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Reconstructive Medicine and 3d Bio-Printing, What Future?

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Abstract:- Bio-printing is a biomedical application of additive manufacturing processes to artificially produce biological tissues. Bio-printing can be defined as the spatial structuring of living cells and other biological products by stacking and assembling them using a computer-assisted layer-by-layer deposition method to develop living tissues and organs for tissue engineering, regenerative medicine, pharmacokinetics, and more general biological research1. It is a recent innovation that simultaneously positions living cells and biomaterials layer by layer to make living tissues2. The main use of printed organs is transplantation3. Research is currently being conducted on artificial structures of the heart4, kidneys, liver and other vital organs. For more complex organs such as the heart, smaller structures such as heart valves have also been researched. Some of the printed organs have already reached clinical implementation but mainly concern hollow structures such as the bladder as well as vascular structures.

Keywords:- *3D Bioprinting; Regenerative Medicine.*

I. 3D PRINTING

Reconstructing human tissue in the manner of the salamander regenerating an amputated limb is an ancient myth already evoked in Greek mythology about Heracles and the Hydra of Lerna. This snake regenerated its heads as Hercules cut them off. In the absence of being able to regenerate human tissue, the most commonly accepted solution is the replacement of damaged structures with a prosthesis, graft or transplant. The first prosthetic fingers were found in Egyptian mummies dating from 900 to 700 BC [1]. Classically, antique or modern prostheses are made by machining or moulding. However, for a little over 20 years a new manufacturing process by stacking successive layers has been developed: additive manufacturing, which today is assimilated to three-dimensional (3D) printing. Formerly confined to high-tech industries such as aeronautics, 3D printing is developing and gaining ground in the medical world. It has several advantages: low production costs for limited series or prototypes, optimization of the raw material which is deposited only where it is needed, manufacture of complex objects, personalization, etc... One of the applications of 3D printing is the production of custom-made bone substitutes [36] (→) that adapt better to defects than substitutes or grafts sculpted by the surgeon. 3D printing technologies are penetrating the world of medical prosthetics. Recently, a network of passionate volunteers created an open source e-NABLE'S (http://enablingthefuture.org/) website to create custom finger and hand prosthesis kits. The principle is the free provision of digital files to "print" the different parts of the prosthesis. Assembly tutorials are associated with it. The cost of the prostheses produced by this type of printing is approximately $100 to $150 compared to $4,000 to $6,000 for a commercial prosthesis. Approximately 1,500 of these prostheses have been created [2]. American physicians have made custom-made tracheal implants (splints) using 3D printing of bioresorbable polyester for the treatment of children with severe bronchotracheomalacia1. The first child who was implanted is now 3 years old and the splint is being resorbed without any adverse effects [3]. 3D printers are becoming more and more common in the operating room. They allow the surgeon to prepare complex operations by replicating the tissues to be operated on [4] and the inexperienced surgeon to prepare an operation but also to modify the indication for treatment thanks to a reality that is increased compared to traditional imaging examinations. Recently, a fetus with a mass that could compress its airways was not treated by emergency surgery, which presented a high iatrogenic risk for mother and child, but received a facial implant made using 3D printing [5].Today there are many commercially available rapid prototyping methods for tissue engineering applications: printing, extrusion, laser polymerization.

- Printing methods use the printing of a "glue" that joins together powder particles placed in a receiving tray. Each layer is progressively assembled to form a 3D structure. Powder residue often remains in the finished materials, which is a major limitation to the use of this technique in tissue engineering [6].
- Methods based on the interaction of lasers with matter operate on the principle of photopolymerization of photosensitive materials. Materials in liquid or solid form (powders) are available for these applications. Selective laser sintering uses a CO2 laser to bind the powder layer by layer. The resulting materials have a controlled internal and external architecture [7]. Stereolithography is one of the first rapid prototyping methods using this method. It involves liquid resins that are polymerized by a UV laser, layer by layer. At the end of the manufacturing process, the object is baked in an oven to finalize the polymerization. The resolution obtained is relatively low but this method is already widely used to make planning models or surgical guides [8].

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- Extrusion methods of polymer materials use print heads mounted on axes that are movable in all three spatial planes. Depending on the material, the material is deposited at room temperature or melted. After deposition, it solidifies by evaporation of a solvent or by reducing the temperature. By modifying the diameter of the extrusion nozzle and the path of the print head, a wide variety of morphologies can be obtained. The materials used are mainly synthetic polymers [7].

