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# MODELING ON THE THREE-DIMENSIONAL PRINTING OF HUMAN ORGANS

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**Abstract**. This work demonstrates how information technology can be employed onto biofabrication, specifically showing a computational modeling and simulation. Organ printing is an emerging solid scaffold-free biofabrication technology or layer by layer additive bioprinting of functional 3D human tissue and organ constructs from self-assembling tissue spheroids. Organ printing comes as an alternative way to the tissue engineering. Damaged and diseased organs could be replaced by the new organ fabricated by a 3D printer. The biofabrication cycle is too complex. On the beginning, the input is feed by medical images or an organ design. At an intermediary level, the blueprint and bioprinting further the bioreactor for the organ maturation take place. At the end, the clinical phase. In order to have a final 3D bioprinted organ, the complete biofabrication cycle has to be accomplished. All of these phases are associated to the information technology (IT) since image treatments, computational simulations, organ representation through a virtual model are straightly involved. At this moment, the focus is on the maturation phase. Mathematical modeling and computer simulation have been used to estimate proper design parameters and maturation scenario. After 3D printing the human organ, this needs to be matured in a bioreactor in order to be implantable. Bioreactors are used to accelerate tissue maturation through the control of their mechanical, biochemical and electrical conditions maintaining the viability of the engineered tissue. This work presents an incipient study that reproduces elements included in the bioreactor approach with some variables considered at the simulations based on the finite element method running on Ansys CFX software.

## 1 INTRODUCTION

Organ printing or 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. First of all, they should maintain the viability of the engineered tissue. Following, they are many times employed as equipment to the cell seeding and can be also applied to experimental and monitoring of maturation processes. The creation of a representative environment inside the bioreactor is too complex since it can enclose a large range of variables.

Simulating this scenery is essential to the developments. The success of tissues and organs bioprinting and their real usage are straight linked to the set of a suitable environment in the bioreactor that assures 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. As an example, the perfusion and fluid diffusion within the organs in maturation process in bioreactor is fundamental for understanding of the phenomenon. Computational fluid dynamic software packets have been increasingly developed during the past decade and are powerful tool to calculate flow fields, shear stresses and mass transport within and around 3D constructs, including a bioreactor environment.

## 2 ORGAN BIOFABRICATION

Diseases, accidents, and congenital malformation are critical issues that move the tissue engineering developments around the world. During last decade organ printing has been rapidly emerged as a potentially superior alternative of classic solid scaffold-based approach in tissue engineering. It is becoming increasingly obvious that organ biofabrication could not be reduced anymore to just simple one step bioprinting process and it rather represents an integrated complex of enabling technologies which could be arranged into organ biofabrication line.

Some highlightable motivations for Biofabrication development are:

- ✓ World shortage of donors for tissue and organs
- ✓ Limitations on man-made biomaterials
- ✓ Recent advances in biology and possibility to mass production

Nowadays, donor´s tissue and organ transplantation are the main solutions however some aspects are obstacles and limitations that, on the other hand, stimulate the development of more promising therapies and the development of biological substitutes without necessity of donors such as:

- ✓ Intense immunological response
- ✓ Use of immune suppressor drugs for the life
- ✓ Diseases cross transmission
- ✓ High costs

Organ printing is the layer by layer additive biofabrication of functional human tissue and

organ constructs using self-assembling tissue spheroids as building blocks (Mironov et al. 2009a). Organ printing cycle (see Figure 1) includes three main sequential steps that were drawn from rapid prototyping technology: the design of the organ "blueprint" with special software (pre-processing), the actual printing with robotic bioprinters (processing) (see Figure 2), and the accelerated tissue maturation with bioreactors (post-processing) (Mironov et al. 2008).

