

Emergence of bioprinting in tissue engineering: a mini review

Abstract

Tissue regeneration of damaged tissues or complete reconstruction of the organs is the major concern in the human health-care-systems. The role of scaffold, cell, and growth factors decide the final fate of engineered tissues similar to native tissues depending on how they are formulated, arranged and fabricated. In the last decade, 3D printing technique has attracted a great attention to print scaffolds quickly, efficiently, and mass production of fabricated scaffolds with reproducibility as compared to conventional techniques. Further, based on this concept, 3D bioprinting followed by 4D bioprinting have emerged by precise positioning of biomaterials, cells, and biochemical with spatial control in making 3D bio-construct. In this mini-review, the overview of the basic principles of emerged bioprinting techniques, applications, limitations and future perspectives in tissue engineering field are precisely reviewed.

Keywords: engineering, regenerative medicine, 3d bioprinting, 4d bioprinting, organ printing

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Abbreviations: 3D, three dimensional; 4D, four dimensional; NC, nanocellulose; Alg, alginate; PEG, polyethylene glycol; PCL, polycaprolactone; ECMs, extracellular matrices; CAD, computer-aided designing; RGC, retinal ganglion cells; hTMSCs, human nasal inferior turbinate tissue-derived Mesenchymal Stem Cells

Introduction

Organ transplant is the major concern these days worldwide due to large number of accidents and chronic diseases every year. But, there is high shortage of organs because of the available donors. Therefore, the alternative is to fabricate artificial structures for the formation of new tissues or organs to compensate this gap of supply. Tissue engineering is the concept of regeneration of damaged tissues or reconstruction of the organs by taking advantage of physics, chemistry, mathematics, biology, and engineering sciences. This concept was reported first time in 1993 describing the characteristics and applications of biodegradable scaffolds.¹ For proper formation of new tissues, it needs scaffold with well-interconnected highly porous network providing and mimicking native three-dimensional (3D) microenvironment for cell migration and infiltration.²

For this purpose, several conventional fabrication techniques (e.g., freeze drying, electro spinning, etc.) have been used to fabricate 3D porous scaffolds, but not able to provide ideal or optimum 3D microenvironment by mimicking the targeted native tissues, in terms of controlling architecture of scaffold, pore-size and shape, degradation behavior (based on exposed surface area) for tissue regeneration process depending on particular cell or tissue type *in vitro* and *in vivo*.^{3,4} This is because of mismatch in characteristics of scaffolds and new tissue formation. To overcome this drawback, in the last decade, the concept of 3D printing was proposed to fabricate cell free-3D scaffold with precise control over pore size and shape and their interconnectivity⁵⁻⁹ and minimize the fabrication time and fast regeneration of tissues compared to conventional fabrication techniques. Depending on the biomaterial types, it can be performed in two ways:

A. Direct 3D printing and

B. Indirect 3D printing. Indirect 3D printing was developed for the fabrication of natural polymer

based 3D scaffolds because of the difficulty in direct printing of natural polymers.

This technique involves preparation of a negative mold (usually from support material) for casting particular natural polymer or polymers followed by drying step (e.g., freeze drying) to obtain desired 3D scaffolds.^{7,10-12}

Emergence of 3D bio printing

This concept of 3D printing significantly improved the tissue regeneration process and resulted in an entirely new way of one step printing of cell-laden 3D structure as 3D bioprinting or Organ Printing. In this technique, viable living cells can be incorporated with biomaterials to fabricate 3D complex multi cellular tissue constructs, and called as 3D bioprinting of complex functional tissues and can be applied in various biomedical applications. In addition, this technique presents fine flexibility in the design and fabrication of patient-specific complex 3D tissue-engineered structure or artificial organ.¹³

Compared to 3D printing, 3D bio printing needs a different technical approach showing compatibility with depositing living cells. However, no single bio printing technique facilitates the fabrication of scales (e.g., macro- and micro-scale) and complex construct of synthetic tissues.¹⁴ Originally, this concept of 3D bioprinting¹⁵ as 'organ printing' was proposed by Mironov et al.¹⁶ by defining as computer-aided jet-based engineering of 3D tissues of living human organs. This includes computer-aided designing (CAD) of targeted organ to provide fabricated model followed by the printing. In brief, it starts with the imaging of the targeted damaged tissues or organs of the body (step 1), designing approach (step 2), selection of materials (step 3) and cells (step 4) for specific tissue form and function, integration of these components through 3D bioprinting technique (step 5), and finally testing and application (*in vitro* and *in vivo*) (step 6) for targeted site.¹⁷ The combination of a hydrogel pre-polymer solution and cells is called as 'Bioink'. In this case, hydrogel serves an essential role in bioprinting process by providing structural support to the cells and

physical-chemical properties of the bioink.¹⁸

For bioprinting, ideal hydrogel should have desired

- A. Printability and cross link ability
- B. Structural and mechanical properties, and
- C. Bio-compatibility and controllability of degradation by-products and degradation kinetics, and
- D. Bio mimicry with targeted native tissues.

