

3D Bioprinters – Future of Implants Biofabrication

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BIOGRAPHICAL NOTES

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KEY WORDS

Additive manufacturing, bioprint, bioprinter, scaffold, organs, tissues.

ABSTRACT

In a last decade has additive manufacturing passed a long way, where was reached an impressive advance in rapid prototyping technology of fabrication. From plastic and ceramic materials through metals to at the moment most interesting technology of bioprint, where the material which is used for building, directly consist of human tissues. Organ printing, which is based on computer-aided 3D tissue engineering, offers the wide range for

research and development in this area. This article summarizes the present advance in this new and not entirely explored field of bio-additive manufacturing. With the help of this technology can be produced the real 3D models of organs and tissues, that should help surgeons in preoperative planning or can be used like spare "parts" for transplantations. The main emphasis is placed on tissue engineering technology which has the best assumptions to solve transplantation issues. Also this article includes the comparison of devices and materials which are possible to engage within the bio-manufacturing.

1. Introduction

The change in the field of biology and bio-engineering has built an background in which the improvements in the life sciences are not only more accessible, but call for the active fellowship of engineering design and fabrication to reach solutions for complex biological problems. This progress, along with the development of new design and fabrication, biomaterials, biology and biomedicine, has advanced the additive manufacturing technology to a broad application in biomedical engineering [1].

Tissue engineering has reached more attention in the past decade, owing to its success in enabling tissue regeneration for therapeutic purposes [2]. Tissue engineering introduces the interdisciplinary area in which are applied the principles of biology and engineering to the evolution of substitutes that repair, improve or restore function of tissue. The main target is to fabricate patient-specific biological substitutes in an attempt to sidestep the restrictions of existing clinical treatments for damaged tissue or organs. The main regenerative tissue engineering approach involves transplantation of cells onto scaffolds [2]. These restrictions include deficiency of donor organs, chronic refusal and cell morbidity. The principal approach includes injection of cells alone, evolution of encapsulated systems and implantations of cells onto scaffolds [2]. The utilization of additive manufacturing (AM) in combination with computer-aided (CA) technologies has offered new possibilities for medical modeling, with using of computer models or additive manufacturing fabricated physical models for representation of patient specific anatomical geometry [1].

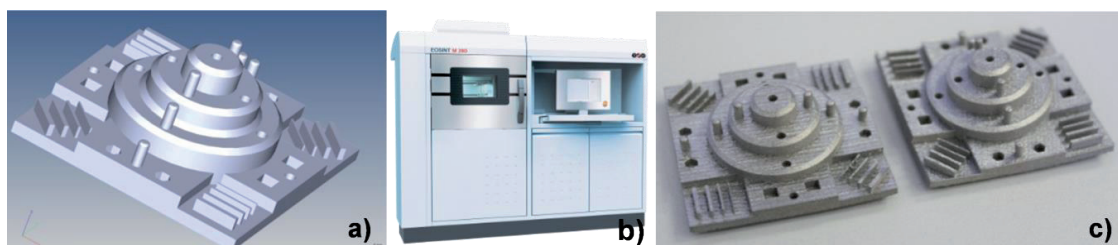


Fig. 1: Example of rapid prototyping fabrication. DMLS method which uses the additive principle to build the real parts. a) .STL model of part, b) technology used for manufacture, c) final physical model

2. Additive Manufacturing

A. Rapid Prototyping

Rapid prototyping also called as solid freeform fabrication and layered (additive) manufacturing technology allow scientist to create physical part in a short period, directly from 3D models created via computer-aided design (CAD), computer-aided engineering (CAE), and computer-aided manufacturing (CAM) programs as is shown on a Fig. 1 [3, 4, 5]. The additive fabrication refers to a class of manufacturing processes, in which a part is built by adding layers of material upon one another [6]. It offers several advantages as speed, part complexity, wide range of materials to use (plastics, metals, ceramics, composites and even material with properties similar to wood) and low-volume production because there is no need to produce custom tooling. Generally the AM technologies bring the special and unique possibilities for customization, upgrades in product performance, multi-dependence, and lower overall manufacturing costs.