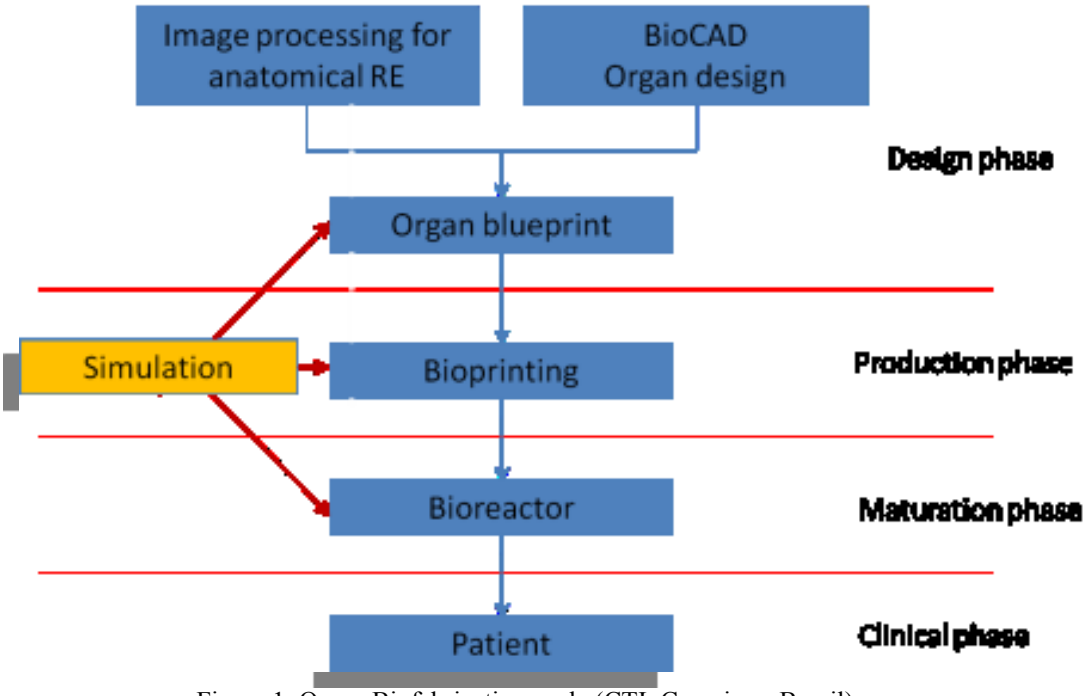



Figure 1. Organ Biofabrication cycle (CTI, Campinas, Brazil).

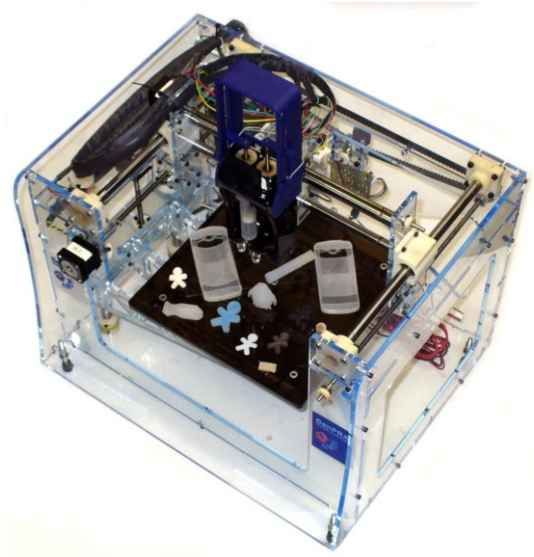


Figure 2. The layer-by-layer additive robotic manufacturing Fab@CTI.

## 2.1 Computer Assistance

The biofabrication cycle begins either from medical images or an organ design made by CAD. In terms of image treatment, normally, the most common types of images used to feed the biofabrication process are those ones come from computer tomography (CT) or magnetic resonance (MR), which are in fact a sequence of 2D images. CT and MR can provide rich details of the patient since the existent difference of colours (gray scale) provides information about the kind of tissues, if they are soft or hard, for example.

These images are input for InVesalius which is open-source public software originated at CTI. This software is capable to join all the 2D images transforming them to a perfect 3D model.

Other input is the direct 3D modeling of a body structure. Much software is available in the market to create 3D designs with the highest complexity as such as Rhinocerus, Solidworks, Pro-Engineer etc. The generated model is then used as a pattern to the production of human organs shapes or tools as, for instance, a skullcap, a heart valve or kidney or, also, a syringe for the bioreactor and a mold to be used as structure for cell seeding, which will be employed during the biofabrication cycle

Before producing a physical 3D model, it is fundamental to generate a virtual environment that represents the real conditions where the model will be inserted and employed. Software as Abaqus, FEMAP, NEi Nastran and ANSYS are available in the market for this purpose. There is a very wide range of situations that can be simulated and also many variables embedded in the process. It can be simulated a static case of how dental structures to be used as replacements for a mandible will behavior under tension or shear stresses. Also, a dynamic approach can be done for the behavior of fluids through an artery. The possibility to use numerical methods as finite elements powers the project since this can lead to the reduction of errors, rejections, bad-engineered models and increase the accuracy. Focusing at the bioprinting, the human organ just fabricated is not completely ready to be implanted. It needs to be carried out into a bioreactor in order to get an improvement in terms of its functionality. The organ can be put under special conditions similarly to the host environment where the organ will take place *in vivo* to become more mature. The organ must receive substrates as nutrients and oxygen for its survival and at the same time perform the excretion putting out organic residues. The end step in the cycle is the clinical phase when the patient receives the new matured organ.

