

Chapter 23

Towards Effective and Efficient Biofabrication Technologies

Andrés Díaz Lantada

Abstract The artificial production, in laboratories, of biological structures and even complete organs, by adequately placing and combining ex vivo cells, synthetically produced tissue patches and supporting biomaterials, including but not limited to tissue engineering scaffolds, is no more a matter of science fiction but a present relevant research challenge already providing promising results, included under an innovative area called “biofabrication”. If larger biological structures and complete organs could be synthetically obtained, patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced ex vivo from the own patient’s cells, and aspects such as organ piracy would be limited. It is important to highlight that nowadays around 10 % of organs used for transplantation worldwide comes from illegal activities. The socio-economical impact of synthetic organ production is comparable to that of the whole pharmaceutical industry, what explains the interest it has arisen in the last decades, with several new companies and research centres worldwide aiming at improving state-of-the-art tissue engineering procedures for starting 3D tissue construction and organ biofabrication. In addition novel scientific journals and book series are being devoted to these advances and related concepts and techniques are starting to be included in the syllabuses of teaching programs at universities, what will for sure be very positive for the evolution of this area. This chapter provides a brief introduction to this field of research, discussing most relevant advances on materials science, design tools and manufacturing technologies that being combined for making biofabrication a viable alternative to conventional therapeutic procedures. Main present difficulties and remarkable research challenges are also discussed. It constitutes an updated version of “Chap. 14: Biofabrication: Main advances and Challenges” from Springer’s “Handbook on Advanced Design and Manufacturing Technologies for Medical Devices” also by Andrés Díaz Lantada (Díaz Lantada 2013).

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23.1 Introduction: The Manufacture of Biological Systems

We have seen that in the context of medical device development and the biomedical industry, one of the major areas of application for these rapid prototyping technologies is tissue engineering. Since the 1980s, outstanding researchers like Eugene Bell and Robert S. Langer, both professors at the MIT, began looking at how to produce scaffolds with materials and geometries that were suitable for cell culture and tissue growth and could be used in surgical operations (Langer and Vacanti 1993).

The gradual progress in the field of biodegradable polymers together with the advances in more flexible rapid manufacturing technologies, means that at present, complex geometry scaffolds can be obtained to which living cells with growth factors adhere, and which multiply until they cover the scaffold. Having reached this stage, the set (scaffold + coating) is implanted into the damaged parts of the body. After being implanted, the cells adapt to their environment and reproduce the functions of the surrounding tissue, while the scaffold is gradually reabsorbed (Hollister 2005; Gómez Ribelles et al. 2010). All this has led to important changes in the approach to solving many surgical problems.

Further research efforts, with a view to obtaining three-dimensional biological structures, will one day lead to the additive fabrication of complex human organs (Atala and Yoo 2015). The firm Envisiontec GmbH has already developed the Bioplotter[®] with which small three-dimensional structures are being obtained by the “layer by layer” deposit of cells together with biocompatible material, and the initial use of the concept of a “bioprinter” looks promising. The company Lithoz stands out for high precision ceramic manufacture in an additive way, with which bone-like structures can be manufactured with micrometric precision. A larger number of printing technologies based on appropriately modified conventional RP technologies are being sought, which is progressively leading to more affordable machines (DigiLab Inc., Formlabs...).

This progress may open up new horizons to the treatment of many diseases by combining synthetic and biological materials to produce veins, capillaries, arteries, bones and soft organs, or at least part of them. By using machines with several heads that can deposit different materials biological tissue could be directly obtained with synthetic implants pre-integrated into them. This would endow the newly generated tissue with mechanical consistency.

However, there is still a long way to go, not only regarding the precision of these “bioprinters” and the biological and biomedical materials they are capable of depositing, but also regarding the manufacture of structures larger than 1 cm³. It would appear that the development of a capillary network to provide the newly generated three-dimensional cell structures with nutrients is currently one of the major limitations (Mironov et al. 2009; Bartolo and Bidanda 2008; Bartolo et al. 2009). There is also an important need for further progress in the design field, so as to obtain more adequate biomimetic CAD files, for subsequent manufacture of biostructures.

Relevant progresses in the field of high-precision medical imaging, together with software for handling such medical images as design inputs, are proving to be a key for further developments in the field.

Organising specific work sessions to facilitate information exchange among researchers is a particularly useful idea, usually within a framework of Bioengineering congresses, where rapid prototyping applications in the medical sector, especially those oriented to biofabrication, can be discussed, and people can join forces to go forward together. Worth mentioning are the “World Bioprinting Congresses”, the “International Workshop on Bioprinting and Biopatterning” and the “International Conferences on Biomedical Electronics and Devices—Biodevices 2008–2015”. Relevant journals in this novel field include “Bioinspiration and Biomimetics” and “Biofabrication”.

