Strategies in biomanufacturing

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Recent advances in additive manufacturing have allowed, even in a preliminary stage, 3D printing of biomaterials and/or cells. Each day, biomanufacturing process has been adapted to produce, in the future, different kinds of human tissues in a wide variety of shapes and structural complexities, which have specific biomechanical properties such as the vasculature characteristics, aiming biomodels creation that mimic tissues with parameters in accordance with real tissues. The big challenge in this area of knowledge takes place in developing models/biomodels representing more faithfully the required biological parameters. This will only be achieved with the integration of several researchers and multidisciplinary studies and techniques.

Aiming a multidisciplinary group formation in additive manufacturing area, with focus in 3D printing of virtual and physical models that incorporates biological concepts, the Divisão de Tecnologias Tridimensionais (Division of Three-dimensional Technologies - DT3D), located in the Technology Center of Information Renato Archer (CTI) that responds to Ministry of Science,

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Technology and Innovation (MCTI) has been developing studies related to all stages of biomanufacturing process, integrating different strategies.

1. TISSUE ENGINEERING STRATEGIES / BIOMANUFACTURING

The biomanufacturing is classified into three approaches and strategies as following:

1st Strategy: based on Scaffolds

The Tissue Engineering by Biomanufacturing with the use of additive manufacturing initially started from a focused approach to scaffolds use (biodegradable and biocompatible three-dimensional structures serving as a support for adhesion and cell proliferation) for physical packaging cell, which subsequently, are responsible for generating new tissue. The scaffold is very useful in adhesion and three dimensional structure of cells. This structure is very close to a living tissue. Therefore, it is expected that the solid scaffold offers suitable conditions for regeneration project of tissues with mechanical properties, geometrical and porosity. At the end, the scaffold must have been fully degraded / absorbed by the body when a new tissue has been formed. Scaffolds fabrication techniques (1st Strategy) have advanced significantly. Additive manufacturing is a great tool option to enable building 3D structures from complex geometries with high precision and controlled pore size and porosity.

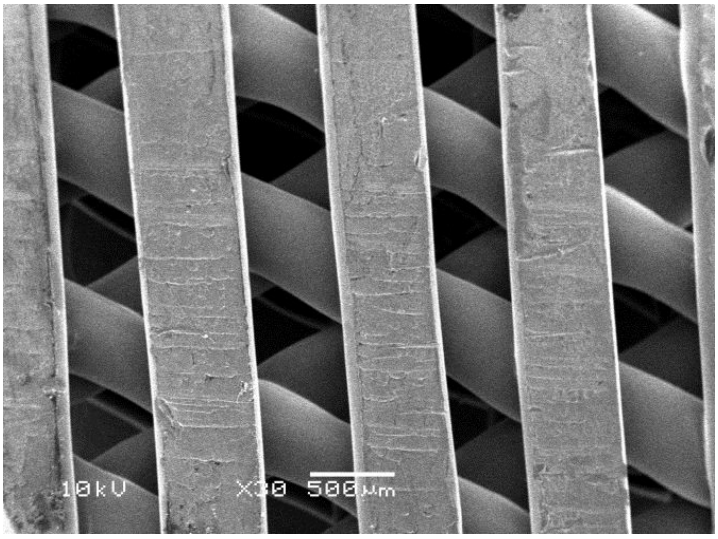

Figure 1. Example of a scaffold made by additive manufacturing in DT3D / CTI seen by microscopy.

2nd Strategy: based on Spheroids Tissue

A new approach has been developed based on spherical 3D structures with cells and without solid scaffold use (solid free scaffold) construction. In other words: cells are used in different way and in as shown in Figure 2 (Mironov, et al., 2003).

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It means that the cells that were previously selected and / or differentiated were manipulated and controllably deposited in an appropriate cell density to form cell aggregates, which are encompassed by the hydrogels as a biomaterial. This second approach demonstrates that biological tissues can be designed with specific compositions and shapes, by exploiting cell-cell characteristic adhesion and culture cells ability to develop their own extracellular matrix. Therefore, it helps to reduce and mediate inflammatory responses (Jakab, 2010).

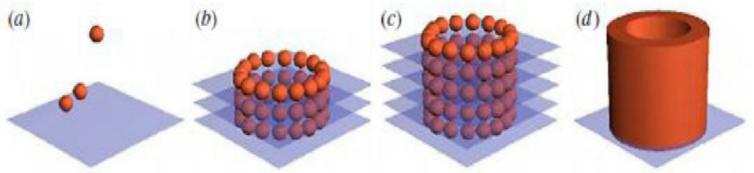

Figure 2. Second tissue/biomanufacturing engineering strategy: tissue spheroids as building blocks in bioprinting (Mironov, et al., 2009).