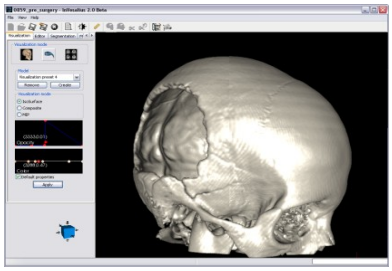
## 2.2 Blueprints for organ printing

Organ printing as any rapid prototyping technology in essence is an information technology because it transforms virtual reality of computer-aided design (CAD) or “organ blueprint” into physical reality or bioprinted organ. Although CAD is well established technology in additive manufacturing with standard software, there is no detailed “blueprint” for printing complex 3D human organs. Human anatomy is also well established discipline with detailed knowledge microscopic organization of all organs and tissues. Clinical bioimaging technologies are constantly improving and resolution of Magnetic Resonance (MRI), Computed Tomography (CT) and Micro-CT is constantly improving or already approaching closely the desirable level of resolution which is sufficient enough to get necessary anatomical and histological information for organ bioprinting. Computer-aided reconstructions of serial histological sections of human organs can provide information about histological organization of human organ which is not yet possible to get using in vivo bioimaging. Finally, mathematical modeling of anatomical and histological structures is increasingly used as

additional powerful tool to design blueprint. Thus, the real challenges in designing CAD of human organs is to not absence of required anatomical information, but rather the ways how to transform this accumulated knowledge of human anatomy and histology into a viable blueprint with sufficient and necessary instruction for robotic printer how to print human organ.

On the beginning, it is necessary that a virtual model is designed. This design can be based on medical images and also in 3D modeled. Figure 3 shows an example of both the cases.

a



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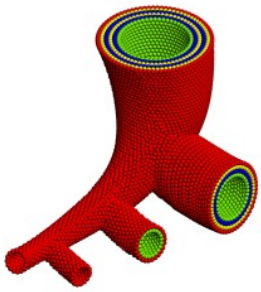

Figure 3. Input for biofabrication cycle used as models also for the simulations: Software for organ printing (CTI, Campinas, Brazil): (a) 3D model by medical imaging obtained with InVesalius by CTI, and (b) Computer-aided design of a vascular branch.

## 3 BIOREACTORS

Bioreactor is also some sort of container which allows keeping tissue engineered constructs in wet environment and thus maintains their viability and also serves as packing and transportation device for matured tissue engineered constructs (Rezende et al. 2011a). Bioreactor is an essential component of classic tissue engineering. There are several reviews (Martin et al. 2004; Mironov et al. 2006) and at least two books specially devoted to bioreactor technologies (Chaudhuri & Al-Rubeai, 2005).

Bioreactors also allow studies of mechanical stimuli on 3D tissue structures. Mechanical stimuli, such as shear stress due to flow characteristics, have been shown to have a great effect on the development of tissues (Ellis et al. 2005). For example, cardiac muscle in vivo encounters strong pulsatile flows, whereas bone constantly encounters mechanical stress and compression (Langer et al. 2008).

Bioreactors for stem cells propagation are necessary if amount of human cells which is possible to get from human cell source are not sufficient in numbers in order to bioprint human organs for example human dental pump stem cells or bone marrow derived stem cells. In essence it is an issue of scalability. Commercially available bioreactors for stem cells propagation are already reality as shown in Figure 4.

Figure 4. Bioreactor for stem cells propagation (Aastrom Inc, Ann Arbor, MI, USA).

### 3.1 Maturation of Organs

It is important to realize that outcome of bioprinting is not a functional tissue construct immediately suitable for implantation. In order to transform bioprinted 3D tissue and organ construct into functional tissue the bioprinted construct must undergo accelerated issue maturation. This process is called accelerated tissue maturation (Rezende et al. 2011a).

