ENEBI 2015
ENCONTRO NACIONAL DE ENGENHARIA BIOMECÂNICA
ENGENHARIA BIOMECÂNICA PARA VIVER SEM LIMITES
05 a 08 DE MAIO UBERLÂNDIA-MG

3D STRUCTURED HUMAN ORGANS BY BIOPRINTING

Rodrigo Alvarenga Rezende, Divisão de Tecnologias Tridimensionais (DT3D)/Centro de Tecnologia da Informação Renato Archer (CTI), rodrigo.rezende@cti.gov.br
Júlia Adami Nogueira, DT3D/CTI, julia.nogueira@cti.gov.br
Viviane Ferreira Lara, DT3D/CTI, viviane.lara@cti.gov.br
Verónica Egidia Passamai Romero, veronica.passamai@cti.gov.br
Janaina Dernowsek, DT3D/CTI, janaina.dernowsek@cti.gov.br
Vladimir Mironov, DT3D/CTI, vladimir.mironov54@gmail.com
Jorge Vicente Lopes da Silva, DT3D/CTI, jorge.silva@cti.gov.br

Abstract. *Organ printing or Bioprinting is a 10 years-old technology. It is an interdisciplinary field involving many sciences and technologies among life sciences, biology, computer sciences, physics and engineering. The modern world mainly at urban areas has generated a high number of patients waiting for organs because of diseases, congenital problems and accidents. The number of available organs for transplantation is not enough causing a shortage of organs. The time is too short. The patient may not wait very long. The shortage is also impacted by the compatibility issue. Even when there is an organ available, it can be not compatible for certain patient. Almost thirty years ago, the 3D printing technique appeared. From that time on, the advances were important. Tissue engineering started then to look at 3D printing as a partner. First, some biocompatible and biodegradable 3D structures for cell seeding called by scaffolds started to be fabricated with different materials and studied in vitro and in vivo. However, the use of scaffolds faces some limitations such as the homogeneity and density of the cells seeded. Organ Printing or simply Bioprinting has come as a promising approach to overcome this limitation assuring higher cell density and more homogeneity to the new alive 3D structures. Other advantage is that this construct is solid scaffold-free. Tissue spheroids (bioink) are the fundamental material in the digital bioprinting. Pre-sorted cells are put together and protected externally by some hydrogel. The tissue spheroids can be deposited by 3D bioprinters controllably side by side and layer by layer originating a 3D living structure. This paper presents quickly the bioprinting, its current state-of-the-art and the coming developments.*

Keywords: *Bioprinting, Biofabrication, Information Technology, Tissue Spheroids, Organ Printing*

1. INTRODUCTION

Bioprinting is a variant of the biomedical application of rapid prototyping technology or layer-by-layer additive biofabrication of 3D tissue and organ constructs for replacement, repair and regeneration of damaged and diseased human organs and tissues. Since its inception (Mironov *et al.*, 2003) the concept of organ printing using robotic bioprinters for the layer-by-layer additive biofabrication of functional 3D tissues and organ constructs using self-assembling tissue spheroids has undergone progressive development (Jakab *et al.*, 2010; Mironov *et al.*, 2009a; Visconti *et al.*, 2010) and gradually gained recognition as a reasonable bottom-up solid scaffold-free alternative to the classic top-down or solid scaffold-based approach to tissue engineering (Nichol and Khademhosseini, 2009). As Dr. David Williams an editor of journal "Biomaterials" and President of TERMIS (Tissue Engineering and Regenerative Medicine International Society) stated in recent influential review: ''There is obviously some way to go before such a paradigm (directed tissue self-assembly) could be translated into a practical reality, but many steps have been taken'' (Williams, 2009). The report on the 4th International Bioprinting and Biofabrication Conference (2009) that took place in Bordeaux, France, stated that 'bioprinting is coming of age'. The increasing number of papers and reviews, publication of the first books, the rapid development of new bioprinting and biofabrication research centers around the world, creation of the new "Biofabrication" journal and International Society for Biofabrication (2010), annual editions of the "International Conference on Biofabrication", "First International Bioprinting Congress" (2014) and, most importantly, the development of commercially available bioprinters are all important progress milestones.