Bioprinting is an emerging variant of biomedical application of additive manufacturing or 3D printing (Bartolo, et al., 2011).

Great efforts have been focusing on biodegradable materials and cells combination, due to a limitation from conventional solid structures use and in approaches that use only non-dependent cells of solid scaffolds (solid free scaffolds). The use of spheroids tissues is advantageous from the perspective of high cell density, homogeneous 3D structure, vascularization construction "from inside to out". In other words: vasculature is already being constructed at the same time as spheroids are deposited.

3rd Strategy: based on Scaffolds + tissue spheroids

The First two strategies have advantages and disadvantages.
A third approach or third strategy of tissue engineering / biomanufacturing was drawn by thinking optimally and taking advantage of each one of the first two strategies, trying to compensate the limitations of them. This third strategy involves solid scaffold mechanical structure integration and incorporates on its inner tissue spheroids that are more consistent than cells that are deposited alone. A new type of microscaffold, dubbed lockyball emerges as an original idea developed in DT3D / CTI.

The lockyball is a hollow sphere with outer surface composed of pentagons with elevations and containing multiple hooks as seen in Figure 3. These hooks imitate (mimic) Natural structures such as burrs or Velcro, making it possible that a lockyball can cling to a neighboring generating a three-dimensional mesh of packed cells.

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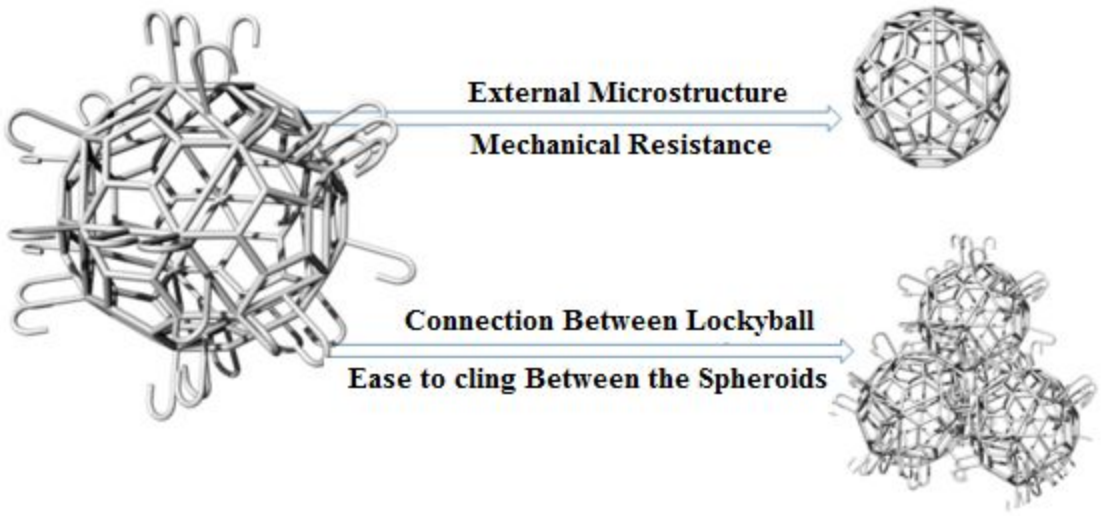

Figure 3. *Lockyballs* as third strategy for tissue engineering and/or biomanufacturing.

Early in development, the first step was to design a range of lockyballs virtually. These models were printed by additive manufacturing in a scale of approximately 6 cm in diameter, and tested for their ability / facility to connect with other lockyballs of the same type. Having identified the most efficient project in terms of connectivity, the same was sent to partners in Europe which printed the microscale device, since Brazil does not have the equipment. The process used for lockyballs manufacturing is known as "two-photon polymerization". The original Royal lockyball scale is around 200 micrometers in diameter (Rao, et al., 2012).

In vitro tests have already been carried out in partnership with Inmetro of Rio de Janeiro (brazilian National Institute of Metrology, Quality and Technology). The first step was verified and validated confirming the viability of cellularization of interconnected lockyballs (Danilevicius, et al., 2015).