Initially bioreactors have been used as tool for enhancing cell seeding on solid scaffolds. Perfusion bioreactors additionally have been used as a tool for providing mechanical conditioning of tubular tissue engineered constructs. 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. The bioprinted tissue construct even with "build in" vascular system is not ready for immediate intravascular perfusion because it takes certain time for vascular tissue spheroids to fuse and resulted vascular tree to be sufficiently mature and ready for intravascular perfusion. 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. (see Figure 5).

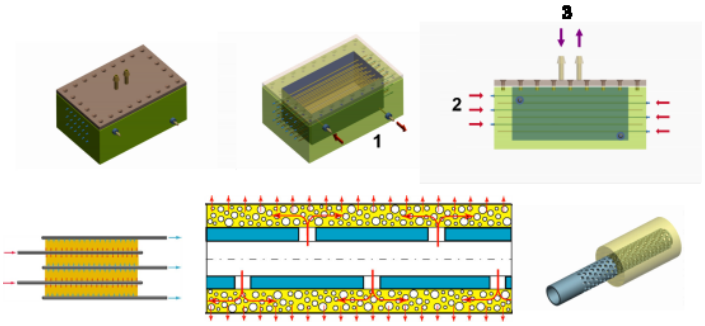


Figure 5. Conceptual design of irrigation dripping tripled perfusion bioreactor for bioprinted organs (Mironov et al. 2009).

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. 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 (Rezende et al. 2011b). The design of porous removable minitubes for irrigation bioreactor is shown on Figure 6.

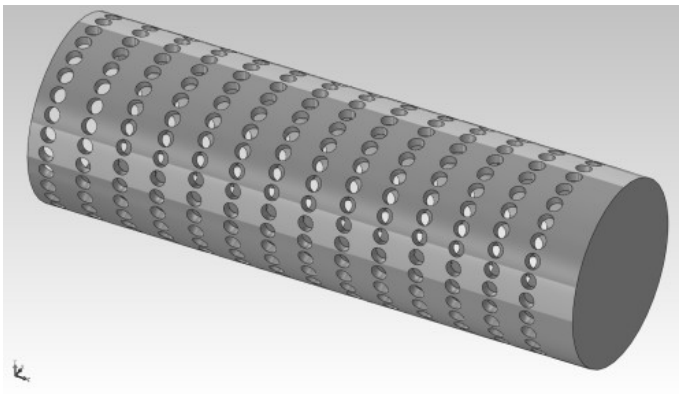

Figure 6. Computer-aided design of porous syringe with 14 rings for irrigation dripping tripled perfusion bioreactor.

The removable minitubes must be as thin as possible and novel strong material (composite with carbon nanotubes) must be probably used for manufacturing of such minitubes. Finally, these minitubes must be coated with non-adhesive porous inert Teflon-like biomaterial which will avoid cell adhesion and consequently their non-destructive removal after bioprinted tissue maturation and switching from interstitial to intravascular perfusion. Rationale design of irrigation dripping tripled perfusion bioreactors is first step on the way to build such novel type of bioreactor. Selection and testing proper materials for removable porous minitube is a second step. Finally, fabrication and testing bioreactor is third and last step. It is interesting that removable porous tube can have additional functions: they can as biosensors of functional maturation of bioprinted tissue, they can be used for delivery soluble extracellular matrix molecular and even cells for accelerating tissue maturation, and finally they can be use for providing electric stimulation and other physical methods enhancing functional tissue maturation. The design, manufacturing, testing and further optimization of such novel type of perfusion bioreactor are not trivial but feasible engineering task. At least the conceptual design of such bioreactor has been already accomplished. Economic irrigation dripping approach which was already successfully used in agricultural irrigation will allow dramatically reduce cost of perfusion by more rational using of expensive perfusion cell culture media. Thus, it is obvious that development of perfusion bioreactor is critically important and it is essential enabling technology for organ printing.

One of principal and not properly addressed question in emerging organ printing technology is how to estimate that bioprinted 3D tissue and organ construct is became functional and ready to implantation. For example, biosensor can estimate impedance or tissue electroconductivity and thus judge tissue maturation. Non-invasive and non-destructive bioimaging technology such as ultrasound and MRI can be also employed (Rezende et al. 2011a).

## 4 COMPUTATIONAL FLUID DYNAMICS (CFD)

Computational simulations are widely welcome during biofabrication cycle. The simulations were carried out in ANSYS CFX®. The work aimed at the simulations to evaluate the needles positioning and distribution in the bioreactor environment including the distance between the needles, the number of pores in each one, the pores diameter, in order to optimize those parameters for a better tissue perfusion. This present and preliminary study concerns the evaluation of just some parameters at the needle such as the pores diameter, the number of rings at long the needle and the number of pored per ring. Future studies can include the association of many needles with controlled temperature, nutrient and oxygen taxes, stress and strain, for example.