Apart from prostheses, few cases of implantation of biomaterials produced by rapid prototyping have been described in humans. In a preclinical model of an alveolar bone defect in pigs, Yeo et al. used a PCL-TCP (polycaprolactone/tricalcium phosphate) bone graft manufactured by a hot extrusion process and compared it to an autograft. They were able to show that the biomaterial was very well adapted [9] illustrating that rapid prototyping can provide a benefit in terms of material adaptation in a bone defect. On the other hand, this biomaterial is not very effective for bone regeneration in the absence of growth factors or cells. Rapid prototyping methods, used to manufacture macroporous biomaterials, have a resolution suitable for macroscopic clinical applications. However, this resolution is not suitable for controlling the microenvironment at the cellular level. It is probably necessary to combine several technologies to satisfy these two contradictory objectives: a porous macrostructure with a controlled microstructure [10].

II. BIOMANUFACTURING

Returning to the grail of tissue regeneration mentioned at the beginning of this review, prosthesis manufacture is only palliative and never restores the functionality of the tissue ad integrum. It is in this context that tissue engineering was developed, defined in 1993 by Langer and Vacanti as "the set of techniques and methods inspired by the principles of engineering and life sciences, used to develop biological substitutes that can restore, maintain or improve tissue functions" [11]. 11] In conventional tissue engineering, a biological or artificial matrix that is inoculated with cells and/or growth factors is usually used. This tissue engineered product can then be implanted or, prior to implantation, matured in a bioreactor. The purpose of this product is to be integrated into the tissue to restore or enhance function. Today, the concept of tissue engineering goes beyond regenerative medicine. It tends to cover the field of biological models of physiological or pathological tissues in order to reduce the need for animal experimentation and thus develop personalised pathological models allowing different molecules to be tested before being used in a patient. One of the major obstacles to the use of these products remains the lack of control and reproducibility: of the matrix (geometry, porosity, etc.), of the distribution of biological elements in this matrix (cells or growth factors), of the in vitro vascularisation of tissues or organoids, and of the complexity of the tissues to be reproduced.

One of the answers to these technological locks could be biomanufacturing. This technology has taken a major place in recent years. The term biomanufacturing was introduced in 1994, in connection with the manufacture of flat beads [12]. Beyond the natural phenomena of biomineralization, the term biomanufacturing is used in many technological disciplines such as biotechnology or synthetic biology. The broadest definition of biomanufacturing is the use of a process to produce a product with a biological function. In the field of tissue engineering, biomanufacturing includes bio-printing and bio-assembly [13]. Both techniques are based on a "bottom-up" approach, in contrast to conventional tissue engineering, which is "top-down". The bottom-up approach consists in producing three-dimensional elements layer by layer, whereas the top-down approach uses 3D matrices that are secondarily colonized by cells or growth factors. Bio-printing and bio-assembly are nevertheless differentiated by the units assembled and the manufacturing technologies used.

Bio-assembly consists in generating multicellular units in the form of fibres, aggregates or sheets, or with complex structures (organoids, microtissues) using extracellular matrix (ECM). Bio-assembly therefore consists of manufacturing hierarchical structures that are modular and have a 2D or 3D organization through automated assembly of cellularized elements. These elements can be fabricated by cellular self-assembly or by using building blocks that are composed of cells associated with biomaterials (Figure 1). These units are generated mainly from micro-fluidic or casting techniques that can be coupled with 3D printing of materials [13].

