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Review

3D Bioprinting:principles, fantasies and prospects

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ABSTRACT

Conventional three-dimensional (3D) printing techniques have been growing in importance in the field of reconstructive surgery. Three-dimensional bioprinting is the adaptation of 3D printing techniques to tissue engineering, through the use of a bio-ink containing living cells and biomaterials. We hereby describe the principles of bioprinting, its main current limitations, and the prospects of this technique. A PubMed/MEDLINE search was performed. A total of 40 publications were included. To date, most of the tissues have been printed with promising results in vitro (e.g., skin, cartilage, and muscle). The first animal studies are promising for small-scale defects. Vascularization issues are the main limitation to printing large constructs. Once the barrier of vascularization is overcome, printing organs and composite tissues of any size could be possible, opening the doors for personalized medicine based on medical imaging. Printing custom-made autologous grafts or flaps could minimize donor site morbidity and maximize the morphological results. Considering the potential future applications of bioprinting in the field of reconstructive surgery, one has to be aware of this tool, which could drastically change our practice.

1. Introduction

The importance of three-dimensional printing techniques has been increasing in the medical field [1]. Several applications are now routinely used in facial surgery, including printed anatomical models that are used for teaching and surgery planning [2] and industrial printing of metallic objects to allow for prototyping of personalized surgical guides and plates [3]. The main point of interest in current applications for reconstructive surgery is better precision, but the morbidity of donor sites remains the same as in classical techniques.

Three-dimensional bioprinting is the combination of 3D printing and tissue engineering. The potential therapeutic interest in this type of 3D printing could change the face of reconstructive surgery, increasing precision and suppressing the need for donor site or immunosuppressive treatments.

We hereby describe the principles of bioprinting, its main current limitations, and the exciting potential of this technique.

2. Methods

We performed a detailed literature search in the PubMed/MEDLINE database of all publications in the English language up to May 2018. The search terms used were "bioprinting AND facial surgery", "bioprinting AND reconstructive surgery", "bioprinting AND regenerative medicine". The abstracts were reviewed, and pertinent publications were included. Supplementary references were selected among the bibliographies of included articles. This review was based on a total of 40 publications.

3. Discussion

3.1. Principles of tissue engineering

Tissue engineering is a branch of regenerative medicine [4,5]. The aim of this discipline is to use the patient's own cells to create an autologous graft [6].

In the 90s, Robert Langer, a researcher in biotechnology, and Joseph Vacanti, a pediatric surgeon, were at the origin of the term

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tissue engineering. They described it as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" [7].

The three pillars of tissue engineering are cells, scaffold, and signals (growth factors). The success of in vitro tissue culture is judged on self-synthesis of the matrix and multiplication of cells.

There has been major advancement in tissue engineering techniques in the field of skin substitutes. Today, skin substitutes are obtained in vitro from the culture of keratinocytes, issued from a small skin biopsy. After 4 to 6 weeks of culture, the autologous epidermal sheets can be grafted. This has changed the management of severe extended burns [8].

Unfortunately, this type of in vitro substitute is not available for other tissues. For instance, in the field of bone reconstruction, autologous full-thickness grafts (cortical or cancellous bone) are harvested directly from the patient [9]. Xenografts and synthetic biomaterials might be suitable for small bone defects. Nevertheless, autologous grafts are still considered the gold standard for medium or large defects, responsible for donor site morbidity [10].

3.2. Concept of 3D bioprinting

Three-dimensional bioprinting is the use of 3D printing techniques for tissue engineering. Murphy and Atala described 3D bioprinting as "layer-by-layer precise positioning of biological materials, biochemicals and living cells, with spatial control of the placement of functional components (extracellular matrix, cells and pre-organized microvessels) to fabricate 3D structures" [11].

Classic 3D printers are adapted to receive cellular inks. Printers can be based on inkjet deposition, laser-assisted desorption, or microextrusion (Fig. 1).

The cells are either differentiated cells or stem cells [12]. They are integrated in a fluidic biomaterial (synthetic or natural polymers) to form what is called a bio-ink.