Software ANSYS CFX® makes use of an element-based finite volume method to discrete the governing equations, which firstly involves to discrete the spatial domain using a mesh. The mesh is used to construct finite volumes, which are used to conserve relevant amounts such as mass, momentum, and energy. A control volume is constructed around each mesh node and these equations are integrated over each control volume (ANSYS CFX® Guide, 2009).

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

The Continuity Equation:

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

The Momentum Equation:

$$\frac{\partial (\rho U)}{\partial t} + \nabla \cdot (\rho U'' \otimes \mathrm{U}'') = -\nabla \mathrm{p} + \nabla \cdot \tau + \mathrm{S\_M} \tag{2}$$

where the stress tensor, $\tau$, is related to the strain rate by:

$$\tau = \mu(\nabla U + (\nabla U)^T - \tfrac{2}{3}\delta \nabla \cdot \mathrm{U}) \tag{3}$$

The resultant flow of a liquid through a needle with many holes or pores laterally and with the final tip completely closed. Some important variables are the diameter of each pore, the number of pores per ring and the distance between the rings. All of these parameters were considered in this work.

See Table 1 which describes the needle geometry data taken.

| Non-newtonian system | |
|---|---|
| Needle length | 1500 |
| Uniform pore diameter | 40 |
| External needle diameter | 470 |
| Internal needle diameter | 420 |
| *All the units in μm* | |

Table 1 – The needle geometry parameters adopted.

A natural polymer, the hydrogel alginate is the material inside and outside the needle (within a volume) as shown in Figure 7 and water being injected into the needle with velocity equals to 1 mm/s trying to push alginate out.

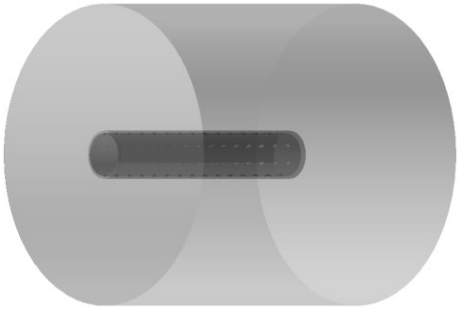

Figure 7. A needle involved by a volume of hydrogel.

Based on Rezende et al. (2009) data, an intermediary alginate concentration level of 3% was taken, 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. These parameters are connected in the equation 4 that represents the Power-Law rheological model:

$$\tau = k \cdot \dot{\gamma}^{n} \tag{4}$$

where $\tau$ is the shear stress (Pa), $\gamma$ is the shear rate ($s^{-1}$), k is the consistency index (Pa.sn) and n is the Power-Law index (dimensionless).

## 5 RESULTS

Two approaches were considered: linear and logarithmic which are exploited during the results discussions.

**Linear setting**

First of all, it was considered a condition in which the needle was composed with 28 linear rings (see Figure 8) containing 30 pores each one along the length of the needle.

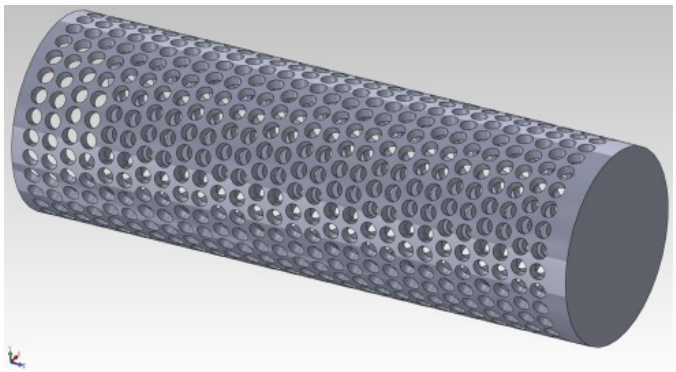

Figure 8. Model of needle with 28 rings.

The simulation represents a real period of 12 seconds. What it can be also noted is that the

water volume leaves the needle practically through the pores at the first ring of pores and the water does not get to push the alginate content which is more dense along the needle length. The exit at first ring is the easiest way the water finds to escape to outside. Figure 9 shows that it is possible to subtly observe the effect of gravity (according to y axis) having the middle line as a reference.