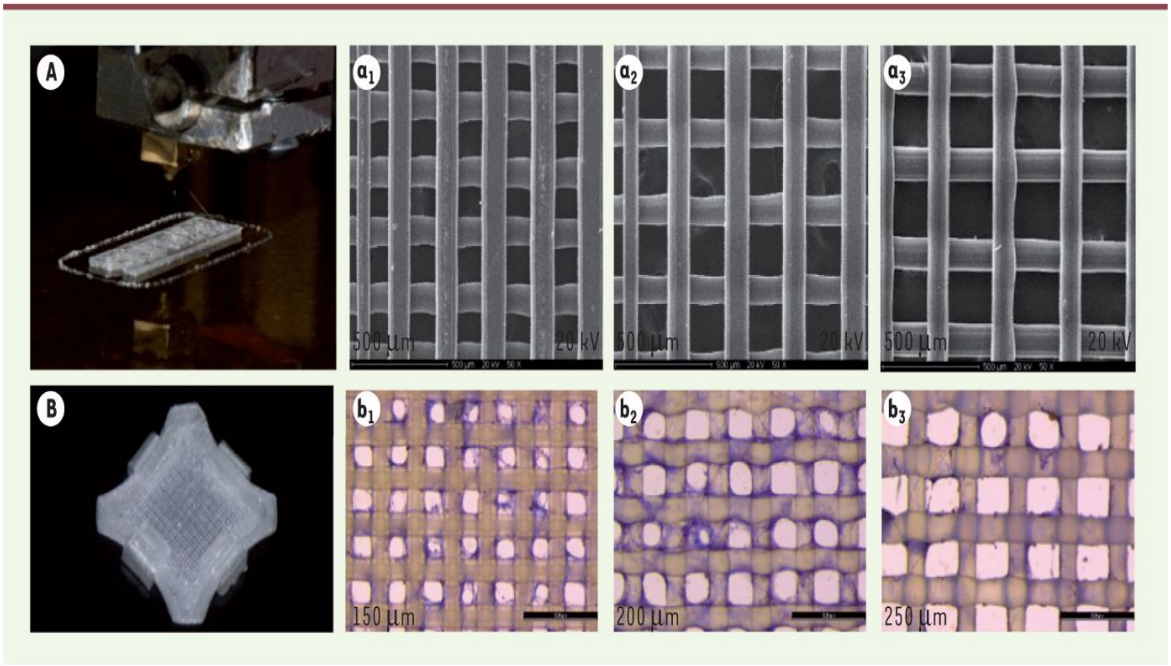

Fig 1:- Biomanufacturing process using the "sandwich technique". 3D printing of PLA (plastic material) grids (A) of increasing porosities (a1, a2, a3) . These grids can be superimposed and held by clips (B) which allows cellularisation of the different layers (b1, b2, b3) before assembly to promote cellular colonisation of the printed matrix in a bottom-up approach [35].

III. BIO-PRINTING

In 2010, Guillemot et al. defined bio-imprinting as "the use of computer-aided printing technologies that allow the arrangement and assembly of living and non-living structures, with two- or three-dimensional organization, to produce composite structures that can be used for applications in regenerative medicine, pharmacokinetic studies or fundamental work in cell biology" [14].

A. General Principle

Bio-printing is the two-dimensional (2D) or three-dimensional (3D) printing of living biological tissue. This is what distinguishes it from what is commonly known as 3D printing where materials are printed. Bio-printing is the layer-by-layer or point-by-point deposition of cells, extracellular matrix components (ECM), growth factors and biomaterials using computer-controlled printing technology from a digital file. It is therefore a computer-aided design (CAD) and manufacturing (CAM) process, using an additive manufacturing method by coupling the computer and a printer.

A notable difference between 3D printing, which prints "inert" matter, and bio-printing, which prints living matter, is the evolution of the biological pattern. It will undergo processes of fusion and maturation that will evolve with time, the environment and the printed pattern. This evolution of the bio-printed biological product has introduced the notion of 4D bio-printing where time represents the fourth dimension [15]. More recently, the notion of fourth dimension has also been applied to deformable materials that evolve over time [16]. While this notion of evolution is essential, it should be noted that it is not specific to bio-printing. It also applies to tissue-engineered products, regardless of the manufacturing process. Irrespective of the technique used, the bio-imprinting of a tissue is carried out in three stages: (1) computer-aided design of the printing pattern, (2) printing, and (3) characterization.

B. The printers

Several bio-printers have been developed: inkjet printers, extrusion printers (the print heads are made of micro-syringes) and laser-assisted printers (Figure 2A). Printing technologies will be more or less efficient depending on the volume to be printed and the desired resolution (Figure 2B).

