

## Bio-printing Damaged Tissues: A Novel Approach in Regenerative Medicine

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### ABSTRACT

Regenerative medicine, deals with functional reconstruction of damaged tissues or organs after severe injuries chronic diseases, while body's natural responses are not sufficient. In this field, stem cells due to their exclusive potential in self-renewal and differentiation into other cell types, are the main sources of functional cells in regenerative medicine. However, challenges in stem cell culturing highlighted the need for new methods, which in addition to maintaining cell viability and functionality, can control the precise microarchitecture for cells in a three dimensional structure. In this review we focus on the application of different types of bioprinting technology in regenerative medicine and we overview how this method has been able to make progress in 3D-cell culturing and tissue engineering protocols.

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### Introduction

Bioprinting is an additive manufacturing technique that produces spatially precise controlled patterns for living cells and other nonliving biologic materials (1). In this process, different cell types can be combined with various materials to form bioinks. Among the different types of cells, stem cells have made great attention because of their unique abilities in self-renewal and differentiation, as well as, their numerous applications in tissue engineering and regenerative medicine (2). Bioinks are used to fabricate scaffold-based (i.e., microcarriers, hydrogels and decellularized matrix components) or scaffold-free cell aggregates. Then they are assembled using a computer aided layer-by-layer

deposition method (3, 4). The three most used bioprinting mechanisms are inkjet-, extrusion- and laser-based bioprinting (5). These different inks and techniques are suitable to customize a particular application, including construction of different cellular, tissue and organ-like structures in order to perform drug toxicity assay, evaluate the efficacy of treatment, assessment and validation of diagnostic methods and last not least the transplantation of functional engineered tissues or organs. The advantages of bioprinting techniques consist of accurate control of cell deposition, high resolution and scalability compared to traditional 3D tissue engineering, have specially made this technology

attractive for different applications in regenerative medicine (6, 7).

### **Inkjet bioprinting**

Inkjet bioprinting was the first bioprinting technology developed and is a noncontact printing process that deposits controlled picoliter droplets of cell-laden bioinks to fabricate multiscale architectures (8). Based on the method that the device uses to produce droplets, inkjet bioprinters can be classified into three types: 1) Thermal (by heating up to hundreds degrees in a few microseconds, evaporates biomaterial and produces bubble, which leads to dropwise ejection of bioink), 2) Piezoelectric (by a piezoelectric actuator that generates acoustic waves and results in droplet production), and 3) Microvalve (by a electromechanical valve which interrupts the bioink stream and produces droplet) (5).

Many studies have been done on the printing of stem cells by different types of inkjet bioprinters. For example, a thermal inkjet method was used to print human mesenchymal stem cells (hMSCs) encapsulated in acrylated polyethylene glycol hydrogel in a one-step manner. The printed construct showed high cellular viability and functionality including significantly enhanced osteogenic and chondrogenic differentiation for bone and cartilage formation as well as increased mechanical properties (9, 10). Furthermore, in order to provide a continuous supply of basic fibroblast growth factor to enhance bone marrow stem cell (BMSCs) proliferation, a piezoelectric inkjet photo printer was used and demonstrated higher cell proliferation rate (11). Another study used a microvalve-based inkjet system to print highly viable human embryonic stem cells (hESCs) and was indicated that valve-based printing process is able to produce spheroids of uniform size and maintain stem cells viability and pluripotency (12, 13).

### **Extrusion-based bioprinting**

Extrusion-based bioprinting (EBB) is a contact printing process and has made substantial progress in the past decade. The main principle of this technique is to extrude continuous filaments of bioinks by coordinating the pressure of a pneumatic- or mechanical- (piston or screw) based system (4). Pneumatic-based bioprinters utilize a

pressurized air supply while mechanical-based systems provide required pressure by applying the force of piston or screw on the bioink surface. Continuous filaments under the control of a computer software are deposited in two dimensions layer-by-layer to form 3D complete tissue constructs (6).