Computer-aided design and computer-aided manufacturing (CAD-CAM) tools are used for controlling both the pattern of layer-by-layer deposition (microarchitecture) and the overall shape (macroarchitecture) of the object to be printed [13]. The CAD-CAM step can be based on medical imaging (such as computed tomography) and involves image segmentation and mesh generation [14].

The printing step (Fig. 2) is similar to classical 3D printing but needs a high control of printing parameters to guarantee both the suitable rheology of the ink and the survival of the cells [15] (viscosity, speed of extrusion, and temperature of the extruder, temperature of the seal, and temperature of the plate receiving the object).

Once printed, the final object is kept under specific conditions inside an incubator and will go through a maturation step consisting of regular addition of growth factors and daily culture medium supply. Some authors described time as a fourth dimension, leading to the term 4D bioprinting [16].

The success of the process is judged on the survival of cells and their ability to synthetize their extracellular matrix.

The enhanced control of microarchitecture is the main interest of bioprinting compared with classical tissue engineering [17]. Indeed, in bioprinted samples, the cells and the particles are spread with a uniform distribution, whereas classical deposition leads to accumulation of cells and particles in the bottom of the sample due to gravity [18].

3.3. State of the art

Multiple laboratories have been working on the development of 3D bioprinting. Both academic and industrial teams are involved in research on this topic.

Every type of tissue has been studied in vitro through small size constructs [12,19].

Every cell type was tested (differentiating cells and stem cells) [17]. A cell type can be used on its own in association with other cell types.

A very high number of bio-inks were tested, usually by mixing resorbable and nonresorbable biomaterials [20]. A broad spectrum of polymers can be used to compose bio-ink [21]. The main natural polymers are alginate, hyaluronic acid, silk fibroin, collagen, and gelatin [22–24]. The main synthetic polymers are polylactide-co-glycolide, polyethylene glycol, poly-L-lactic acid, and polycaprolactone [25].

Intercellular signals such as specific growth factors (bone morphogenetic protein or vascular endothelial growth factor, for instance) can be added during the preparation of the bio-ink [26].

The most promising in vitro results concern the printing of skin tissues [27,28]. Full-thickness printed skin is obtained after 21 days of maturation using fibroblasts and keratinocytes, whereas 45 days were needed using traditional tissue engineering [24].

The first animal studies have already been launched for several types of applications [29].The constructs can either be printed first and implanted in a second phase [30] or printed directly on the animal.

Owens et al. [31] bioprinted a synthetic nerve graft composed of Schwann cell tubes and bone marrow stem cells subsequently

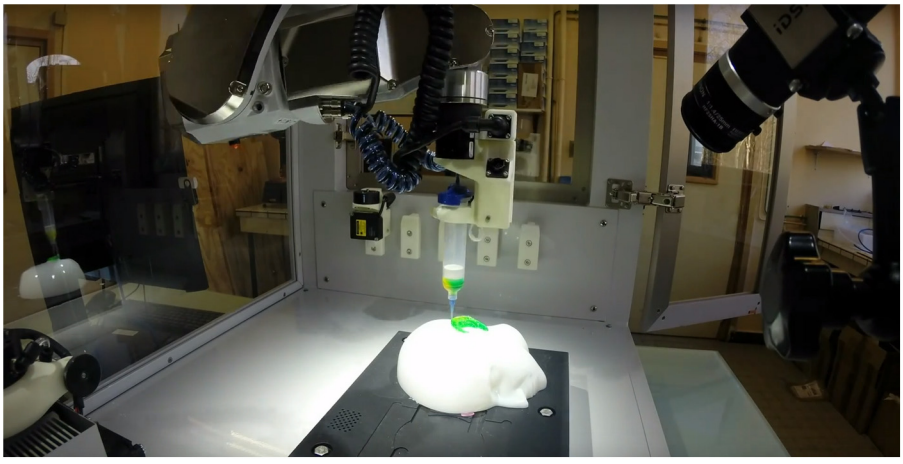

Fig. 1. Example of micro-extrusion bioprinter at work.