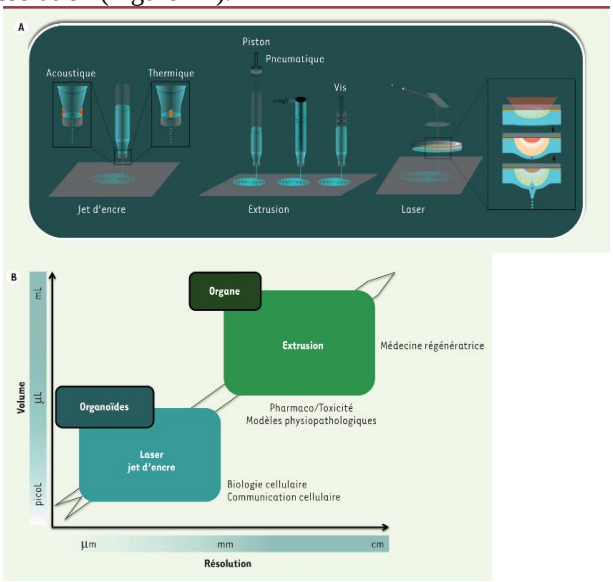

Fig 2:- A. Main technologies used in bio-printers. B. The choice of technology depends on the resolution requirements and the volume to be printed depending on the application [35].

The first micro-printing of biological elements (based on fibronectin) was carried out by Klebe in 1988 by inkjet printing [16]. In 2006, Boland et al. used modified desktop printers to perform cell microprinting [17]. Today, thermal (or piezoelectric) printers are used. Thermal inkjet printing relies on a thermal cell that produces a vapour bubble whose pressure generates a droplet through a 30 to 200 µm diameter hole. Piezoelectric inkjet printers use a voltage pulse that generates a change in the shape of a crystal that contracts the ink reservoir. When the piezoelectric crystal expands, the drop is ejected. These printers can print living cells in predefined patterns. The main disadvantage of these printers is the low usable cell density (less than 5 million cells/mL), which is necessary to prevent blockage of the printheads. In tissue engineering, inkjet printers have been used in situ to regenerate skin and cartilage, or in vitro to produce bone tissue engineering products.

Microsyringes have been developed to print biological elements by extrusion. Biomaterials (alginate, agarose, matrigel) are continuously extruded through nozzles a few hundred micrometers in diameter. The advantage of these techniques is to print scaffolds and cells at the same time. Micro-syringes have been used to make aortic valves, vessels, tumour or pharmacological models.

Laser-assisted bioprinting (LAB) techniques are based on a pulsed laser source, a donor (target) blade covered with a thin layer of bio-ink to be printed, and a receiver blade placed a few micrometers or millimeters away from the first one that receives the printed elements. Nano or femto-second2 pulsed lasers with wavelengths in the infrared (1 064 nm) or ultraviolet (193, 248, 266 and 355 nm) were used. The laser energy can be directly absorbed by the ink (MAPLE-DW [matrix assisted pulsed laser evaporation direct write] technique), resulting in the formation of a jet that vaporizes the first molecular layers of the bioink at the point of focus. When the laser energy is not absorbed by the bioink, a transducing/absorbing layer, which converts the light energy into thermal energy (in the case of BioLPs, biological laser printers) and/or mechanical energy (BA [blister-actuated]- and AFA [absorbing film assisted]-LIFT [laser induced forward transfer]), must be used. The absorbing layer is made of metal (gold, titanium, silver) of a few tens of nm for the BioLP, while it is made of polyimide for the BA-LIFT. The principle of drop ejection by LAB depends on the formation of a gas pocket secondary to the interaction of laser energy with the absorbing layer. In 2002, Ringeisen et al. demonstrated the possibility of printing proteins by MAPLE-DW without damaging the epitopes they contain, a double strand of DNA, or a functional alkaline phosphatase [18]. The process has been improved by printing complex bio-inks composed of hydroxyapatite, cells and extracellular matrix [19-28]. Compared to plating the same number of sheets by simple deposition (top-down approach), printing cell patterns layer by layer on polycaprolactone sheets (bottom-up approach) increases cell proliferation in vitro and in vivo [21]. Current research is focusing on laser-assisted in situ bio-imprinting, which consists of printing cell and matrix components directly on the patient according to a defined organization to promote tissue regeneration.

C. Applications of 3D bio-imprinting

Tissue bio-imprinting has two types of applications: the creation of cell and tissue models, and the manufacture of tissue engineering products for regenerative and restorative medicine.

