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ORGAN PRINTING: PAST, PRESENT AND FUTURE

RODRIGO REZENDE*, SERGEY BALASHOV, JORGE DA SILVA, VLADIMIR MIRONOV#
*Renato Archer Information Technology Center, Division of 3D Technologies and Microsystems,
Rod. Dom Pedro I (SP-65), km 143,6, CEP:13069-901, Campinas, SP, Brazil*
#3D Bioprinting Solutions, Moscow, Russian Federation

VLADIMIR KASYANOV
Riga Stradins University and Riga Technological University, Riga, Latvia, European Union

*Corresponding author

ABSTRACT: Organ printing technology could be defined as an automated, robotic and computer-aided layer by layer additive biofabrication of functional 3D tissue and organ constructs using living tissue spheroids as building blocks. The concept of organ printing has been introduced a decade ago as a potentially superior alternative to conventional solid scaffold-based tissue engineering. The organ printing technology consists of three main steps: (i) pre-processing or design of blueprint for bioprinting of human organ; (ii) processing or actual 3D bioprinting using bioink or self-assembling tissue spheroids, biopaper or bioprintable, biocompatible and sacrificial hydrogel and bioprinter or automatic computer-aided robotic dispenser; and finally, (iii) post-processing or bioreactor-based accelerated tissue maturation. During last decade 3D bioprinting technology has been advances and broadly recognized a new research paradigm and new exciting research direction in tissue engineering and regenerative medicine. Moreover, commercialization of 3D bioprinting technology is already an ongoing process. The design of next generation of 3D bioprinters and industrial scale complete organ biofabrication line will be presented and discussed.

DEVELOPMENT OF ORGAN PRINTING TECHNOLOGY

The concept of organ printing was conceived one decade ago. In pioneering paper (Mironov et al., 2003) we outlined concept of organ printing and then present conceptual basis in sequential publications (Mironov et al., 2008; Mironov et al., 2009; Viscont et al., 2010; Mironov et al., 2011) (Table 1).

Table 1. Conceptual framework for organ-printing technology.

The most important assumption is a developmental biology-inspired belief in the possibility of creating functional human tissue and organs without solid biodegradable scaffolds or template-based approaches.
High cell density is essential for fast tissue assembly and accelerated tissue maturation.
Minitissue or tissue spheroids can fuse and be assembled into macrotissues through the tissue fusion process or directed tissue self-assembly.
Standardized size and viable minitissues or tissue spheroids with different compositions and organization, including microvascularized tissue spheroids, can be rapidly fabricated in large quantity.
Due to their size, the material properties and shape of minitissue or tissue spheroids represent a processible “ bioink ” suitable for precise, automated dispensing or digital ‘punching’ onto sprayed hydrogel layers (“ biopaper ”) and sequential bioassembly and biofabrication into macrotissues and living organ constructs with the help of sophisticated computer-aided robotic devices (“ bioprinters ”).
Self-assembled vascular tissue spheroids allow generation of a “built-in” intraorgan branched vascular tree within 3D thick tissue or organ constructs, suitable for perfusion.
Microvasculature can be formed by the self-assembly process and be seamlessly integrated with an intraorgan branched vascular tree by self-assembly, tissue fusion and sprouting processes.

It is possible to develop accelerated methods of tissue maturation.
A microfluidic-based, irrigation type tripled perfusion bioreactor with temporally removable porous microtubes will enable the construct to maintain shape and buy time necessary for tissue maturation of the perfusable intraorgan branched vascular tree.
A minitissue-based solid, biodegradable scaffold-free tissue engineering approach enables automated, robotic, large-scale industrial tissue engineering using computer-aided design of organs (“blueprints”).

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 (Williams, 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 specialized journal **Biofabrication**. There are regular international conferences including for coming **First International Bioprinting Congress** in Singapore (<http://selectbiosciences.com/conferences/index.aspx?conf=IBC2014>). 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 (<http://www.nasdaq.com/symbol/onvo>). The first industrial report of market for 3D bioprinting has been recently published (http://www.researchandmarkets.com/research/lvbb88/3d_bioprinting). Finally, **Human Organ Project Inc.** (<http://humanorganproject.org/home/index>) 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 (<http://www.economist.com/node/21553017>).

BIOLOGICAL ASPECTS OF ORGAN PRINTING TECHNOLOGY

The advances in the development of organ printing as a truly cross-disciplinary or multidisciplinary technology depend on progress in many different disciplines and biology is obviously one of them. There are several fundamental biological issues related to the organ printing technology. The processing of living cells in form of tissue spheroids is an unique characteristic of organ printing technology. The success of organ printing is possible only on integrated development of biological aspects of bioprinting technology. Below we will try to outline and summarize the main biological issue related with organ printing (Table 2)

Table 2. Biological aspects of organ printing technology.