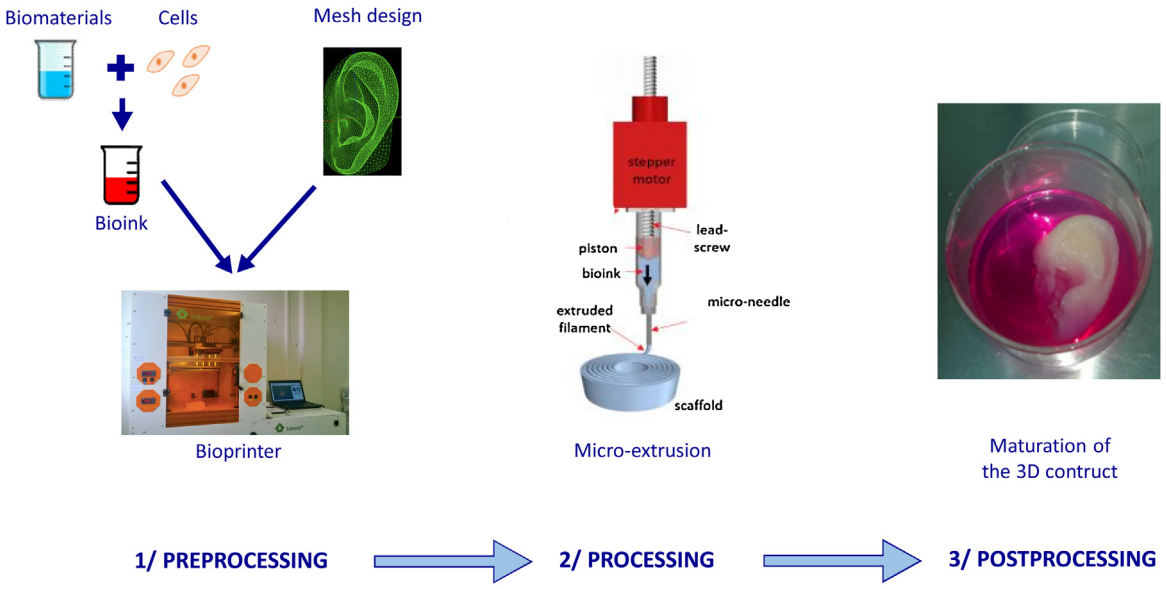

Fig. 2. The process of bioprinting is divided into three main steps: 1/ Pre-processing (generation of the mesh and preparation of the bioink) ; 2/ Processing (printing of the 3D object), 3/ Post-processing (maturation of the printed construct and transformation into a functional tissue).

implanted in rats for sciatic nerve repair. Motor and sensitive electrophysiological testing as well as histological findings showed results similar to autologous grafts.

Keriquel et al. [32] used in vivo bioprinting in a preliminary study to create hydroxyapatite-based constructs directly in calvarial bone defects on mice, in the perspective of custom-made robotic surgery.

Michael et al. [33] engineered cellularized skin substitutes containing keratinocytes via laser-assisted bioprinting. These substitutes were transplanted into full-thickness skin defects in mice, resulting in migration of fibroblasts, blood vessel formation, and collagen production.

Laronda et al. [34] used additive manufacturing in surgically sterilized mice by printing microporous hydrogel scaffolds of 15 × 15 mm in which mouse follicles were inserted. Follicle-seeded scaffolds were implanted and became highly vascularized and ovarian function was fully restored. Moreover, pups were born through natural mating and thrive through maternal lactation.

One has to be careful with interpretation of animal studies measuring healing after implantation of a printed construct. Indeed, characterization of natural healing versus benefit related strictly to printed tissue is hard to highlight in small tissue defects. To date, considering the technical limitations for large scale constructs printing, there have been no human studies.

The promises of bioprinting are well illustrated by the number of publications growing quickly year after year. The first field-specific journal was launched in 2015 (*International Journal of Bioprinting*). Four other journals dedicated to bioprinting were launched in 2016 [35]. Another indicator of the dynamism of research about bioprinting is the economic trend. Financial forecasts estimate that the 3D bioprinting market will reach $1.3 billion by 2021 [36].

3.4. Limits

One of the main limitations of bioprinting is the lack of a consensus because of the very high number of parameters [30]. There are so many options in bio-ink composition (cells and biomaterials), printing conditions (printer type, temperature, oxygen rate, speed of deposition), and maturation procedure (signals and bioreactors) that defining a gold standard for each tissue is a very hard task.