The application of computational tools in biomanufacturing has evolved mainly structures lockyballs creation from scenarios simulations with parameters that represent tissue structure related to its growth, aiming tissue metabolism functionalization. Thus, computer simulation integrated with scripts, and even more efficient specific tools, opens new possibilities for more accurate determination of parameters for new solutions. Regarding lockyballs, Figure 4 illustrates, in a general way, a computer simulation carried out with the objective of verifying the effects in terms of mechanical properties related to changes in geometry or internal structure of lockyball (Danilevicius, et al., 2015).

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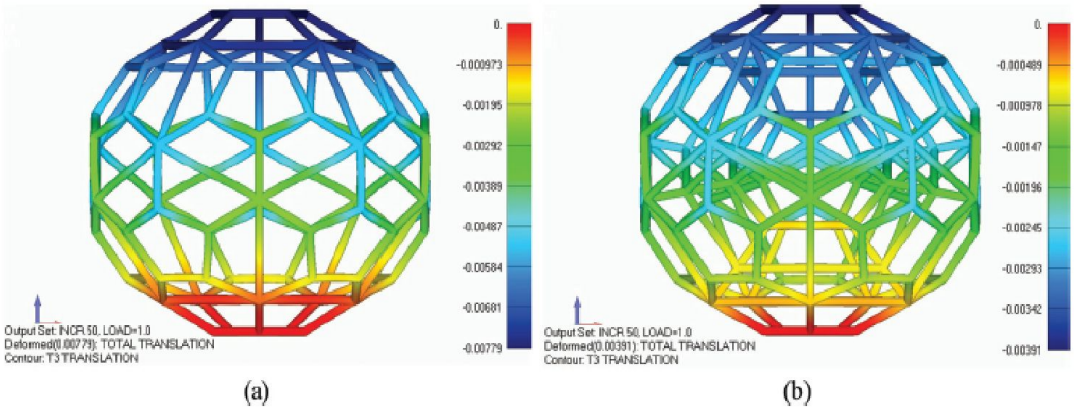

Figure 4. Mechanical properties related to changes in a lockyball geometry (a) without internal structures and (b) with internal structures.

The lockyballs may prove to be a very interesting solution for many areas of tissue engineering. The original lockyball shown in Figure 3, is the starting point of a family of microstructures that has emerged in accordance with specific applications, such as lockyballs, oriented to capillary regeneration. The ultimate goal is that lockyballs filled with tissue spheroids can ensure the formation of a more enhanced three-dimensional mesh and also more time for spheroids to merge and therefore enable a new tissue construction.

2. STAGES OF BIOMANUFACTURING

Regardless of the strategy employed, biomanufacturing consists in a coordinated sequence of steps, named pre-processing, processing and post-processing (Figure 5). These steps are briefly discussed below.

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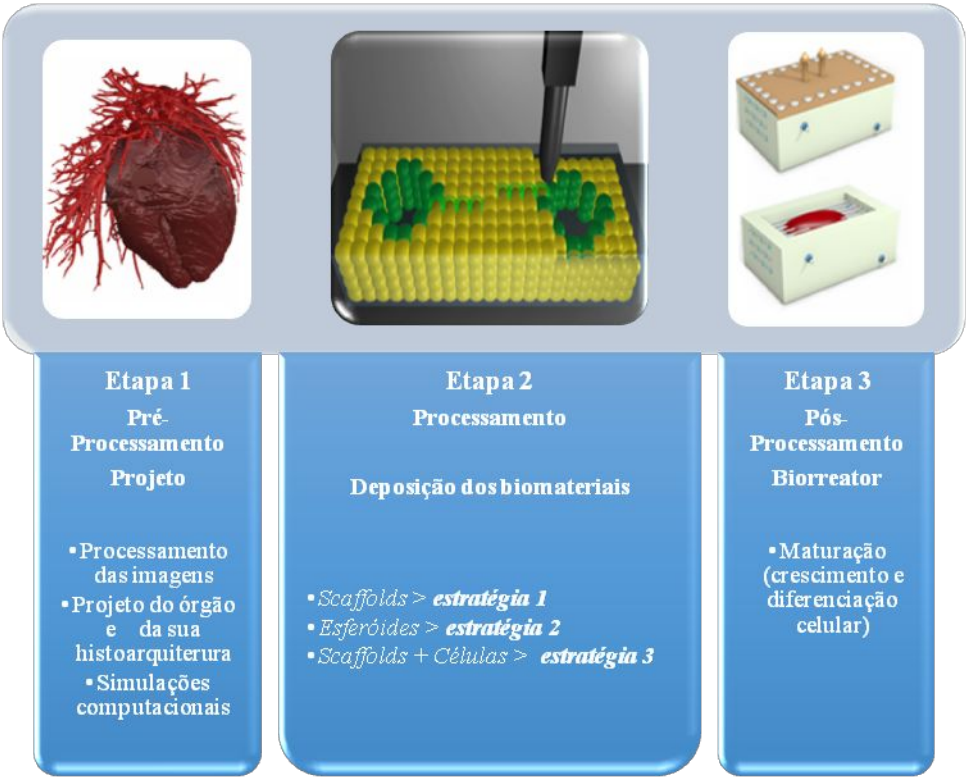