This chapter provides a brief introduction to these topics, after discussing main applications of biofabrication for the biomedical field in next section.

23.2 The Potential of Biofabrication and Its Application Fields

The final objective of research in the biofabrication area is the artificial production of organs and biological structures in laboratories, by adequately placing and combining *ex vivo* cells, synthetically produced tissue patches and supporting biomaterials. If organs could be artificially produced patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced *ex vivo* from the own patient’s cells, and aspects such as organ piracy would be limited. The applications in Medicine, if this final objective is achieved, are endless, however partial results, in the way to final achievement, are already providing interesting applications briefly discussed here.

Advances in the field of biofabrication are actually improving tasks and procedures linked to Tissue Engineering, as novel machines allow for the combined manufacture of biosubstrates with incorporated living cells and nutrients, hence enhancing cell growth and tissue formation for transplantation (Jakab et al. 2010).

Some biodevices for surgical interventions, such as sutures, are being seeded with cells (normally hMSCs) with the help of 3D printers designed ad hoc, thus accelerating tissue repair and recovery from surgical procedures (Kanani 2012).

New materials and biomaterials are continuously being discovered, in the search for more adequate substrates and supports for cell growth, and special attention is being paid to unconventional biomaterials as candidates for Tissue Engineering, as well as for other fields of technology, such as secretions from animals and plants (spider silk, plant resins...) (Lenaghan et al. 2011).

In addition, progresses on imaging technologies, aimed initially at improving diagnosis, if adequately combined with design and modeling tools, are also being of

help for promoting biomimetic designs, but also for replicating the structures of novel bio- and meta-materials and *in silico* assessing their behaviour, as detailed in next section.

23.3 Advances and Challenges Linked to Biomaterials

Materials Science has devoted great efforts in the last decades of 20th Century to the development (mainly synthesis/extraction and processing) of new materials and material families (such as polymers, polymer-matrix composites, metallic foams, super alloys...) and main advances during the first decade of the 21st Century focus also on that direction (artificial muscles, biopolymers, materials from natural origin...). These advances have completely changed the engineering world and reshaped the whole product development process, with outstanding impact in several fields including automation, aeronautics, architecture, design, electronics, information and communication technologies, energy and biosciences.

Parallel advances in design and simulation tools are providing very adequate resources for modeling such novel and often complex materials, whose behaviour is in many cases not yet fully characterized or understood. Therefore, besides the continued search for new materials capable of producing biocompatible devices, additional challenges linked to characterization and precise simulation are also needed for promoting the global biodevice development process.

Some relevant characterization tools (both oriented to biomaterials and to more specific biodevices) are normally oriented to an assessment of overall long-term mechanical performance and stability. More linked to modeling tasks are advances in medical imaging technologies, especially micro-CT, whose current precision, reaching around 25–50 μm , is high enough for the detailed reconstruction of most corporal structures (Shi et al. 2008; Guo et al. 2010).

The use of micro-CT technology to the 3D reconstruction of complex materials (and biomaterials), for subsequent modeling and simulation linked to studies in the field of Materials Science, is already common place. Once reconstructed, these materials can also be used, thanks to Boolean operations, for designing the inner structure of several biomimetic biodevices and prostheses. The linkage between medical imaging, CAD programs and FEM-based simulation modules can be a great help for assessing the adequate performance of a biomimetic structure, once adapted to the geometry of novel prostheses and biodevices, before the investing in the manufacture of prototypes for pre-production validation trials.

Reconstructions of the glass fibers of composite materials, of nickel foams and of porous woods can be found as cases of study in the website of SkyScan micro-CT company. Similar results can be obtained from micro-CT of polymers, ceramics, biopolymers and other biomaterials such as bone, as well as biostructures, what can help to design biomimetic scaffolds for tissue engineering and supports for biofabrication strategies.

23.4 Advances and Challenges Linked to Biodesign Tools

Advances for promoting biofabrication approaches are not only linked to finding more adequate materials and processing technologies compatible with cell deposition, but also to additionally exploring design processes capable of providing alternative approaches or complementary solutions, to those based on medical imaging-based reconstructions.

The term “Biomimesis” (from Greek “bios”—life and “mimesis”—imitation) is linked to the study of Nature’s models, principles, designs and processes to imitate them or find new inspiration for solving human problems (Benyus 2002). Main applications of biomimesis are aimed at finding new ways of producing food or energy, novel methods of manufacture, innovative therapeutic solutions and overall management of mankind and its relations.