The sequential development during last decade demonstrated that originally proposed conceptual basis of organ printing technology is valid and 3D bioprinting of human organ is technologically feasible. Moreover, organ printing or solid scaffold-free directed tissue self-assembly was recognized as new paradigm (Wiliams, 2009) and 3D bioprinting is now considered as new research direction in tissue engineering and regenerative medicine (Derby, 2012). Organ printing technology is now one of potentially superior strategies or emerging technological platforms for organ level tissue engineering (Rustad *et al.*, 2010; Melchels *et al.*, 2012; Ren and Ott, 2014). The number of research centers focused on development of 3D bioprinting technology, biofabrication and 3D bioprinting is growing. Nanyang Technological University offers first course on biofabrication. In several countries, thesis on different aspects of biofabrication, 3D bioprinting and organ printing have been already prepared and successfully defended. Thus, education and training specialists for biofabrication, 3D bioprinting and organ printing is an ongoing process. There are

already the International Society for Biofabrication and the specialized journal Biofabrication. There are regular international conferences including forcoming First International Bioprinting Congress in Singapore. The commercialization of organ printing and different aspects of 3D printing technology is already ongoing process. There are several companies producing tissue spheroids (Insphero, 3D Matrix, 3D Nano, USA) and commercial bioprinters (Envisiontech, Germany; RegenHu, Switzerland; Sciperio/nScript, USA; Organovo, USA; 3D Bioprinting Solutions, Russia (Figure 1). Organovo (USA) – a first company focusing on organ printing is already publically traded company with market capitalization $1 billion dollars. The first industrial report of market for 3D bioprinting has been recently published. Finally, Human Organ Project Inc. foundation has been formed. These milestones strongly indicate that concept of organ printing was adapted and accepted by scientific, educational and industrial community as a perspective technology platform. Emerging and rapid development of organ printing and 3D bioprinting is a direct manifestation of ongoing Third Industrial Revolution which is focusing on digitalization of manufacturing, according to the british weekly newspaper "The Economist".

3D bioprinting has already been used for the generation and transplantation of several tissues, including multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures. Other applications include developing high-throughput 3D-bioprinted tissue models for research, drug discovery and toxicology (Murphy and Atala, 2014).

Many developments in the interdisciplinary areas related to bioprinting must come in the next years. Only this will allow bioprinting go ahead. Many countries as South Korea, China, Singapore and The Netherlands besides others who have been investing strongly in bioprinting such as United States are injecting high amounts of money and giving the suitable conditions to their scientists progress at larger steps. Singapore, for example, created the NTU Additive Manufacturing Centre where they have employed 3D printing also for living solutions. The University of Manchester has just created the Manchester Biomanufacturing Centre, a multidisciplinary researching complex involving many engineering schools and hospital at the university and containing the most advances equipment available for the researches at biomanufacturing.

Concerning Brazil, it still needs more investment in this area. In 2008, CNPq launched the edital "INCT" by CNPq and in 2008 the Brazilian Institute of Biofabrication (INCT-BIOFABRIS) was created. Many researching groups in Brazil are affiliated to this institute including UNICAMP (headquarter), USP, INT, UFRGS and CTI Renato Archer. CTI created the bioprinting research group. However, there are still limited resources to expand this area in Brazil. It is needed that the science foundations and government recognize the importance of the bioprinting research and then higher financial support is provided. Brazil may not wait for also import bioprinted human organs in the future.

2. BIOPRINTING TECHNOLOGY

The potential competitive advantage with the use of self- assembling tissue spheroids for organ printing has been recently reviewed (Mironov *et al.*, 2008; Mironov *et al.*, 2009a; Visconti *et al.*, 2010). It has been suggested that the bottom-up solid scaffold-free approach can enhance the development of tissue engineering technology by enabling the automated and robotic industrial scale organ biofabrication (Mironov *et al.*, 2009b). History of the automobile industry and the emergence of microelectronic industry have taught us that an automated robotic approach is required for the successful development of new commercially profitable industries. The combination of computer-aided robotics and tissue engineering will not only enable tissue and organ bioassembly at large industrial scale, but will also provide the necessary level of flexibility for patient specific, customized organ biofabrication.

It is become increasingly obvious that, from a systems engineering point of view, it will take more than just bioprinters to biofabricate complex human tissues and organs. Indications suggest that the development of series of integrated automated robotic tools, or an organ biofabrication line (OBL) is required. Components of the OBL must include a clinical cell sorter, stem cell propagation bioreactor, cell differentiator, tissue spheroid bio- fabricator, tissue spheroids encapsulator, robotic bioprinter, and perfusion bioreactor.

Organ printing is a rapidly emerging technology that promises to transform tissue engineering into a commercially successful biomedical industry. It is increasingly obvious that similar well established industries implement automated robotic systems on the path to commercial translation and economic success. The use of robotic bioprinters alone however is not sufficient for the development of large industrial scale organ biofabrication. The design and development of a fully integrated organ biofabrication line or development of series of integrated automated robotic tools is imperative for the commercial translation of organ printing technology. Development of integrated line of automated robotic tools for biofabrication at industrial scale requires a complex multidisciplinary approach and close research and development collaboration of mechanical engineers, experts in rapid prototyping technology, computers scientists, chemical engineers and material scientists with biologists and tissue engineers.