IV. ORGAN OR TISSUE MODELS

Organ or tissue models are tools for reproducible and repeated testing of the pharmacological action of drugs. They represent an important issue in the selection of molecules according to their efficacy and toxicity. The development of complex three-dimensional models is an important issue for pharmacological research in the 21st century. Today, most of the available models only very partially reproduce the in vivo situation because their architecture does not take into account the complexity of tissue interfaces and vascular perfusion. Microfluidics chips partially resolve tissue exposure to mechanical fluid stimulation and perfusion, but they do not reproduce, in three dimensions, the complexity of tissues. In this context, research is being developed to reproducibly bio-print complex organoids in 3D that are perfusable. Another hope lies in the printing of organoids from different tissues to study their interactions. Few results are currently available. In 2008, R. Chang [29] published a model combining microfluidics and 3D printing. Organovo in the United States will release its first liver model in 2014, which integrates hepatocytes, stellar cells and endothelial cells printed in a matrix. This model is said to be more discriminating than 2D cultures. The main limitation is the small volume of the liver structures, which does not exceed a few hundred microns. They are therefore far from the real model. Three-dimensional tumour models are currently most often carried out using spheroid techniques suspended in gels (collagen, alginate, matrigel), reproducing the extracellular environment, in support structures (chitosan, polycaprolactone). Their limitations include the absence of interaction with immunity and angiogenesis and insufficient nutrient supply, which has been partially resolved by microfluidic systems. Xu et al. printed cancerous ovary cells and fibroblasts in matrigel but could not show the superiority of their model [30]. A breast cancer model was made by 3D printing of cancer cells and fibroblasts. This model was validated in terms of response to treatment [31]. More recently, HeLa (cancer line) cells were printed with gelatin, fibrinogen, and alginate, mimicking the 3D environment. After assembly, 90% of the cells were alive and tended to form spheroids, whereas cells grown in 2D remained as monolayers. Compared to 2D cultured cells, these cells expressed more metallo-proteases and showed greater resistance to chemotherapy, bringing them closer to the clinical situation [32]. Current research is attempting to model the behaviour of tumour cells in their environment. The development of these 3D tumour models by bio-imprinting therefore represents a great hope for pharmaceutical research and the development of personalised treatments.

V. REGENERATIVE AND RESTORATIVE MEDICINE

The second area of application concerns regenerative and restorative medicine. In this field, a distinction is made between ex vivo bio-imprinting and in situ bio-imprinting.

Ex vivo bio-imprinting is the creation of tissue engineered products using bio-printers. Several tissues of varying complexity have been reconstructed using this technology. It requires the use of complex multi-head bioprinters to print different biological inks to accommodate the complexity of the tissues. The production of complex organs such as the kidney is still a distant reality, but several groups are currently capable of producing skin. In the medium term, vessels will be able to be partially bio-printed. One of the current challenges of tissue engineering is the production of a venous, arterial and capillary network associated with printed organs. Vessels could be created by 3D printing by printing endothelial cells, fibroblasts and fibrin in a tunneled collagen gel using a heat-labile ink. A capillary network developed between the endothelialized vessels. The second approach is to directly imprint a tubular vascular network around a tube or on a support material (agarose) [33]. Vessels are formed by the maturation and fusion of cell aggregates within a few days. Recently, Atala et al. have demonstrated that it is possible to print human tissues of a size compatible with clinical use. This was made possible by a combination of several techniques: imaging of the anatomical defect and computer-aided design of the tissue to be reconstructed, creation of a degradable acellular mould to shape the tissue, printing of cross-linked cellularized hydrogels after printing, and creation of a network of microtunnels to promote the passage of nutrients [34]. They were thus able to demonstrate, through combined techniques integrated in a single bio-printer, that it was possible to reconstruct : (1) a portion of mandible with a morphology adapted to substance loss (3.6 × 3.2 × 1.6 cm), and the manufacture of a calvaria3 bone that allows bone regeneration in rats; (2) a skeletal striated muscle (15 × 1 × 5 mm) innervated and responding to electrical stimulation after implantation in vivo in rats ; (3) ear cartilage (3.2 × 0.9 × 1.6 cm), which has a complex shape, which was maintained for two months after implantation for maturation at the subcutaneous site in athymic rats [34].

In situ bio-imprinting involves bio-imprinting cells, matrix, growth factors directly at the site of tissue loss to promote regeneration of damaged tissue. The arrival of bio-printers to print the missing tissue or organ in situ in the operating theatre is obviously a long-term prospect that will require coupling printers to imaging systems in order to visualise the tissue defect. The advantage of this approach, however, is to dispense with the in vitro maturation steps which are long, costly and promote the risk of contamination. The current limitation of in situ bio-imprinting is that it can only be used for localized surface tissue losses such as skin or calvaria. This approach has been used with inkjet printers to print stem cells from amniotic fluid for the treatment of burns [35]. Our group has used a laser printer to print hydroxyapatite in calvarial defects [25]. More recently, we were able to print collagen, hydroxyapatite and mesenchymal stem cells and observed that the printed cell pattern could guide healing.