Since the extrusion bioprinting can print uninterrupted filaments rather than bioink droplets, many studies have switched to EBB. For instance, a pneumatic-based extrusion bioprinter was utilized to print HepG2 and human umbilical vein endothelial cells (HUVECs) in gelatin and collagen type I hydrogels to fabricate a liver-on-a-chip platform. They demonstrated the viability and functionality of hepatocytes and endothelial cells in the 3D construct (14). The same system was used to print chondrocytes and hMSCs in a nanofibrillated cellulose and alginate hydrogel scaffold in order to obtain a functional cartilage tissue (15). In another study a screw-based extrusion bioprinter with the aim of constructing biomimetic bone grafts was applied. The hMSCs were cultured in propylene fumarate dimethacrylate (PFDMA) ink with a dense shell of thermoplastic polyester resulted in a scaffold with superior strength along with increased permeability and osteoconductivity that promote cell viability and proliferation (16). A 3D double printed construct was built by two print-heads of an extrusion-based bioprinter. One head printed hyaluronic acid via pneumatic pressure and another one extruded poly( $\epsilon$ -caprolactone) (PCL) through screw mechanical pressure and finally a hybrid hydrogel was made. This construct was used to generate cartilage tissue by directed differentiation of MSCs to chondrocytes (17).

### **Laser-Based Bioprinting**

Laser-Based Bioprinting (LBB) is a nozzle-free, non-contact technique, which consists of three parts: 1) A pulsed laser source, 2) A donor layer and finally 3) A receiving substrate. During the printing process, a laser beam is focused on the donor layer or 'print ribbon' which contains an energy-absorbing layer (e.g., titanium or gold) and a transfer layer of bioink. The laser energy causes evaporation and creates a high-pressure bubble in the absorbent layer. This bubble can propel the bioink layer and leads to ejection of a cell-

suspended material droplet on the receiving substrate surface (18). Because of the unique advantages of this method, such as mechanical stress reduction and high printed cell viability (>95%) and the ability to print highly viscous materials and higher resolution, recently, this method is used in some studies.

For example, a type of laser-based printing named Laser-Induced-Forward-Transfer (LIFT) was applied to prepare a cardiac patch seeded with HUVEC and hMSC in a specified pattern for cardiac regeneration. This construct showed increased angiogenesis and improved cardiac function of infarcted heart, compared to control patch in which there was no pattern for cell seeding (19). LBB was also utilized to control both the size and density of the initial printed mouse ESCs to investigate the effects of these two parameters on the embryoid bodies. This study indicated that EB diameter was affected by printing density, while there was no such a relevance between the diameter of printed colony and EB size (20). A similar study, in order to figure out the effect of MSCs and endothelial cells (ECs) co-culture on the migration potential, used a LBB system and showed that co-printing can reduce the ECs migration and results in initial pattern conservation (21).

### **Challenges and future prospects**

The great potential of 3D bioprinting methods in tissue regeneration provides a promising solution for repairing lesions even in situ (22, 23) as well as addressing organ shortage for transplantation with minimal risk for immune rejection. In addition, the application of this technique in drug screening provides more realistic and reliable human models rather than animal models. This approach not only can reduce the overall costs but also can accelerate new drug development procedures. Furthermore, 3D bioprinting along with iPSC technology and novel gene editing methods e.g., CRISPER/Cas9 could be able to play an important role in treatment of many genetic diseases.

The question is why, despite all above mentioned possible applications, among more than 40 clinical trials submitted with the subject of 3D printing ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)), none of them used functional cells along with biomaterials to print a living construct? In these studies, application

of the 3D printers is limited to manufacturing of prosthesis and hard scaffolds for 3D modeling and supporting scaffolds. This technology is in its early development, but certainly other challenges such as legal hurdles have a profound effect on the progress speed of this technique. Under current EU and US legislations, cell-based medicinal products in which engineered cells combined with a supporting biomaterial for culturing new tissues, are regulated as biologics in the US and advanced therapy medicinal products (ATMPs) in EU. Currently, there is no distinct rule, explicit clear direction or guidance from regulatory authorities regarding the approval of medicinal products that constructed using 3D bioprinting technology (24). Therefore, it is a necessity for regulatory bodies to develop and approve relevant laws to ensure rapid development of 3D bioprinting technology in the near future.

### **Conclusion:**

Bioprinting is a promising technology that has made remarkable progress over the past decade. Advances in bioprinting have established the ability to print stem cells with high viability, preserved function, and maintained pluripotency that can generate new modeling systems and tissue constructs for a wide range of regenerative medicine applications. However many challenges should be addressed, such as co- or multi- printing of multiple cell types in an ordered spatial structure, mimicking human scale tissues and vascularization within the fabricated structures.

### **Conflict of Interest**

Authors declared no conflict of interest.

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