intravascular perfusion; (d) Scheme demonstrating the fluid flow pattern between temporally removable porous tubes in the triple perfusion bioreactor; (e) Scheme demonstrating a longitudinal section through the wall of temporally removable porous tube and the flow of fluid through its porous wall; (f) Computer-aided design of a temporally removable porous tube for the triple perfusion bioreactor (according to Mironov et al., 2011); (g) a general needle.

3. Behind the simulations

Computational simulation allows optimal design of the bioreactor, improved mass transfer, and guarantee that all cells will receive adequate oxygen and nutrient supply. 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 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 behaviours, computational fluid dynamics

software was adopted for the creation of similar sceneries and simulation of phenomena involved. Computational Fluid Dynamic software Ansys CFX® 13.0 has been used. The needle (Figure 3) is filled with a fluid with water-like viscosity. The needle is inserted in a volume with gel (alginate-like viscosity) (Rezende et al., 2007). 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/cm^3 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.

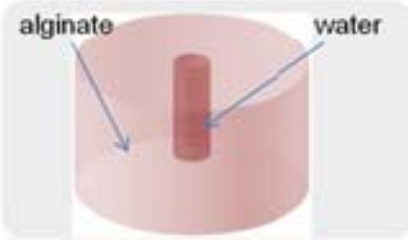

Figure 3. Needle with water within an alginate volume.

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

The Continuity Equation:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho U) = 0 \quad (1)$$

The Momentum Equation:

$$\frac{\partial (\rho U)}{\partial t} + \nabla \cdot (\rho U'' \, \square U'') = -\nabla p + \nabla \cdot \tau + S\_M \quad (2)$$

where the stress tensor, τ, is related to the strain rate by:

$$\tau = \mu(\nabla U + (\nabla U)^T - \frac{2}{3}\delta \nabla \cdot U) \quad (3)$$

Figure 4 shows examples of a previous work aimed at verifying the flow in isolated needles before simulations comprehending a set with input and output needles.

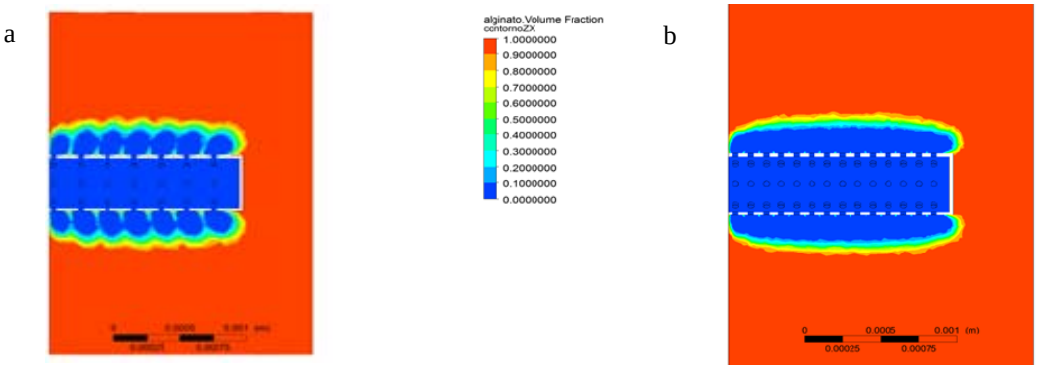

Figure 4: Flow of water into alginate in one single needle: (a) with 7 rings, (b) with 14 rings (more homogeneous spreading).

Following, the Figure 5 shows the flow of water through alginate inside a domain containing four input and four output needles with an outflow with velocity equals to 5 mm/s. Complementarily, Table 1 presents the parameters set up for these simulations.

Table 1: Input data for the simulations (1 mm/s).