Further, for forming highly mimetic tissue or organ bioprinted, cells must be proliferated. For the selection of cells, two main factors are considered as (a) how closely the bioprinted cells mimic the *in vivo* cells physiological state and (b) what degree of *in vivo* functions of bioprinted cells under optimized 3D micro-environment.¹⁷ In this case, artificial tissues are seeded by either printing primary cells with supporting cells^{19,20} or printing progenitors or stem cells for further differentiation.^{21,22} Complexity in bioprinting may be increased by direct printing of primary cells. Multiple cell types incorporated into same or different hydrogel systems need to be printed in parallel and many bioinks need to be prepared for each print.¹⁴

The main advantages of this 3D organ printing are automation, high cell density, and ability to deliver organized and complex tissues compared to conventional scaffold-based approaches.^{23,24} The first bio-printed organ transplant is likely to be the thyroid gland of a mouse in 2015 in the world.²⁵ However, there is main challenge in proper positioning and culturing multiple cell types in a single process at a targeted location.^{26,27}

Table 1 Comparison of conventional and 3D printing techniques

| Techniques | Fabrication type | Advantages | Disadvantages | Ref. |
|----------------|--|---|---|-------------|
| Conventional | Fabrication of 3D cell-free scaffold | Able to fabricate porous scaffolds for possible cell attachment, proliferation and differentiation | Time consuming and not able to provide ideal or optimum 3D microenvironment by mimicking the targeted native tissues due to uncontrolled architecture, porosity and pores properties for proper cell infiltration and viability | 3,4 |
| 3D printing | Printing of 3D cell-free scaffold | Automation and precise control over pore size and shape and their interconnectivity, and minimize the fabrication time and fast regeneration of tissues compared to conventional fabrication techniques | Not able to provide ideal or optimum 3D microenvironment by mimicking the targeted native tissues due to mismatch in scaffold properties and native tissue in 3D microenvironment | 5–9 |
| 3D bioprinting | Printing of 3D cell-laden Scaffold | Automation and viable living cells can be incorporated with biomaterials with fine flexibility in the design and fabrication of patient-specific complex 3D tissue-engineered structure | Focuses only on the initial form or stage of the printed-object (static and lifeless) and by assuming printed cells can form and assemble tissues rapidly followed by facilitation of tissues with appropriate geometrical shape and mechanical performance | 13 |
| 4D bioprinting | Printing of 3D cell-laden Scaffold integrated with 'time' as new 4 th dimension | Automation with gradual transformation and Maturation of printed bio constructs over post-printed period (time) and leads to forming functional tissue construct within particular time duration | Especially the inferior mechanical strength compared to native bone and other. various challenges and problems | 25,28-32,56 |

Emergence of 4D bioprinting

In tissue engineering applications, 3D bioprinting has been applied potentially because of the printing in precise manner (layer-by-layer) and control in geometrical shape. Actually, it focuses only on the initial form or stage of the printed-object, and assuming it static and lifeless. In brief, 3D bioprinting involves the process, where it is assumed that the printed cells can form and assemble tissues rapidly through attachment, sorting, and fusion-processes of the cells followed by the synthesis of the extracellular matrices (ECMs) that facilitate tissues with desired geometrical shape and mechanical performance.

To overcome this drawback, recently new concept as '4D bioprinting' has emerged with 'time' involvement as the fourth dimension integrated with 3D bioprinting. Here, 'time' demonstrates the gradual transform of printed programmable biomaterials or living cellular bio constructs over post-printed period (time) (post-printing time-dependent shape change). This 4D bioprinting can be categorized in two ways:

- A. Printing of smart materials, usually responsive materials having functionalities or inherent properties that respond to external stimuli and are able to reshape or transform themselves.^{25,28–30}
- B. Maturation of complex constructs of tissue (cell-laden microgels) after printing through cellular coating, self-organization of the cells, and/or gradual matrix deposition and leading to forming functional constructs of tissue within a particular time duration.^{31,32} The comparison of conventional and 3D printing techniques is given in Table 1.