These possibilities include [4]:

- *Shape complexity,*
- *Material complexity,*
- *Hierarchical complexity.*

B. Standard 3D printing methods

Between 3D printing methods which are up to now known, and they differ among themselves by used technology, we classify for example: direct metal laser sintering (DMLS), selective laser sintering (SLS), jetted photopolymer (JP), laminated object manufacturing (LOM), fused deposition modeling (FDM), stereolithography (SLA), three-dimensional printing (3DP) by ThermoForm fabrication, precision extruding deposition (PED) and micro-syringe based polymer deposition. Several of these methods are used for the bio-additive manufacturing, because they meet the principles and require-

ments for printing the living human tissue.

3. Bio-Additive Manufacturing

Bio-additive manufacturing (BAM) utilize the principles of standard additive manufacturing to build the physical model which have the same or nearly the same properties like real tissue or organ. BAM should be described as biofabrication using cells, biologics or biomaterials as building blocks to fabricate biological and bio-application oriented substances, devices and therapeutic products through a broad range of engineering, physical, chemical and/or biological processes [1]. The research and development (R&D) of anatomical and biological models represents the most significant area which is necessary for integration of RP in biological engineering. On the Fig. 2 is shown the example procedure of BAM process.

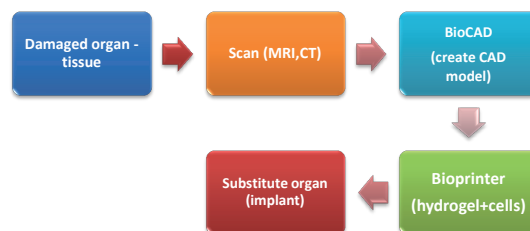


Fig. 2: Example of BAM process which can be modified according to real needs of biofabrication.

Classical tissue engineering refers to seeding isolated cells on solid scaffolds as introduced by Langer and Vacanti almost two decades ago and is still a cutting-edge technology [7].

Biomaterials can offer ideal biocompatible properties for scaffolding and generating cell-scaffold constructs demonstrate promising alternatives for autologous grafting and organ replacement

[7]. Biofabrication allows cells to be situated in a controlled way in and together with biomaterials. Biofabrication includes various techniques: bioprinting, bioplotting, inkjet printing and stereolithography.

Definition is described as production of complex living and non-living biological products by placing proteins, peptides, DNA, cells, hormones or ECM molecules together with biomaterials [7]. During the BAM process is necessary to ensure fulfillment requirements which are shown on Fig. 3.

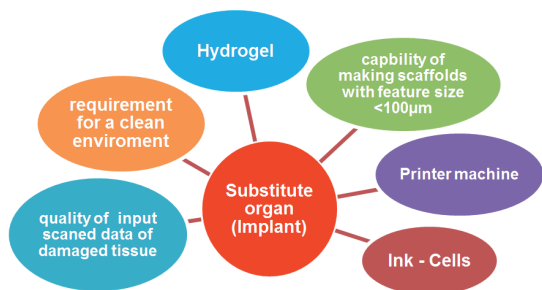


Fig. 3: Requirements for bio-additive manufacturing.

As part of the requirements is necessary, to provide taxonomy of communication between scientists from different disciplines (engineering, biology, materials and science) validation of biological tissue for production, biological function and stability of bio-material before and after the manufacture of the product.

A. Rapid Prototyping

Inkjet 3D printing is a non-contact method which uses digital data from a computer and reproduces it layer-by-layer by putting ink drops on previously printed successive layers [7]. Bioprinters may be constructed in various configurations. However, all bioprinters output cells from a bioprint head that moves in three dimensional (3D) space (x,y,z) in order to place the cells precisely where required. In addition to outputting cells, most bioprinters also output dissolvable water based hydrogel to support and protect cells during printing [10]. Several experimental bioprinters have already been built. The Fig. 4 presents the timeline of bioprinting evolution and its pioneers over the years.

The NovoGen MMX Bioprinter (USA) includes two robotically controlled accuracy print heads: one for placing human cells, the other for placing a hydrogel, scaffold, or support matrix. The NovoGen

(USA) bioprinter is schematically shown on Fig. 5.

One of the most complex challenges in the development of the bio-printer was to perfect a means to consistently position the cell dispensing capillary tip attached to the print head within microns [10]. Invetech (USA) developed a computer-controlled, laser-based calibration system to achieve the required repeatability. The process uses inkjet based 3D printers and an ink made of human cells (as with cloning, using the patient's own cells are the best bet) mixed with a dissolvable gel, often cellulose [10].