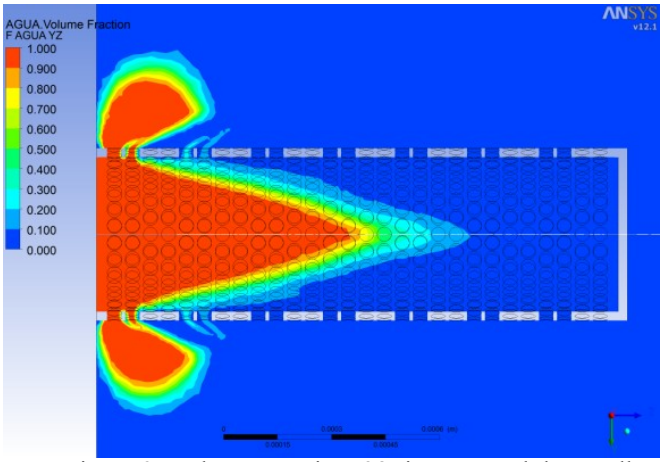


Figure 9. Volume Fraction: 28 rings around the needle

In Figure 10 the number of rings is half-reduced (as previously illustrated in Figure 6) and kept the diameter of the pores.

The water content reached less volume than in the case presented in the Figure 9 and therefore more water escaped through first ring. This case considers the same real period of 12 seconds.

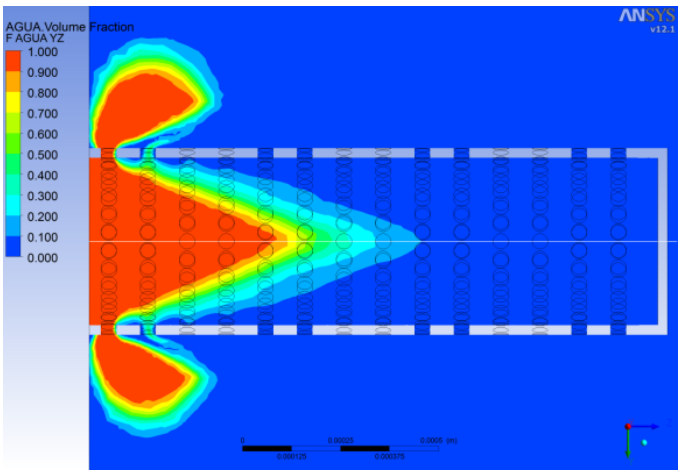


Figure 10. Volume Fraction half of the original rings

The flow field created outside the needle difficult the exit through other pores.

Figure 11 shows the resizing of number of pores per ring. Instead of 30 pores, now the scenario considers 8 pores. This case and the next were simulated for real 5 seconds, so less

than the cases before.

In Figures 11 and 12, after 5 seconds (much less than in Figure 10), the water content achieved the centre of the fifth pore and between fifth and sixth pores, respectively. This demonstrates that as less as the amount of porous per ring, more the water content is spread along the needle.