VI. CONCLUSION

Additive manufacturing encompasses a number of popular technologies that are attracting the interest of biomaterials and tissue engineering researchers. Additive manufacturing as applied to regenerative medicine covers two main fields: 3D printing of inert or bioactive material and biomanufacturing. While 3D printing has penetrated the world of regenerative medicine, bio-assembly and bio-printing techniques are still in their infancy. The aim of this article is to provide a non-exhaustive review of these various complementary aspects of additive manufacturing in the service of restorative, regenerative medicine and tissue engineering.

- *Conflicts of interest:*
  None.

REFERENCES

[1]. Finch J. The ancient origins of prosthetic medicine. Lancet 2011 ; 377 : 548–549. [CrossRef] [PubMed] [Google Scholar]
[2]. Kuehn BM Clinicians embrace 3D printers to solve unique clinical challenges. JAMA 2016 ; 315 : 333–335. [CrossRef] [PubMed] [Google Scholar]
[3]. Zopf DA, Hollister SJ, Nelson ME, *et al.* Bioresorbable airway splint created with a three-dimensional printer. N Engl J Med 2013 ; 368 : 2043–2045. [CrossRef] [PubMed] [Google Scholar]
[4]. Malik HH, Darwood AR, Shaunak S, *et al.* Three-dimensional printing in surgery: a review of current surgical applications. J Surg Res 2015 ; 199 : 512–522. [CrossRef] [PubMed] [Google Scholar]
[5]. VanKoevering KK, Morrison RJ, Prabhu SP, *et al.* Antenatal three-dimensional printing of aberrant facial anatomy. Pediatrics 2015 ; 136 : e1382–e1385. [CrossRef] [PubMed] [Google Scholar]
[6]. Billiet T, Vandenhaute M, Schelfhout J, *et al.* A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. Biomaterials 2012 ; 33 : 6020–6041. [CrossRef] [PubMed] [Google Scholar]
[7]. Leong KF, Cheah CM, Chua CK Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials 2003 ; 24 : 2363–2378. [CrossRef] [PubMed] [Google Scholar]
[8]. Bai S, Bo B, Bi Y, *et al.* CAD/CAM surface templates as an alternative to the intermediate wafer in orthognathic surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010 ; 110 : e1–e7. [CrossRef] [Google Scholar]