2.1 Stages of Bioprinting

Bioprinting is normally splitted into three parts - pre-processing, processing and post-processing – as can be seen in the Fig. (1).

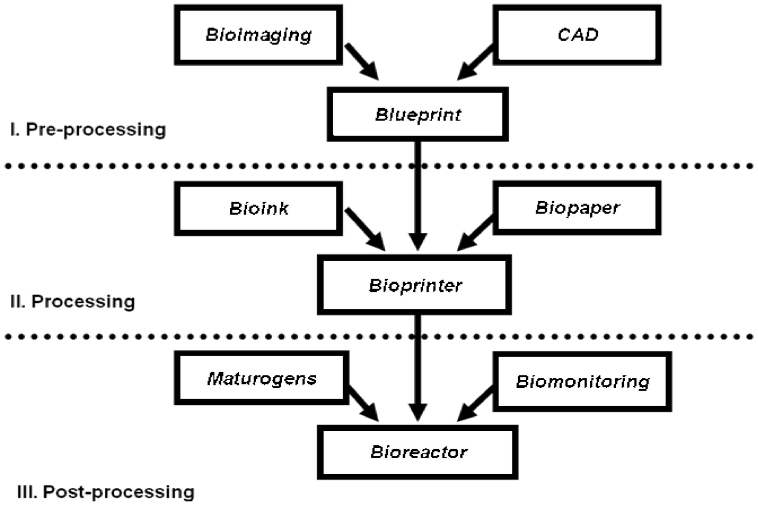

Figure 1. Three main steps in organ printing technology.

The pre-processing can be defined as development of computer-aided design or "blueprint" of 3D human tissue and organ based on using clinical imaging modalities and special additive manufacturing. Blueprint in STL file is actual instruction for robotic bioprinter how to print 3D tissue construct. It is not possible to bioprint human organ and tissue without development of CAD based "blueprint". A new method to represent three-dimensionally human anatomy digitally has been developed by the company Uformia from Norway (a CTI's partner) using mathematical functions for generate digital representations (Fig. (2)) what will surely enhance the fashioning of the blueprint.

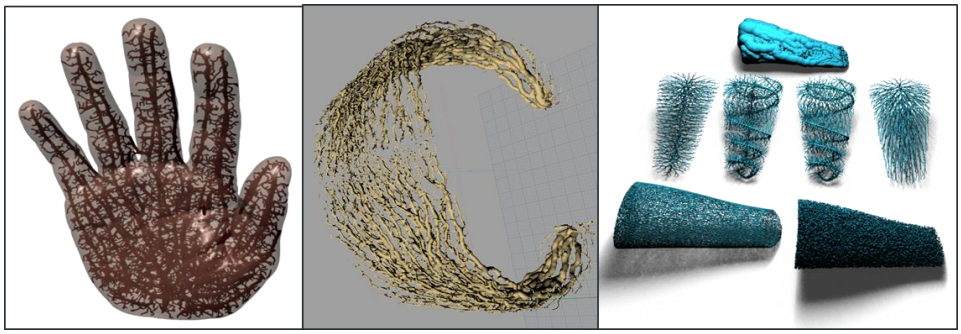

Figure 2. Blueprint by function representation (courtesy of Uformia, Norway).

The processing or actual computer-aided robotic bioprinting include preparation of "bioink" or self-assembled tissue spheroid (Rezende *et al.*, 2011a), development of "bio-paper" or processible and biocompatible hydrogel and using "robotic bioprinter" or computer controlled robotic precised dispenser (Fig. (3)). There are already several commercially available 3D bioprinters and robotic dispenser. CTI has Biofab@CTI bioprinter.

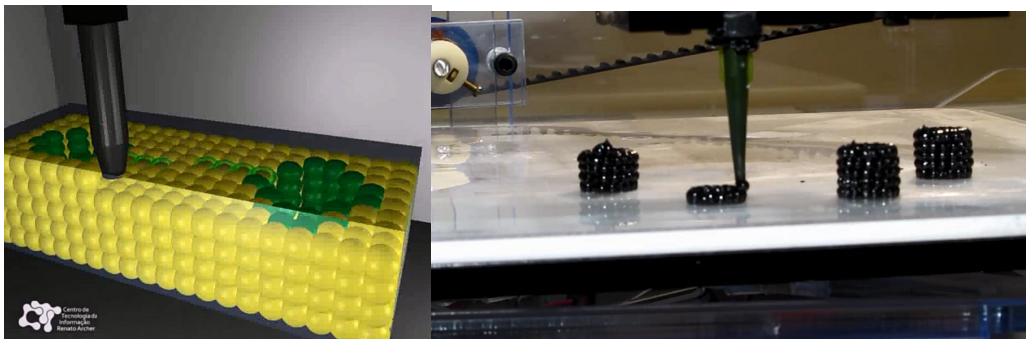

Figure 3. Virtual (left) and real (right) digital (droplet) printing.