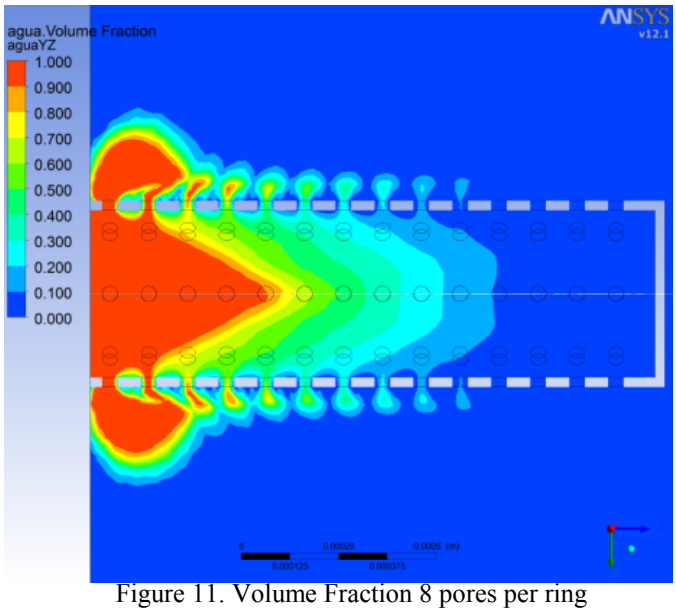


Figure 11. Volume Fraction 8 pores per ring

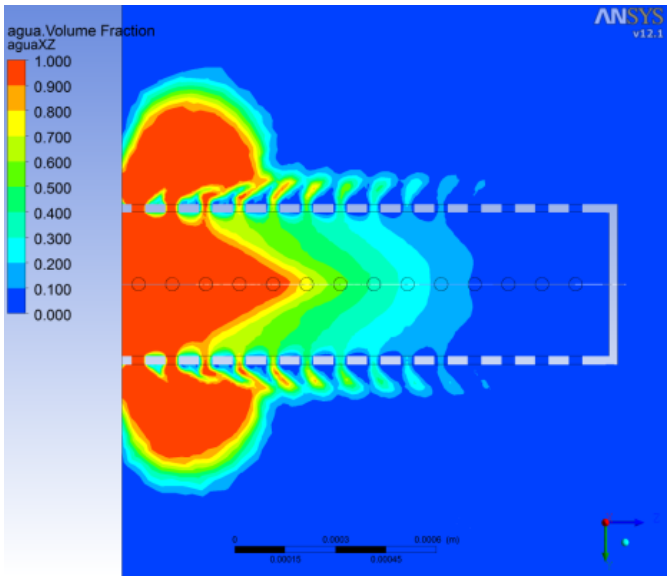


Figure 12. Volume Fraction 4 pores per ring

It is interesting to observe that as less the number of pores per ring as much the spread outside the needle and also more pores are crossed, that is, in Figure 12, the field of water volume generated outside the needle is larger and it is feed now from new pores.

**Logarithmic setting**

As it has been observed that the water content trends to leave the needle through the initial pores, it was though a way to make easier the flow through next pores. Then, a logarithmic distribution was considered for the number of pores at long the needle increased as shown in Figure 13 and also for the pore diameter varying at long the needle (being constant at the same ring). The logarithmic scale was chosen since there is a fast increase on the beginning in order to compensate the high flow escaping from the first ring (see Table 2 – column: number of pores per ring).

| Ring | Number of pores per ring | | Pore Diameter (mm) |
|---|---|---|---|
| | Non-discrete | Discrete | |
| 1 | 5.00 | 5 | 10.2 |
| 2 | 7.77 | 8 | 11.8 |
| 3 | 8.43 | 8 | 13.6 |
| 4 | 8.82 | 9 | 15.3 |
| 5 | 9.11 | 9 | 17.2 |
| 6 | 9.33 | 9 | 19.2 |
| 7 | 9.49 | 9 | 21.3 |
| 8 | 9.66 | 10 | 23.5 |
| 9 | 9.79 | 10 | 25.8 |
| 10 | 9.91 | 10 | 28.2 |
| 11 | 10.01 | 10 | 30.9 |
| 12 | 10.11 | 10 | 33.7 |
| 13 | 10.19 | 10 | 36.7 |
| 14 | 10.27 | 10 | 40.0 |

Table 2. Number of pores per ring (non-discrete and discrete) and pore diameter adopted values.

Figure 13 presents the volume fraction while Figure 14 shows the alginate velocity spreading from the needle to outside.

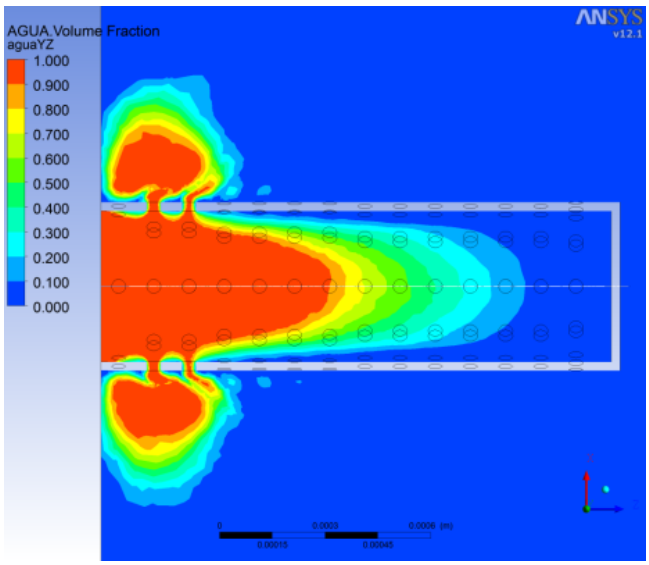


Figure 13. Volume Fraction with logarithmically pores distribution per ring.