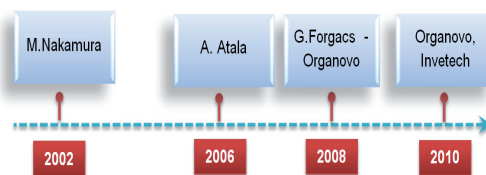


Fig. 4: 2002 – utilization of inkjet principle to bioprint, 2006 – artificial bladder, 2008 – bioprinter NovoGen MMX, 2010 – creation of blood vessels by bioprinter.

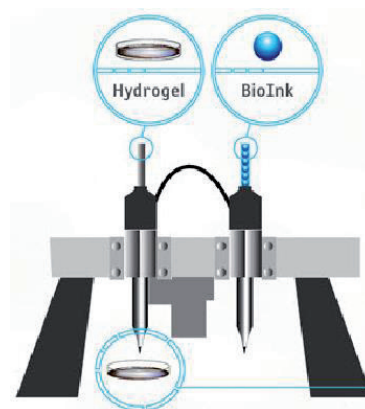


Fig. 5: NovoGen MMX Bioprinter (USA) [15].

The fabrication process is similar to other (AM) techniques. The printer puts down a layer, which is then cured with heat, chemicals or ultra-violet (UV) light, before moving on to the next layer.

To create its output, the NovoGen (USA) first lays down a single layer of a water-based bio-paper made from collagen, gelatin or other hydrogels [12]. Bioink spheroids are then injected into this water-based material. This process is shown on the Fig. 6. After that bioink spheroids slowly fuse together. As this occurs, the bio-paper dissolves away or is removed, than leaving a final bioprinted body part or tissue [10].

B. Organ printing

Organ printing is a biomedically similar variant of RP, which is based on tissue fluidity. Computer-assisted deposition (printing) of natural materials (cells or matrix) is done one layer at a time until a particular 3D form is achieved. However, recent attempts using rapid prototyping technologies to design solid synthetic scaffolds receive from the inability to precisely place cells or cell aggregates into a printed scaffold [13]. Organ printing involves three sequential steps: -pre-processing or development, processing or actual organ printing and post processing or organ conditioning [13].

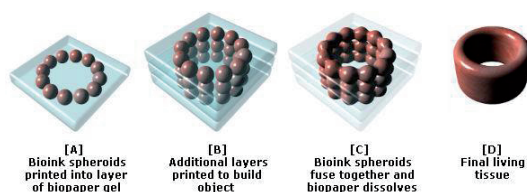


Fig. 6: Principle of bioprint on the cell level [11].

Organ bio-printing applies microfluidic design to cells and cell aggregates starting biological effect such as fusion [10].

C. Bioplotting

3D bioplotting is a bio-fabrication method, based on the extrusion of continuous filaments. It is sometimes also referred to as direct write system, compared to drop-based deposition in inkjet technology [10].

During the bioplotting, the ink is embedded in a syringe or a similar device and plotted in filaments spatially controlled by an X, Y, and Z robotic system.

"One of the first devices for biofunctional and cell compatible printing was developed at the Freiburg Materials Research Center in 2002 under the guidance of Rüdiger Landers and Rolf Mülhaupt and in cooperation with Envision Technologies (Envisiontec GmbH, Gladbeck, Germany)" [7].

D. Scaffolds

The scaffold trying's to reproduce the function of the natural extracellular matrix. The primary roles of scaffold are [5]:

I. Serve as an adhesion substrate for the cell, facilitating the localization and delivery of cells when they are implanted.

II. Provide temporary mechanical support to the newly grown tissue by defining and maintaining a

3D structure.

III. Guide the development of new tissues with the appropriate function.

Scaffolds can be produced in a variety of ways, using conventional techniques or advanced processing methods.

E. Scaffolds Requirements

Scaffold produced by bioprinting must fulfill these requirements [5]:

- *Fit complex anatomic defects,*
- *Mechanical strength (e.g., compressive stress 0.5 – 10 MPa) for temporary load bearing,*
- *Three dimensional (3D) interconnected macro-porous microstructures,*
- *Controllable biodegradation and bioresorption,*
- *Suitable surface chemistry,*
- *Good biocompatibility and biofunctionality.*

F. Conventional and Advanced scaffold – fabrication methods

"Conventional methods for manufacturing scaffolds include solvent casting and particulate leaching, gas foaming, fiber meshes and fiber bonding, phase separation, melt molding, emulsion freeze drying, solution casting and freeze drying" [2].