The post-processing comprehends the step where the construct from the bioprinter must undergo a maturation time inside a bioreactor (Fig. (4)). Bioreactors are used to accelerate tissue maturation through the control of their mechanical, biochemical and thermal 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 preparation 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 study (Rezende *et al.*, 2011b). The success of tissues and organs bioprinting is straight linked to a set of an appropriate 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 phenomena within the organs in maturation process in bioreactor is fundamental for understanding of the phenomenon. Moreover, 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.

Post-processing is probably the most essentially crucial step in organ printing technology, and effective post-processing or accelerated tissue maturation will require the development of new types of bioreactors, more efficient accelerated tissue maturation technologies as well as methods of non-invasive and non-destructive biomonitoring.

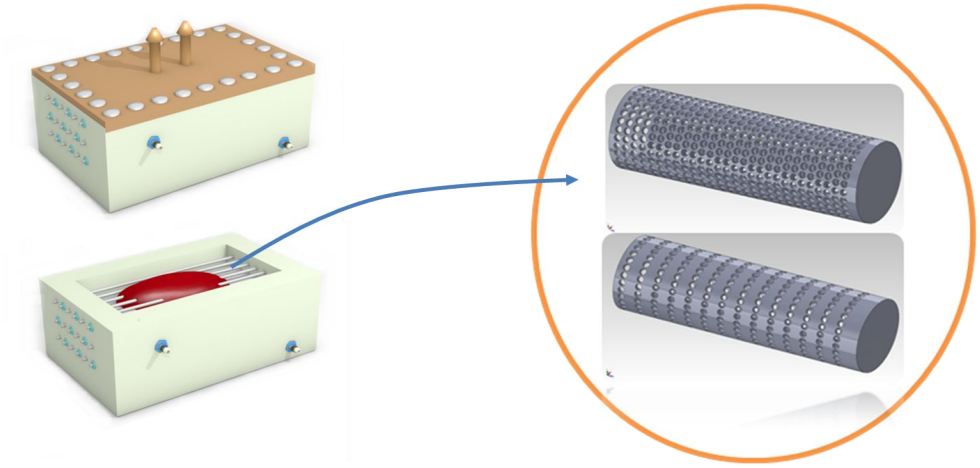

Figure 4. Scheme of the perfusion bioreactor

3. WHAT COMES NEXT

Three forefronts on the bioprinting direction deserve to be addressed:

in silico 3D bioprinting is rapidly emerging as a powerful information technology tool for virtual biofabrication and predictive computer simulation of 3D bioprinting and organ printing. *in silico* bioprinting includes design of blueprint for organ printing using computer-aided design softwares, virtual organ biofabrication line or virtual simulation of all steps of the bioprinting process and computational simulation of tissue spheroids fusion using Surface Evolver software and Molecular Dynamics softwares and other approaches as well as computational modelling of bioprinted constructs perfusion during post-processing using Computational Fluid Dynamics (CFD) software (Rezende *et al.*, 2013) and especially Lattice Boltzman approach. It is safe to predict that first human organ will be initially bioprinted *in silico*.

4D bioprinting is the programmable self-assembling and self-folding biomaterials will enable post-printed tissue and organ self-assembly. Using magnetic forces tissue engineering, pre-stretched electrospun biomaterials and novel stimuli responsive hydrogel will allow designing self-folded and self-assembled tissue and organ constructs. At this case we will move from directed self-assembly to self-directed self-assembly already employed in some areas of nanotechnology (Lin *et al.*, 2005).

***in situ* or *in vivo* 3D bioprinting** has been emerged on interface of relatively simple hydrogel and cell spraying technologies, robotic surgery and 3D bioprinting. The bioprinting of cartilage, bone and skin as well as human hair will be short term applications.

4. CONCLUSIONS

Past (1), present (2) and future (3) of bioprinting respectively can be summarized as follows:

(1) The conceptual framework of organ printing or solid scaffold-free bottom up directed tissue self-assembly have been invented decade ago as a potentially superior alternative to conventional solid scaffold based tissue engineering.
(2) Organ printing or 3D bioprinting technology has been recognized as new research paradigm, promising technological platform and new research direction in tissue engineering and regenerative medicine.
(3) The commercialization of described technology is already ongoing process and further development including for coming clinical translation will depends on our progress both in biological and technological aspects of organ printing technology.