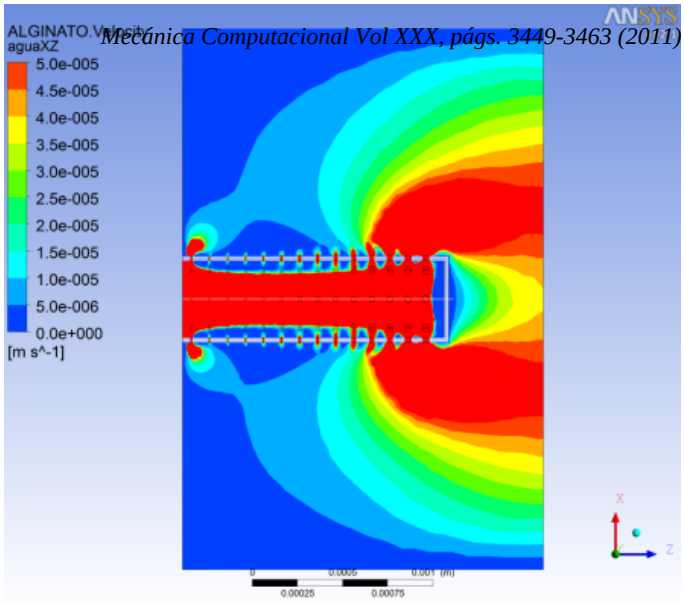


Figure 14. Velocity of alginate leaving the needle (with logarithmically pores distribution per ring).

Figure 15 presents a distribution where the pore diameter dimensioning was also logarithmic according to the Table 2 (column: pore diameter), where all the 14 rings are composed from 5 to 10 pores each one progressively. In this case, there is an inversion about the exit flow profile. The larger diameters into the end created a way with fewer barriers to water spread itself. Now, all the pores are used flow water.

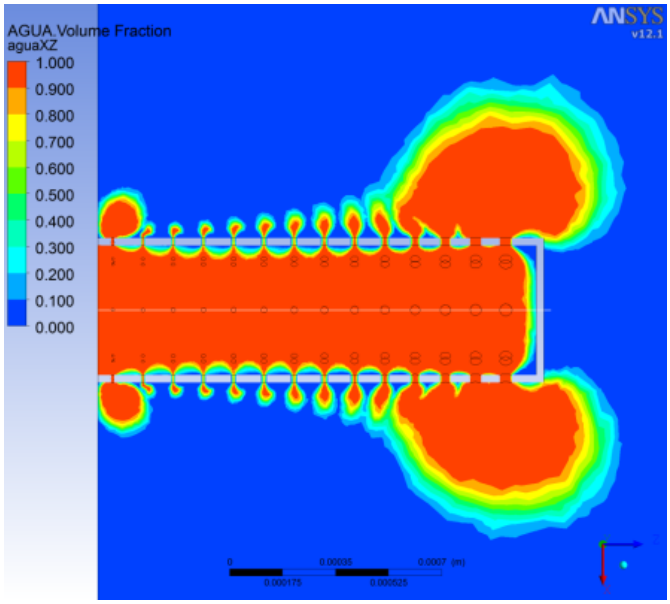


Figure 15. Volume Fraction (with logarithmically pores diameter at long the needle).

## 6 CONCLUSIONS

It is also important to underline that bioprinting and biofabrication technologies as all rapid prototyping technologies in essence are information technologies, because they are transforming virtual information or "organ blueprint" into physical biological reality - bioprinted organ.

Biofabrication technologies as any other modern manufacturing technologies are heavily depended on computer-aided design, computer simulation, mathematical and computational modeling and *in silico* or virtual testing. Thus, it is safe to predict that the first complex human organ such as kidney will be bioprinted at first.

Incipient analyses on the phenomenon of diffusion through a needle within the bioreactor show the interaction between two fluids, water and alginate hydrogel, and how the lesser dense fluid is spread into this hydrogel. Different geometric settings were adopted as such as variations on number and distribution of porous and the pores diameter.

Regarding the simulations where on the beginning alginate was inside the needle, it was verified that the resistance the hydrogel offers to the water (lesser viscous), forces the flow to leave the needle already by the pores of the first rings. When the compensation in terms of distribution and diameters of pores is incorporated, different results are obtained being possible to check that the part of the flow crosses the needle length reaching the last rings.

In general terms, in order to build a suitable real bioreactor, the efforts initially should be done computationally and moreover regarding isolated parts, as the case of the syringes and later integrate all the studies performed in the same environment representing the complete bioreactor.

## 7 ACKNOWLEDGEMENTS

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