Viability of tissue spheroids during processing and post-processing
Structural determinant of material properties of tissue spheroids
Tissue spheroids fusion process
Tissue spheroids differentiation before processing
Vascularization of bioprinted constructs using solid and lumenized vascular tissue spheroids
Accelerated perfusion bioreactor-based bioprinted tissue maturation using maturogens and its non-invasive and non-destructive biomonitoring
Control of size of bioprinted 3D tissue and organ constructs
Bioprinted organ with regenerative capacities
Estimation functionality and integrity of matured bioprinted organs
Prevention of possible malignization of ioprinted organs

TECHNOLOGICAL ASPECTS OF ORGAN PRINTING TECHNOLOGY

Technological aspects of organ printing technology includes (i) design of organ for bioprinting, (ii) development of bioink (tissue spheroids), biopaper (or bioprintable hydrogel), 3D bioprinters and (iii) design of complete industrial organ biofabrication line. According to our best knowledge the blueprints of human organ have not been yet developed. The bioprinter is an essential element of organ printing technology and there are already some commercial 3D bioprinters (Figure 1) (Mironov et al., 2008). There are scalable technologies for biofabrication of tissue spheroids or bioink of standard size and shape and different internal structure and composition is also under rapid development (Mehesz et al., 2011). Biopaper or hydrogel for bioprinting is now called bioprintable hydrogel. The specifications for ideal bioprintable hydrogel are presented at Table 2.

The bioprintable hydrogels which enable rapid polymerization such as fibrin (based on combination of thrombin and fibrinogen) alginate (based on combination of alginate and ions of calcium) and well as stimuli-sensitive, photo-sensitive and light-triggered hydrogel are obvious choices. In our recent publications we are arguing that development of only 3D bioprinters is not enough for biofabrication of human organ at industrial scale. We recently introduced a concept of automatic robotic Organ Biofabrication Line (Figure 2) which included cell sorters, robotic tissue spheroids biofabricators, tissue spheroids encapsulators, robotic 3D bioprinters and perfusion bioreactors (Mironov et al., 2011; Rezende et al., 2013a; Rezende et al., 2013b; Rezende et al., 2013c). In essence, it is a laboratory prototype of future organ biofabrication plant. The history of technology strongly indicates that the introduction of automated and robotics manufacturing processes will enable development of cost-effective and profitable organ biofabrication industry. The new generation of 3D bioprinters must enable rapid bio-assembly of functional and matured 3D tissue and organ constructs suitable for safe implantation. Ink-jet printers, laser based 3D bioprinters and dispensing based 3D bioprinters are subject of intensive development.

Figure 1. Some of the commercial 3D Bioprinters.

Table 3. The specifications for ideal bioprintable hydrogel.

Bioprocessible (dispensable and fast solidification)
Biomimetic (functional arginine-glycine-aspartic acid [RGD] peptides for improving viability)
Biocompatible (nontoxic, high cell viability)
Intelligent (stimuli-sensitive)
Tissue fusion permissive (optimal physicochemical properties)
Shape maintainance (Preventing construct melting and distortion)
Hydrophylic (efficient diffusion)
Biodegradable (removable on demand)
Naturally derived hydrogels (collagen, fibrin, hyaluronan based)
Pro-angiogenic and loaded with survival and angiogenic factors (enhancing bioprinted construct viability)
Affordable (relatively low cost)
FDA approvable (noncancerogenic and nonimmunogenic)

We are now working on new type of bioprinters based on principles of light-triggered polymerization, magnetic tissue spheroids levitation and surface acoustic wave (SAW) manipulations. The integrated microchip with SAW interdigital transducers for rapid biofabrication, encapsulation and ejection of tissue spheroids is designed and under development (Figure 3).

Figure 2. Organ biofabrication line room.

Figure 3. Surface acoustic wave based microchip for rapid tissue spheroids biofabrication and bioprinting: (a) Surface acoustic wave based microchip for tissue spheroids biofabrication and ejection, (b) Dehydration of tissue spheroids using hydrophilic substrate and (c) Rapid biofabrication of tissue spheroids using surface acoustic wave mixing.

NEW DIRECTIONS IN THE DEVELOPMENT OF ORGAN PRINTING TECHNOLOGY

In situ or *in vivo* 3D bioprinting is one of the most promising new directions in the development of described technology. *In situ* 3D bioprinting has been emerged on interface of relatively simple hydrogel and cell spraying technologies, robotic surgery and 3D bioprinting. Creative combination of robotic surgery and bioprinting will advance 3D bioprinting and build foundation for

transformation of surgical practice. It will provide surgeon with new tools which will enable rapid biofabrication of tissue *in situ* in operation room. The bioprinting of cartilage, bone and skin as well as human hair will be short term applications. Endoscopic snake-like robot combined with bioprinting devices will enable biofabrication of tissue in other organs.

4D bioprinting is another possible very interesting and even exciting development. 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. Infrared laser technology which is non-toxic for living cells most perspective way to design remote controlled self-assembled and self-folded 3D printed tissue and organ constructs. In 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 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 software, 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** software and other approaches as well as computational modeling of bioprinted constructs perfusion during post-processing using **Computational Fluid Dynamics (CFD)** software (Rezende et al., 2013d) and especially **Lattice Boltzman** approach. It is safe to predict that first human organ will be initially bioprinted *in silico*.

CONCLUSIONS

Past: 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.

Present: Organ printing and 3D bioprinting technology have been recognized as new research paradigm, promising technological platform and new research direction in tissue engineering and regenerative medicine.

Future: 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.

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