

A Perspective on 3D Bioprinting Technology: Present and Future

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Abstract: Bioprinting, an additive manufacturing technique, has become the cynosure of research and industry world alike. This emerging technology holds the promise of constructing artificial tissues and organs in future. This paper overviews the current state in bioprinting technology, distinguishes between different types of techniques and describes the bio-inks. Current challenges and limitations inhibiting the growth of this field are also discussed.

Keywords: Bioprinting, Additive Manufacturing, 3D Printing, Biofabrication, Bio-Inks

Introduction

Last decade has seen rapid rise in the field of three-dimensional (3D) printing, also known as additive manufacturing (Agarwala and Yeong, 2016; Chua *et al.*, 2003; Goh *et al.*, 2016; Kruth *et al.*, 1998). The immense interest in the technology comes from the fact that structures can be built bottom-up easily, fast, cost-effectively and in customized manner according to the end user. Most importantly, 3D printing can create intricate designs and complex structures with ease using CAD models (Chua *et al.*, 2003). The customizable and easy processing of 3D printing has made it an integral tool in rapid prototyping for both industry and research and with applications ranging from home décor, furniture and toy to aerospace and military.

There has been particular interest in using 3D printing capability for bio-medical field. Although last decade has already seen rise in 3D printed bio-medical devices, appliances and accessories (Giannatsis and Dedoussis, 2009; Lee *et al.*, 2016; Yeong and Chua, 2013), the field is now moving on to print live cells, tissues and even organs (Murphy and Atala, 2014; Mironov *et al.*, 2013). Bioprinting, as it is commonly referred, holds the promise to print standalone or embedded cells on any surface in a customized manner for added functionality. Bioprinting is especially attractive to build tissue model platforms and for tissue engineering field. Traditional approach towards tissue models and engineering is to seed cells on to a porous and mechanically stable scaffold. However, such approach suffers from drawbacks like limited or no

control on cell placement, limited cell density, unable to fabricate complex scaffold shapes etc. Hence, new bio-fabrication approaches are critical to generate 2D and 3D complex biological constructs using living cells and engineered biomaterials. Bioprinting may overcome issues faced by traditional fabrication processes and provide required accuracy, flexibility, viability and reproducibility for biomedical applications (Wang *et al.*, 2015).

Bioprinting directly writes the living cells and biomaterials in a layer-by-layer fashion utilizing the initial CAD model of the final object or design. Hence, bioprinting can allow precise position capability that is required to create tissue model mimicking real human body structures with high cell density. The added in-situ skill can help to realize the dream of printing real body parts to solve the shortage of transplantable organs. This paper provides a general overview of the current state of bioprinting, elaborating on different technologies- laser, extrusion and inkjet. The paper summarizes printing strategies, current state and materials for bioprinting. Finally, challenges of the field and future research and trends are discussed.

Bioprinting Method

3D bioprinting is heralded as the next big revolution in biomedical and biotechnology field. Like other additive manufacturing techniques; namely fused deposition modeling, stereolithography, digital light processing, selective laser sintering and selective laser melting; the 3D bioprinting also works on building the end product from a computer model in layer-by-layer fashion. The

model file could be designed using several ways like Computer-Aided Design (CAD), Computer Tomography (CT) scanning or Magnetic Resonance Imaging (MRI) and used to print on the required printer (Hutmacher *et al.*, 2004; Peltola *et al.*, 2008; Seol *et al.*, 2012). The bioprinting techniques either fall under orifice based or orifice-free methods, which can be further classified in to four main categories depending on their method of operation. Figure 1 shows the schematic diagrams depicting various types of bioprinting techniques.

Inkjet Based

An inkjet-based printer is a non-contact printing technique that places extremely small droplets of ink on the substrate (Xu *et al.*, 2013; Abeyewickreme *et al.*, 2009; Fang *et al.*, 2012). Inkjet printing could be further divided in to continuous or drop-on-demand methodology to create cellular constructs in a desired manner. Continuous inkjet technique produces small droplets of ink from a reservoir through a nozzle in to a continuous and steady stream via instability mechanism (Xu *et al.*, 2013). The ink droplets are subjected to an electrostatic field and drops are deflected to print on the substrate or remain undeflected to be re-used. Drop-on-demand, as the name suggests, produces drops at regular intervals as and when required (Binder *et al.*, 2011). It can be classified in to thermal type or piezoelectric type. In thermal inkjet printing, a heating element helps to generate a bubble and eject the small droplet from the nozzle. A piezoelectric material, controlled by voltage, is actuated and dispenses the ink droplets.

Extrusion Based

Extrusion is a technique where continuous filaments of a material are forced through a nozzle in a controlled manner to construct a 3D structure. There is no droplet generation; instead a continuous stream of bio-inks is dispensed on the substrate. The printer setup normally consists of an orifice made of syringes, a motor that drives the syringes in 3-axis motion and a stage on which the object is constructed (Fang *et al.*, 2012). For extrusion printing of cells, the material usually consists of a highly viscous cell-laden hydrogel that can flow from the nozzle without the need for high temperatures (Fedorovich *et al.*, 2007).

Microvalve Based

The deposition of living cells using microvalve dispensing systems was first explored by Demirci and Montesano (2007), who developed a custom printing tool where four of these dispensers were mounted above a three-axis robotic stage for use in cell printing. Microvalve printing makes use of constant pneumatic

pressure to dispense small droplets through a valve. A controlled pressure system will force the bio-ink to go through the nozzle. The valve can operate under mechanical, electrical and magnetic influence to open and close. The nozzle size is usually in 100-300 μm in diameter. Different pressure control systems are used to print bio-inks of varying viscosities, as each may require different level of control. Microvalve-based bioprinting methods have high printing speed ($10\text{-}50 \mu\text{m sec}^{-1}$) and high cell density.