Application areas of bioprinting techniques

Functional organ-printing is a potentially growing field and have been applied in various tissue engineering areas such as bone,^{33,34} cartilage,^{35,36} cardiac,^{37,38} heart,^{39,40} vascular tissue,^{41–43} lung tissue,⁴⁴ liver tissue,⁴⁵ skin tissue,^{46,47} neural tissue,^{48,49} pancreas tissue,⁵⁰ and composite tissues.^{27,51} For cartilaginous tissues, Lee et al.⁵² printed a bioconstruct composed of polyethylene glycol (PEG)/ polycaprolactone (PCL) having chondrocytes and demonstrated the printing of ear-shaped bioconstructs by using this mixture of materials.⁵² Markstedt et al.⁵³ printed 3D cartilaginous ear by using novel nanocellulose (NC)/alginate (Alg) bioink supported with human nasoseptal chondrocytes. The results showed the biocompatibility of the bioink with patentability for the bioprinting of living tissues and organs and further, the composition of bioink can be manipulated for different mechanical performances based on applications.⁵³ In another experiment, for neuronal tissues, Owens et al.⁴⁹ showed the printing of a synthetic-nerve-graft using cells only (bone marrow stem cells and Schwann cells) for forming a dense nerve-conduit of Schwann cells tubes surrounded by bone marrow stem cell tubes for possible application in animal model studies.⁴⁹ Further, Lorber et al.⁵⁴ demonstrated the feasibility of printing of rat retinal ganglion cells (RGC) and glial cells through inkjet-printing systems. The printing process did not affect the viability of the RGC/glial cells in culture, and retain the growth promoting properties of the glial cells. Pateman et al.⁵⁵ printed PEG-based fine nerve guidance conduit through micro-stereo lithographic technique for nerve repair studies. In addition, 4D bioprinting has also been applied in several studies, for example bone tissue,⁵⁶ vascular tissue^{21,41,57,58} etc. In this concept, responsive cell-laden hydrogel facilitates the shape changes (3D microenvironment) and maturation of complex bio constructs leading to timely formed functional tissue network. Norotte et al.⁴¹ prepared fully biological vascular tubular grafts through self-assembly approach in layer-by-layer bio printing (scaffold-free tissue fabrication) resembling the vasculature by mimicking both compositionally and architecturally. In this study, agarose rods were used as a molding template and various cell types were aggregated into discrete units (either multicellular spheroids or cylinders). These discrete units were fused in post-printing and led to single- and double-layered small diameter vascular tubes.⁴¹ In other study, Hong et al.²¹ demonstrated the cellular behavior in micro-patterned hydrogels activated by maturation with multiple cell types through a 4D bioprinting system resulting in vascularization.²¹ Further, for bone tissues, Pati et al.⁵⁶ investigated the 4D bioprinting of hard-tissue bio constructs by printing grid pattern followed by coating with human nasal inferior turbinate tissue-derived mesenchymal stem cells (hTMSCs) for graft-mineralization. Additionally, this printed bone tissue bio construct matured after a designed culture period and the decellularized bio construct demonstrated improved osteoinductive and osteoconductive performance *in vitro* and *in vivo*. However, further investigations are needed to improve the mechanical performance (strength) of the printed bone tissue-engineered bio constructs due to the inferior mechanical strength compared to native bone.⁵⁶ Although, 3D and 4D bioprinting techniques have shown great potential but remains elusive due to various challenges and problems.

Limitations, future perspective, and conclusion

Bioprinting shows great potential in fabricating organ to alleviate the shortage of the organs for the patients. However, it still has some

limitations in some respect as follows:

- A. The selection of appropriate material with broad range of properties and cell types,
- B. Use of multiple materials and their compatibility and processing with cells or growth factors for the fabrication of complex tissues is also a limiting step in preparing many independent solutions for bioinks.

Further, the possibility of void formation during layer-by-layer printing that leads to collapsing of printed construct geometry. However, this drawback could be rectified by using sacrificial materials (must be cytocompatible) to provide mechanical support and removed in post-processing step, but this may limit further due to the complexity of printing process with rapid materials exchange or different inks loaded in multiple nozzles.¹⁷ The preparation of bioink and processing time of bioink are also an important issue. That time issue can be rectified to make some changes in features of bioprinter (multiple print heads or other refinement in printing process) by making faster bioprinting process.

Bioprinting process is still in its infancy and needs to be improved in materials' characteristics, time for bioink preparation, vascularization and innervation of tissues, on-demand scaffold controllability and maturation of cells for the preparation of human on-a-chip systems and anatomically realistic artificial organs on-demand. 4D bioprinting, compared to 3D bioprinting, shows a great potential in tissue engineering by taking the advantages of stimulus-induced structural deformation and maturation of engineered functional tissue constructs. Although, it is in early stage and several challenges and problems such as

- A. Printability and biocompatibility of responsive-materials against one or multiple stimulus systems.
- B. Control of 4D printing of engineered complex tissue constructs on a micro-scale (e.g., localized control) followed by the change in their shape, orientation, and functionality depending on external-stimulus and/or cellular self-organization, and
- C. Control over the alignment of cells during cell maturation need to be addressed and resolved in future.⁵⁹ In the present status, 4D bioprinting provides a potential and promising solution for the applications in tissue engineering.

In initial stage, 3D bioprinting or organ-printing has shown a huge potential in tissue engineering and become a promising fabrication technique to fabricate complex macro-/micro-scale systems in biomedical applications. In future perspective, this technique is a growing and emerging area for numerous challenges and problems, for example, fabrication of nano-scaled complex biomedical systems.¹⁴

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Conflict of interest

The author declares no conflict of interest.

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