Figure 5. Main steps and strategies used in biomanufacturing and tissue engineering process.

3. PREPROCESSING

Preprocessing consists of the project conceptional fase, structure or organ that is intended to be manufactured. The preparation occurs by softwares combination for images treatment, computer-aided design (CAD) and computer simulations. The representation of the model of interest by structural organization projects, vascular for biological tissues as well as the extracellular matrix, becomes a challenge to this field. The design of a human organ, for example, is very complex. Express biological and physical phenomena of the formation of a new tissue process is not trivial. The domain issues such as embryology and also the fusion of tissue spheroids phenomenon is required. You have to know how aggregated cells will develop after being deposited and placed side by side and start the natural process of fusion. What types of cells and, therefore, what types of spheroids an organ needs to be structured. There are numerous questions to be studied, discussed and answered. In all these wanderings, all this information must be compiled and organized as an input file for 3D printer so that can interprets the data and conduct printing of the desired structure.

4. PROCESSING

To operate a 3D printing in the stage of biomaterials or processing in general depends, among other things, of the project and the availability of material for printing. The design phase has already been discussed in previous section. In relation to materials, there must be sufficient amout, so that the structure can be printed completely and only one time. In the case of human organs probably it will take a few million spheroids tissue. The spheroids have an average diameter of 200 micrometers. The fabrication of the spheroids must be performed in an automated manner. Microfluidics is currently the case with the greatest potential to produce the

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greatest amount of spheroids by time. Another important factor is spheroids packaging, because it should not start fusion process before they are deposited by the printer.

In terms of printer, there are already commercial bioprinters, however, the biggest customer remains the academy. There are manufacturing processes that uses filament deposition of bioink (solution containing cells or tissue spheroids) and laser. In bioprinting case (2nd Strategy), tissue spheroids are digitally deposited, i.e a spheroid at a time, which provides greater control in organ structure. Different types of spheroids can be used according to which constitution organ type is required. The constructed 3D structure can not be considered a finished organ. In fact, it is called construct and necessarily requires a development stage incubated on a device which provides appropriate physiological conditions related to parameters such as temperature, nutrient supply, waste removal, mechanical and electrical stimuli, among others.

5. POST-PROCESSING

The biomanufacturing post-processing step is related to a stage where cellularized scaffolds or newly manufactured organs are subjected to controlled physical conditions, so that the living material has time to evolve in the new tissue formation. It is a period of maturing prior to *in vitro* implantation of the solution. The equipment ensures that this process is known as bioreactor. Many bioreactors have been studied by several research groups. Particularly, in 2nd strategy case, using tissue spheroids, the DT3D has been conducting computer simulations to interpret embedded parameters in bioreactor.

Strategies in biomanufacturing process are complex and include architecture, structural mechanics, surface properties, degradation products and composition of biological components in addition to changes of these factors with the *in vitro* time (Hutmacher and Singh, 2008) . There is certainly a very great need for creation and incorporation of software and more appropriated parameters for this area, it is imperative to integrate several areas, especially in information technology. It is obvious that without a virtual model, it is not possible to progress to next stages of biomanufacturing. Furthermore, the combination with mathematical modeling, computer simulation and biological algorithms can identify potential unexpected problems, and find a better solution to optimize digital models and thus facilitating all biomanufacturing steps. (Rao, et al., 2015).

Taking the three strategies into consideration, in general, automation of the steps involved in biomanufacturing requires a number of discrimination, since the images, used materials / biomaterials, structure printing to the preparation and evaluation of biofabricated tissues . This automation process is part of the construction purpose, DT3D / CTI, a virtual biomanufacturing line which will encompass, for example, computer models and information about the equipment, so that it can scale the effort required and the structural cost for production of human tissue and organs. The virtual biomanufacturing line has also a propose a virtual display of biomanufacturing plant, analysis and optimization of the biomanufacturing process and future research team training.

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