[9]. Yeo A, Cheok C, Teoh SH, *et al.* Lateral ridge augmentation using a PCL-TCP scaffold in a clinically relevant but challenging micropig model. Clin Oral Implants Res 2012 ; 23 : 1322–1332. [CrossRef] [PubMed] [Google Scholar]
[10]. Ovsianikov A, Mironov V, Stampf J, Liska R Engineering 3D cell-culture matrices: multiphoton processing technologies for biological and tissue engineering applications. Expert Rev Med Devices 2012 ; 9 : 613–633. [CrossRef] [PubMed] [Google Scholar]
[11]. Langer R, Vacanti JP Tissue engineering. Science 1993 ; 260 : 920–926. [CrossRef] [PubMed] [Google Scholar]
[12]. Fritz M, Belcher AM, Radmacher M, *et al.* Flat pearls from biofabrication of organized composites on inorganic substrates. Nature 1994 ; 371 : 49–51. [CrossRef] [Google Scholar]
[13]. Groll J, Boland T, Blunk T, *et al.* Biofabrication: reappraising the definition of an evolving field. Biofabrication 2016 ; 8 : 013001. [CrossRef] [PubMed] [Google Scholar]
[14]. Guillemot F, Mironov V, Nakamura M. Bioprinting is coming of age: report from the International Conference on bioprinting and biofabrication in Bordeaux (3B'09). Biofabrication 2010 ; 2 : 010201. [CrossRef] [PubMed] [Google Scholar]
[15]. Gao B, Yang Q, Zhao X, *et al.* 4D bioprinting for biomedical applications. Trends Biotechnol 2016 ; 34 : 746–756. [CrossRef] [PubMed] [Google Scholar]
[16]. Cytoscribing Klebe RJ a method for micropositioning cells and the construction of two- and three-dimensional synthetic tissues. Exp Cell Res 1988 ; 179 : 362–373. [CrossRef] [PubMed] [Google Scholar]
[17]. Boland T, Xu T, Damon B, Cui X. Application of inkjet printing to tissue engineering. Biotechnol J 2006 ; 1 : 910–917. [CrossRef] [PubMed] [Google Scholar]
[18]. Ringeisen BR, Chrisey DB, Piqué A, *et al.* Generation of mesoscopic patterns of viable Escherichia coli by ambient laser transfer. Biomaterials 2002 ; 23 : 161–166. [CrossRef] [PubMed] [Google Scholar]
[19]. Ali M, Pages E, Ducom A, *et al.* Controlling laser-induced jet formation for bioprinting mesenchymal stem cells with high viability and high resolution. Biofabrication 2014 ; 6 : 045001. [CrossRef] [PubMed] [Google Scholar]
[20]. Devillard R, Pagès E, Correa MM, *et al.* Cell patterning by laser-assisted bioprinting. Methods Cell Biol 2014 ; 119 : 159–174. [CrossRef] [PubMed] [Google Scholar]
[21]. Catros S, Guillemot F, Nandakumar A, *et al.* Layer-by-layer tissue microfabrication supports cell proliferation *in vitro* and *in vivo*. Tissue Eng Part C Methods 2012 ; 18 : 62–70. [CrossRef] [Google Scholar]
[22]. Guillotin B, Guillemot F Cell patterning technologies for organotypic tissue fabrication. Trends Biotechnol 2011 ; 29 : 183–190. [CrossRef] [PubMed] [Google Scholar]
[23]. Mézel C, Souquet A, Hallo L, Guillemot F Bioprinting by laser-induced forward transfer for tissue engineering applications: jet formation modeling. Biofabrication 2010 ; 2 : 014103. [CrossRef] [PubMed] [Google Scholar]
[24]. Keriquel V, Guillemot F, Arnault I, *et al. In vivo* bioprinting for computer- and robotic-assisted medical intervention: preliminary study in mice. Biofabrication 2010 ; 2 : 014101. [CrossRef] [PubMed] [Google Scholar]
[25]. Guillotin B, Souquet A, Catros S, *et al.* Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials 2010 ; 31 : 7250–7256. [CrossRef] [PubMed] [Google Scholar]
[26]. Guillemot F, Souquet A, Catros S, *et al.* High-throughput laser printing of cells and biomaterials for tissue engineering. Acta Biomater 2010 ; 6 : 2494–2500. [CrossRef] [Google Scholar]
[27]. Chang R, Nam J, Sun W Direct cell writing of 3D microorgan for in vitro pharmacokinetic model. Tissue Eng Part C Methods 2008 ; 14 : 157–166. [CrossRef] [Google Scholar]
[28]. Xu F, Celli J, Rizvi I, *et al.* A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. Biotechnol J 2011 ; 6 : 204–212. [CrossRef] [PubMed] [Google Scholar]
[29]. Wang C, Tang Z, Zhao Y, *et al.* Three-dimensional in vitro cancer models: a short review. Biofabrication 2014 ; 6 : 022001. [CrossRef] [PubMed] [Google Scholar]
[30]. Zhao Y, Yao R, Ouyang L, *et al.* Three-dimensional printing of Hela cells for cervical tumor model in vitro. Biofabrication 2014 ; 6 : 035001. [CrossRef] [PubMed] [Google Scholar]
[31]. Ozbolat IT Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol 2015 ; 33 : 395–400. [CrossRef] [PubMed] [Google Scholar]
[32]. Kang HW, Lee SJ, Ko IK, *et al.* A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol 2016 ; 34 : 312–319. [CrossRef] [PubMed] [Google Scholar]
[33]. Skardal A, Mack D, Kapetanovic E, *et al.* Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med 2012 ; 1 : 792–802. [CrossRef] [PubMed] [Google Scholar]
[34]. Jordana F, Le Visage C, Weiss P Substituts osseux. Med Sci (Paris) 2017 ; 33 : 60–65. [CrossRef] [EDP Sciences] [PubMed] [Google Scholar]
[35]. J.C. Fricain, H. De Olivera, R. Devillard, J. Kalisky, M. Remy, V. Keriquel, D. Le Nihounen, A. Gremare, V. Guduric, A. Plaud, N. L'Heureux, J. Amedee, S. Catros. 3D bioprinting in regenerative medicine and tissue engineering. Med. Sci. (Paris), 33 (1) (2017), pp. 52-59.