"Even though these methods have some restrictions which offer: little capability precisely to control pore size, pore geometry, pore interconnectivity, spatial distribution of pores and construction of internal channels within the scaffold" [2].

RP methods such as (FDM), 3D printing (3DP) and (SLS) has been shown to be viable for fabricating porous structures for use in tissue engineering. With use of this advance techniques can be produced the scaffolds which reduce of the restrictions of conventional scaffold fabrication methods as is shown on the Fig. 7.

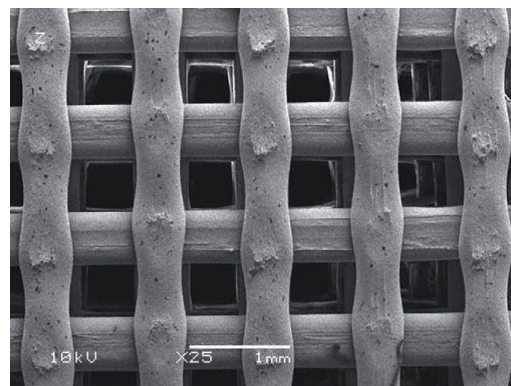


Fig. 7: Structure produced using FDM.

Table 1: Biofabrication of 3D cell-hydrogel construct [7]

Technology	Hydrogel	Cells	Fabrication specifications
Stereolithography			
	PEG	Fibroblasts	Laser beam \varnothing ~250 μ m; layer thickness: ~250 μ m
	PEO, PEGDM	Ovary cells	Ring scaffolds: \varnothing : 5.3 mm; thickness: 1.5 mm; UV laser spot: 250 μ m; resolution per layer: 150 μ m; x-y resolution: 250 μ m
Laser-based biofabrication			
LGDW	Collagen, Matrigel	ECs, hepatocytes	Single cell resolution
BioLP	Matrigel	Osteosarcoma cells	2 layers of cells separated by a 75 μ m layer of hydrogel
LIFT	PEGDA, Alginate, EDTA, blood plasma, Matrigel; Collector slide: agarose	Fibroblasts/keratinocytes, hMSCs, ECs	\varnothing Droplets: 80–140 μ m; speed: 1200 cell droplets/min; scaffold height including 6 layers: 300 μ m; focal spot: 45 μ m; distance between spots: 75 μ m; accuracy: 5 μ m
LIFT-2PP	PEGDA	SMCs, ECs	\varnothing laser spot: 45 μ m; laser transferred droplet size: 80–140 μ m
Inkjet printing			
Inkjet bioprinter	Fibrin gel (fibrinogen + thrombin)	Neural cells	25 orifices with \varnothing 50 μ m, resolution: 85 μ m; 250,000 drops/s; 5 layers
	Alginate, fibrinogen, thrombin	HeLa cells, ECs	Speed: 20 mm/s; ejection time: 800 Hz; pattern \varnothing : 1 mm; size: 5 \times 7 mm
	Collagen Fibrin gel (fibrinogen + thrombin)	SMCs ECs	Size of construct: 5 \times 5 mm, 5 layers; thickness per layer: 16.2 μ m; Size: 10 \times 5 \times 2 mm; 2 layers
3D bioplotting			
Bioplotter	Alginate, Lutrol	BMSCs	\varnothing Nozzle: 100–400 μ m; 4–10 layers;
Envisiontec	F127, Matrigel, agarose, methylcellulose	BMSCs	\varnothing Nozzle: 100–400 μ m; 4–10 layers; thickness per layer: 150 μ m; spacing 300 μ m; speed: 1–30 mm/s; pressure: 0.5–3 bar; size: 20 \times 20 mm
BAT	Polyoxyethylene-polyoxypropylene, collagen I	Fibroblasts, ECs	\varnothing Nozzle: 200–500 μ m; resolution \leq 5 μ m; accuracy \leq 5 μ m; deposition rate: 12 nL/s–1 mL/s; speed: 10 μ m/s–50 mm/s; size: 2 \times 2 \times 1.5 mm; layer height: 50–100 μ m; pressure: 1.2 bar
Fab@Home	Alginate	Chondrocytes	\varnothing Nozzle: 840 μ m, nozzle precision: 25 μ m, width: 1200 μ m; height: 800 μ m; flow rate: 0.6 mL/s; size: \varnothing : 6 mm \times 2 mm height
	Methacrylated hyaluronic acid, methacrylated ethanolamide, PEGs	Hepatoma cells, epithelial cells, fibroblasts	
Bioplotter	Gelatin/chitosan	Hepatocytes	\varnothing Nozzle: 300 μ m; drop volume: 20 nL; lateral resolution: 10 μ m; X-Y velocity: 10 mm/s; extruding velocity 30 mm/min; pressure: 0.3 bar; layer height: 180 μ m
	Gelatin/ alginate gelatin/alginate/fibrinogen gelatin/alginate/chitosan	Neuron cells; Schwann cells, ADSC, hepatocytes	\varnothing Nozzle: 250 μ m, X-Y velocity: 5 mm/s; extruding velocity 15 mm/min; layer height: 150 μ m; width: 380 μ m
Multi-nozzle SFF deposition system	Alginate	ECs, fibroblasts, hepatocytes	Droplet-based or continuous deposition; \varnothing Nozzle: 30–500 μ m; velocity: 10 mm/s; pressure: 0.3–2.8 bar; 40 layers
Cell writing system	Alginate with iron oxide nanoparticles	ECs	Size of construct: 5 \times 5 \times 2 mm Printing pressure: 0.3 bar
Dispensing-based deposition system	Mebiol (Nisopropylamid and polyoxyethylene)	Insect cells	Feed speed: 0.5–0.83 mm/s; pressure: 0.3–0.4 bar; line width: 114–300 μ m; size: 1 \times 1 mm
nScript bioprinter	Collagen I, agarose	Embryonic cardiac cells, ECs, ovary cells, SMCs, fibroblasts	Tubes: \varnothing 900–25,000 μ m, wall thickness 300 μ m