5. REFERENCES

Derby, B., 2012, Printing and prototyping of tissues and scaffolds. Science, 338(6109):921-6.

Jakab, K., Norotte, C., Marga, F., Murphy, K., Vunjak-Novakovic, G., Forgacs, G., 2010, Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication, 2, p. 022001.

Lin, Y., Boker, A., He, J., Sill, K., Xiang, H., Abetz, C., Li, X., Wang, J., Emrick, T., Long, S., Wang, Q., Balazs, A., Russel, T.P., 2005, Self-directed self-assembly of nanoparticle/copolymer mixtures. Nature. Mar 3;434:55-9.

Melchels, F.P.W., Domingos, M.A.N, Travis, J.K., Malda, J., Bartolo, P., Hutmacher, D., 2012, Additive manufacturing of tissues and organs. Progress in Polymer Science, 37 (8) : 1079-1204.

Mironov, V., Boland, T., Trusk, T., Forgacs, G., Markwald, R., 2003, Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol., 21, pp. 157–161.

Mironov, V., Kasyanov, V., Drake, C., Markwald, R., 2008, Organ printing: promises and challenges. Regen Med., 3, pp. 93–103.

Mironov, V., Visconti, R., Kasyanov, V., Forgacs, G., Drake, C., Markwald, R., 2009a, Organ printing: tissue spheroids as building blocks. Biomaterials, 30, pp. 2164–2174.

Mironov, V., Zhang, J., Gentile, C., Brakke, K., Trusk, T., Jakab, K., Forgacs, G., Kasyanov, V., Visconti, R., Markwald, R., 2009b, Designer 'blueprint' for vascular trees: morphology evolution of vascular tissue constructs. Virtual Phys. Prototyping, 4, pp. 63–74.

Murphy, S.V, Atala, A., 2014, Nature Biotechnology, 32, 773–785.

Nichol, J., Khademhosseini, A., 2009, Modular tissue engineering: engineering biological tissues from the bottom up. Soft Matter, 5, pp. 1312–1319.

Ren, X., Ott, H.C., 2014, On the road to bioartificial organs. Pflugers Arch. Apr 2..

Rezende, R., Pereira, F., Kemmoku, D., Mironov, V., Kasyanov, V., Vilbrandt, T., Silva, J., 2011a, Enabling Technologies for Robotic Organ Printing. "Innovative developments in virtual and physical prototyping", P.J. Bártolo et al., published by Taylor & Francis.

Rezende, R., Laureti, C., Mironov, V., Kasyanov, V., Maciel Filho, R., Silva, J., 2011b, Towards Simulation of a Bioreactor Environment for Biofabricated Tissue Maturation. "Innovative developments in virtual and physical prototyping", P.J. Bártolo et al., published by Taylor & Francis.

Rezende, R.A., Nogueira, J.A., Lara, V. F., Mironov, V., Maciel Filho, R., Silva, J.V.L., 2013, Development of a Bioreactor by Computer Fluid Dynamics Simulations for the Maturation of 3D Printed Organs by Rapid Prototyping. Chemical Engineering Transactions, v. 32, p. 1153-1158.

Rezende, R.A., Pereira, F.D., Noritomi, P., Baptista, L., Silva, K.R., Borojevic, R., Kasyanov, V., Ovsianikov, A., Stampfl, J., Farsari, M., Silva, J.V.L., Mironov, V., 2014, Organ Printing: Past, Present and Future. Proceedings of the 1st International Conference on Progress in Additive Manufacturing, Singapore, p.284-290.

Rustad, K.C., Sorkin, M., Levi, B., Longaker, M.T., Gurtner, G.C., 2010, Strategies for organ level tissue engineering. Organogenesis, Jul-Sep;6(3):151-7.

Visconti, R., Kasyanov, V., Gentile, C., Zhang, J., Markwald, R., Mironov, V., 2010, Towards organ printing: engineering an intra-organ branched vascular tree. Expert Opin. Biol. Ther., 10, pp. 409–420.

Williams, D., 2009, On the nature of biomaterials. Biomaterials, 30, pp. 5897–5909.

6. ACKNOWLEDGEMENTS

This work was sponsored by São Paulo Research Foundation (FAPESP), The Brazilian Institute of Biofabrication (INCT-BIOFABRIS) and The National Council for Scientific and Technological Development (CNPq).