As already detailed in cases of study along the Handbook, three important possibilities for promoting the development of biomimetic devices are: (1) the use of multi-scale mathematical modeling of biostructures, (2) the use of computer-aided design resources and recursive procedures towards biomimetic fractal geometries and (3) the use of medical imaging-based reconstructions. Figure 23.1 shows a biomimetic vascular model manufactured by means of additive technologies and designed by means of recursive procedures towards a fractal-like biostructure. The model is just aimed at providing an idea of the complexity of geometries capable of being additively manufactured and of the potential of these resources towards solving the problem of vascularization, which constitutes an unsolved challenge in the field of tissue engineering, repair and regeneration (Boccaccini et al. 2012).

The use of multi-scale manufacturing approaches is also enabling the production of biomedical microsystems with biomimetic features for enhanced interaction at a cellular level (see Chap. 8). Chapter 13 also already introduced the possibility of using fractal models for the design of biodevices with controlled surface properties or geometries imitating those from the body for an optimized biomimetic response.

In any case it seems clear that the incorporation of novel design tools into the conventional computer-aided design resources is a relevant need for supporting industrial designers facing the challenge of designing biomimetic devices. Even resources coming from the cinematographic industry may be of help, as several software used for the incorporation of artificial textures in digitally designed 3D geometries may be adapted to industrial design and rapid prototyping tasks.

It is necessary to note that, thanks to advances in the linkage between medical imaging technologies and CAD-CAE-CAM resources, once more multipurpose and effective 3D bioprinters are developed (capable of manufacturing even whole implantable organs), the reconstructions will probably be carried out on the basis of original information taken from the patient’s body, so as to provide personalized solutions.

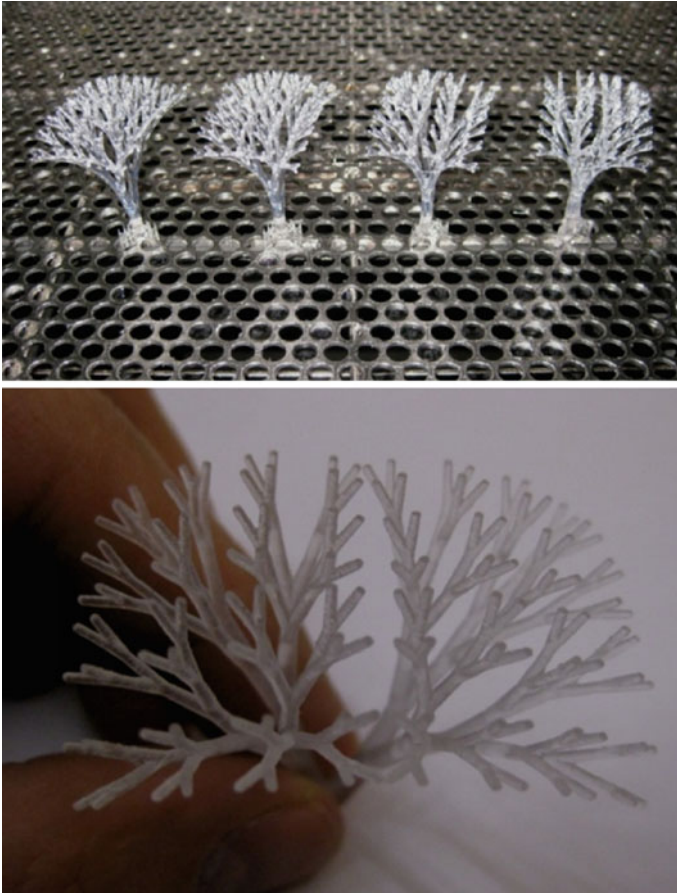


Fig. 23.1 Example of self-supported branched networks based on fractal biomimetic designs carried out by multi-scale computer-aided design

23.5 Advances and Challenges Linked to Biomanufacturing Resources

Conventional desktop-printers deposit micro-bubbles of ink, with remarkable precision, for writing documents and state-of-the-art very simple 3D printers (see information provided by the wiki of the “RepRap” project) are also capable of extruding fused polymers, gels and even molten chocolate, for obtaining three-dimensional prototypes with complex geometries in different materials.

Therefore the technology for depositing cells, coming within a liquid or gel-like matrix, and further constructing sheets and three-dimensional tissues, already exists. A simple combination of already available resources and additional research,

focused on supporting such cell growth, through an adequate vascularization and nutrient supply, are making biofabrication a reality.

Relevant recent results have already been obtained by using alternative methods, such as laser printing of cells into 3D scaffolds, which uses the propulsive force from laser-induced shock wave to propel cells gently into a substrate (Ovsianikov et al. 2010) or layer-by-layer extrusion of gelatin/alginate with seeded stem cells, for bioprinting small 3D biostructures (Norotte et al. 2009; Li et al. 2009; Marga et al. 2012).

The use of concurrent additive manufacture of scaffolding structures based on biodegradable thermoplastics and cells suspended in gels (different extruders would print different materials, as support, and provide also cells and nutrients), has also been proposed (Melchels et al. 2012).