Vascularization of the printed tissues is another challenge [37,38]. The overall outcome of engineered tissue implants depends on the success of microvessel formation, maturation, and patterning [39]. To survive, a cell must be close to the source of nutrients (blood circulation) by a distance less than 400 μm [30,40]. This is the reason why one cannot print living pieces of tissue larger than 1-mm thick. Zhang et al succeeded in printing small-caliber tubes similar to blood vessels containing endothelial cells [41]. Nevertheless, integrating a full vascular network (from large vessels to capillaries) into the printed tissues is still impossible using current techniques.

3.5. Prospects

Once the limit of vascularization is overcome, printing organs and composite tissues of any size could be possible, opening the doors for personalized medicine.

Two main applications are targeted: in vitro cellular and tissue models and tissue engineering constructs for in vivo implantation.

The number of clinical and in vitro applications would be of a paramount scale. In vitro drug tolerance testing would be of a high effectiveness with printing of specific functional tissues [16,29]. Models of pathologic tissues could also be printed to test the efficacy of specific drugs [42]. Printing large functional models would be of a great help for teaching surgery. As simulation on synthetic models is being integrated into medical and surgical education, [43] training on living functional models would permit work in conditions very close to reality.

Most of all, reconstructive surgery would be highly optimized with printed composite tissues [44]. Instead of harvesting a large free flap, only a small biopsy of each type of cells would be necessary, with a great improvement on donor site morbidity [45].

The ideal flap for the patient's tissue defect would be designed from medical imaging (magnetic resonance imaging and computed tomography for deep defects, stereophotogrammetry for superficial defects) after numerical simulation of surgical procedure.

After mixing the patients' cells and biomaterials, the autologous free flap would be printed, including a vascular network connected to the main vascular pedicle placed on demand.

In this way, we could imagine a 2-step management for patients waiting for a reconstructive procedure. In a first 1-day appointment, the patient would have multiple biopsies under local anesthesia and would have a reference imaging. The custom-made free flap could then be printed and disposed in a bioreactor. A few weeks later, once the flap is functional, the surgery to implant the free flap could be performed.

In the same way, autologous organs could be printed, with no need to wait for a donor and no indication for immunosuppressive medication. It would also put an end to illegal trade in human organs.

In the specific situation of face transplantation, this would be of a major benefit to resolve the identity issue by creating a graft similar to the original face.

Considering that the medical profession is aiming toward personalized treatments and that social and technological evolutions are responsible for a decrease in the number of organ donors due to the reduction in accidental deaths, [46] 3D bioprinting might be a very promising solution.

In a more distant but considered ineluctable future, years'-long outer space travel will be the theatre of complex surgery in confined environments where no available tissue or organ donors will be found. This is actually a priority for all space agencies, and programs are already targeting this challenge, such as the Vascular Tissue Challenge launched by NASA in 2016 [47].

Once the current technical limitation of vascularization is solved to make "organ printing" possible for medical use, regulatory and socio-ethical issues might appear. These issues were recently highlighted by an official report from the European Parliament [48].

The emerging applications of bioprinting are "difficult to fit into current legislative pillars or categories. Moreover, one of the key challenges in regulating additive manufacturing is acknowledgement of the fact that biological and non-biological materials are regulated in different ways."

One of the main ethical concerns is that bioprinting "is a costly procedure that is available mostly to those who can afford such treatment. The high cost of the bio-printing manufacturing process and the required production capacity raise social and distributive justice questions and issues of fair or equal access given also the highly individualised character of the products". Moreover, bioprinting "might be (mis-)used to improve organs by adding functions or interbreeding human cells with those of animals to give the patient a competitive edge over other individuals".

These legal and socio-ethical challenges must be anticipated in order to get the best out of bioprinting.

4. Conclusions

Both technological and social evolutions are aiming at regenerative medicine and personalized treatments. The current techniques of facial plastic and reconstructive surgery are still perfectible in terms of morphological results and donor site morbidity. When the current limitations are overcome, 3D bioprinting could be key for these issues. Considering the potential future applications of bioprinting in the field of reconstructive surgery, one has to be aware of this tool, which could drastically change our practice.

Disclosure of interest

The authors declare that they have no competing interest.

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