^a (LGDW = Laser-Guided Direct Writing; BioLP = Biological Laser Printing; LIFT = Laser Induced Forward Transfer; 2PP = Two-Photon Polymerization; BAT = BioAssembly Tool; ECs = endothelial cells; hMSCs = human mesenchymal stem cells; SMCs = smooth muscle cells; BMSCs = bone marrow stromal cells; ADSCs = adipose-derived stromal cells; PEG = poly(ethylene glycol); DM = dimethacrylate; DA = diacrylate).

4. Comparison Between the Different Biofabrication Techniques

Table 1 shows the comparison among various bioprinting technologies, which uses the different scaffold materials and cells, which leads to differences in a final accuracy of printed models. [7]

5. Conclusion

In the last decade has been presented many investigations of new techniques for biofabricating of 3D cellular constructs using complex designs. The amount of research and the creation of new companies indicate the strong growth potential of this new field. 3D biofabrication can be appropriate for the production of required, shape complex scaffold geometries with different materials. It further has the potential to offer a controlled placement of viable cells. Organ printing is currently viable, fast evolving and predicted to be a major technology in tissue engineering. Scaffolds are very important for the fabrication of functional living implants out of cells obtained from cell culture. The scaffold materials should be nonantigenic, nontoxic, and nonteratogenic and possess high cell/tissue biocompatibility so that they will not trigger pathological reactions after implantation.

Requirements of scaffolds are: individual external shape and well defined internal structure with interconnected porosity to host most cell types [5]. In time span of several years, it is expected that 3D bioprinting will be at a level when it will be possible to create complex organs such as kidneys, liver, heart etc. These organs than could be used, apart from the substitutes as educational tool for the study of medicine, for the preparation before major surgery or in the pharmaceutical industry for drug testing without the need for continued use of animals. In the nearest future, it is necessary to solve a number of problems related to bio-additive manufacturing, is there particularly the development of new materials, optimal design of substitutes of associated parts of the human body with the knowledge of physiology of cells, including optimum betting action of cells and vascularization.

6. Acknowledge

Presented paper was supported by project Research of New Diagnostic Methods in Invasive Implantology, MŠSR-3625/2010-11, Stimulus for Research and Development of Ministry of Education, Science, Re-

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