Total time (s)	Time step (s)	Inlet Velocity (mm/s)	Outlet Velocity (mm/s)	External domain (µm)	Needle lenght (µm)	Pores diameter (µm)
30	0.25	5	5	3000	1200	5

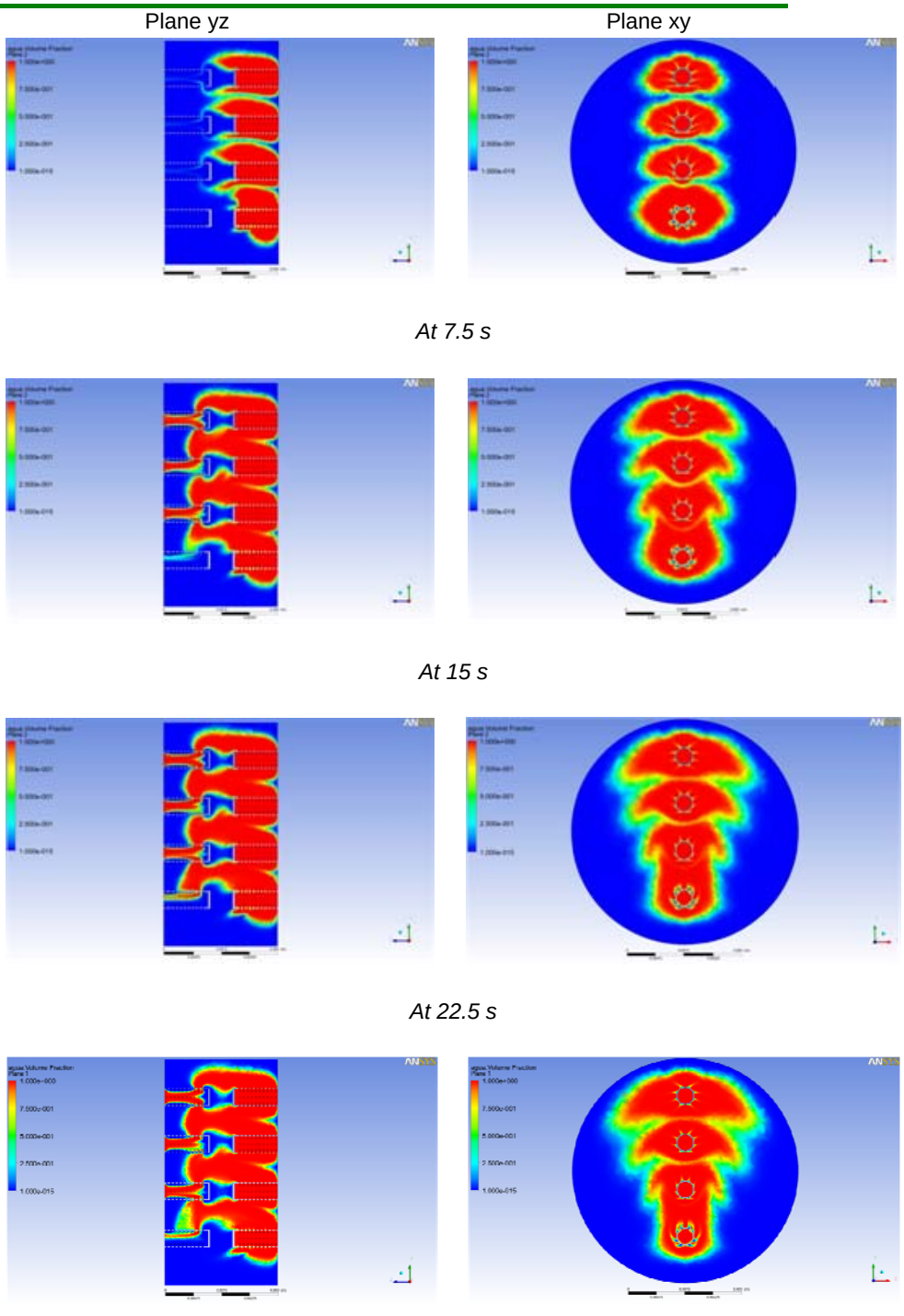

Figure 5: Water volume for outlet velocity equals to 5 mm/s at different moments.

The effect of density is easily perceived. The tendency is that, with higher density, the alginate pushes water up. This must be considered in the next studies in such way, for example, that the geometry of lower needles can be designed offering less resistance to the water passage.

4. Conclusions

Engineering a specific bioreactor is a highly complex work. However, computer simulations can diminish the efforts disposing improbable ways and spending less time on the general project.
Primarily, the study has been carried in isolated needles in order to know the behavior of spread of two different viscous materials. The analysis of the flow through the needles is part of the global concept of the irrigation dripping tripled perfusion bioreactor. The integration of many input and output needles showed at least theoretically that this system can be tuned for different parameters and materials and that from now one new characteristics can be incorporated.

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