In fact some commercially available resources already exist, which are already providing excellent support to research tasks linked to further advances in these directions, as detailed further on.

In Europe, EnvisionTec GmbH provides its “3D-Bioplotter™” (already in its 4th generation). The “3D-Bioplotter™” stands out for its versatility, as it can build parts by combining up to 5 materials with automated tool change, for its fast plotting speed, while maintaining appropriate accuracy, and for the possibility of printing up to 5 types of cells per object. Actually the “3D-Bioplotter™” has the capacity of fabricating scaffolds using the widest range of materials of any singular rapid prototyping machine, from soft hydrogels and biomaterials (agar, alginate, fibrin, chitosan, collagen, gelatin), over polymer melts (PLLA, PCL, PLGA), up to hard ceramics (hydroxyapatite, tricalcium phosphate) and metals (titanium), although these last harder materials require a sintering post-process.

In the United States, Digilab Inc. offers its “Cell Jet Cell Printer”, which stands out for its special focus on gentle cell deposition and for handling and delivering cell suspensions. Some tailoring to final application is also affordable. The current and potential uses of the cell printer include, but are not limited to:

- Delivering cell suspensions into micro-fluidic chips/high throughput cell based assay platforms.
- Dispensing cell suspensions in customized patterns to form microarrays on standard or custom microscope slides (or other substances), most commonly for developing/performing cell based assays.
- Delivering cell suspensions (in cell culture media or hydrogels) at defined locations in 2 and 3 dimensions onto pre-formed scaffolds (such as biological sutures/tissue construct scaffold), in order to populate the scaffold.
- Dispensing cell suspensions in custom patterns on a surface, for migrational studies or to study interaction of cells amongst each other or with growth factors.
- Delivering cell suspensions to micro-wells in a various diagnostic/research devices made of silicon, PDMS, or other substances, where manual delivery of sample is difficult, time consuming or simply impossible.

- Dispensing other reagents or growth factors or biologically relevant substances in a suspension, in addition to cells, to targeted locations/patterns in a similar manner.
- De novo biofabrication of relatively simple tissue constructs.

Researchers wishing to obtain additional information on related advances may wish to visit Digilab's website (www.digilabglobal.com) and have a more detailed look at the conference papers and publications linked to the use of the "Cell Jet Cell Printer" and related "synQUAD Technology" (capable of dispensing drop-by-drop down to 20 nL and up to several microliters of fluids).

Main challenges of bioplotters and cell printers are still linked to constructing more complex and bigger tissue constructs mimicking the complex structures of complete organs. Combined advances in medical imaging, design technologies and materials science will surely find solutions to such challenges, as the "hardware" (automated bio-manufacturing machines) for biofabrication is already working properly and providing effective solutions.

23.6 Main Conclusions and Future Research

The artificial production, in laboratories, of organs and biological structures, by adequately placing and combining *ex vivo* cells, synthetically produced tissue patches and supporting biomaterials, is no more a matter of science fiction but a present relevant research challenge already providing promising results, included under an innovative area called "biofabrication".

If organs could be artificially produced patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced *ex vivo* from the own patient's cells, and aspects such as organ piracy would be limited. The actual socio-economical impact of synthetic organ production is even comparable to that of the whole pharmaceutical industry, what clearly explains the interest it has arisen in the last decade, with several new companies aiming at improving state-of-the-art tissue engineering procedures for starting 3D tissue construction.

This chapter has aimed to provide a brief introduction to this field of research, discussing some relevant advances on materials science, design tools (either based on analytical modeling or on digital reconstruction) and manufacturing technologies that are currently working for making biofabrication a viable alternative to conventional therapeutic procedures.

Even though main challenges of bioplotters and cell printers are still linked to constructing more complex and bigger tissue constructs, mimicking the complex structures of complete organs, combined advances in medical imaging, design technologies and materials science are already providing interesting solutions and the "biomanufacturing machines" are already commercial and effective. Final whole organ printing is just a matter of time.

The promotion of collaboration between researchers may prove essential for reaching final objectives of biofabrication in perhaps a couple of decades, for instance following the example of the “RepRap” project collaborative wiki, which is encouraging many researchers to introduce additive manufacture as an additional support for their research.

These kinds of “do-it-yourself” rapid prototyping machines can also be adapted to 3D printing of biomaterials and cells, as an easy and affordable way of obtaining resources for conceptual validations linked to tissue engineering and biofabrication.

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Some Interesting Related Websites

<http://reprap.org/wiki/RepRap>

<http://www.digilabglobal.com>

<http://www.digilabglobal.com/celljet>

<http://www.envisiontec.de>

<http://www.skyscan.be>