Laser Based

Laser based bioprinting is also called orifice free printing where a laser guides the writing process. The setup consists of a laser beam source, focusing system and stage. The normal mode of operation involves focusing a laser beam to create a light-trap, which is used to guide the cells on the substrate (Malda *et al.*, 2013). Another variation of this system, laser-induced forward transfer (LIFT) is more commonly used for printing cells (Bohandy *et al.*, 1986; Odde and Renn, 2000; Schiele *et al.*, 2010). Here the focused laser causes local vaporization of energy absorbing layer and the generated droplets are received by the substrate facing the bio-ink or biomaterials.

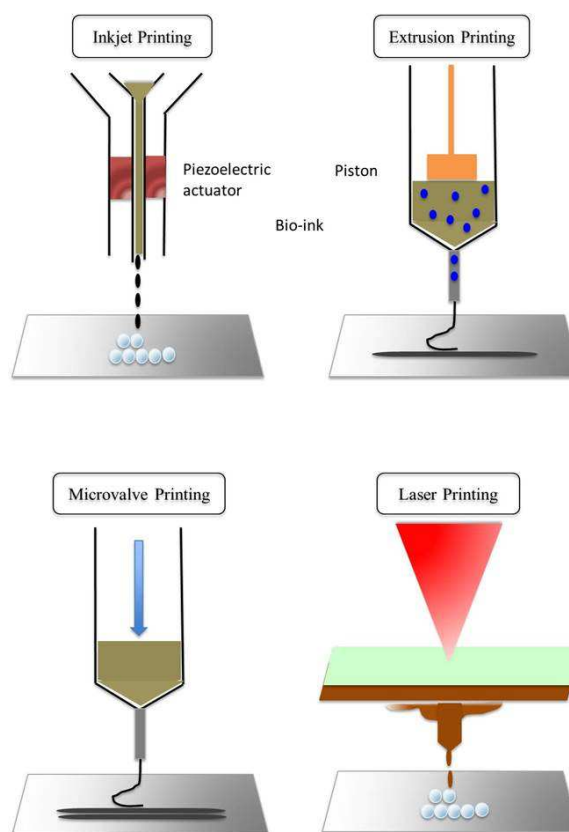


Fig. 1. Schematic showing various bioprinting techniques used

Bio-Inks and Biomaterials

The formulation of right bio-ink to get the desired properties and geometry remains the crucial aspect of bioprinting technique. A lot of applications require bio-inks to be laden with cells and this requires the formulations to have certain properties. The inks need to fulfill biological, mechanical and physical attributes to be printed with cells. Thus, it is important to be aware of the desired characteristics in order to synthesize appropriate bio-ink for various bioprinting techniques.

Hydrogels

Hydrogels are class of materials that are mostly composed of water. They are water insoluble, crosslinked, 3D networks of polymer chains and water. The water mainly fills the voids between polymer chains. Crosslinking facilitates insolubility in water and provides required mechanical strength and physical integrity. Properties like biocompatibility, ability to protect cells, good nutrient transport and in-vivo functions have made hydrogels the star biomaterial. These features also make them ideal candidates to act as Extracellular Matrix (ECM) component and allow cell encapsulation in 3D environment (Fedorovich *et al.*, 2007; Pati *et al.*, 2014). Hydrogels can be classified in variety of ways- depending on cross-linking, physical or chemical bond or physical structure of polymer chains. Another way to categorize these novel materials is on basis of how they are derived- natural or synthetic. The former hydrogels are fully biocompatible and biodegradable (Hunt *et al.*, 2010; Patterson *et al.*, 2010; Spiller *et al.*, 2011), unlike latter that may contain some toxic substances and may lack in some bioactive properties. However one big advantage of synthetic hydrogels is that they can be precisely tailored to give wide range of properties (Censi *et al.*, 2011; Dash *et al.*, 2011). Examples of hydrogels that have natural origin are proteins like collagen and polysaccharides like chitosan, dextran and alginate. Synthetic hydrogels are made from monomers such as vinyl acetate, acrylamide, ethylene glycol and lactic acid. In bioprinting, hydrogels are used as bio-ink materials or as cell delivery vehicles. Many types of cells have been encapsulated within hydrogels and tested, such as fibroblasts, chondrocytes, hepatocytes, smooth muscle cells, adipocytes and stem cells (Lutolf *et al.*, 2009; Savas and Utkan, 2013).

Cell Printing

Crucial for the application of cell printing is a high survival rate and no damage to the genotype and phenotype of the cells. Cell-aggregate-based bio-inks can be homogeneous, containing a single-cell type, or heterogeneous, prepared by coculturing several cell

types. The cell-aggregate-based bio-inks typically used are tissue spheroids, cell pellets and tissue strands. In addition to hydrogels and cell aggregate-based bio-inks, decellularized matrix components have been recently considered as a new bio-ink type. Dong-Woo Cho's group used decellularized adipose, heart and cartilage tissue matrix components and printed with Polycaprolactone (PCL) framework to support tissue analogues in 3D (Pati *et al.*, 2014).

Scaffolds and Constructs

Scaffolds and constructs form the backbone of tissue engineering, as they provide the necessary framework to support cell attachment and tissue regeneration. Printing of scaffolds and constructs laden with cells and materials is most common type of biofabrication carried out. In bioprinting, a substrate is usually used as a bioprocessable scaffolding material, contributing as a biological and structural support for cells to attach, proliferate and differentiate. Biocompatible, nontoxicity, dispensability, rapid solidification and functionality with growth factors for high cell viability are some properties required for the scaffolds. Thus far, various hydrogels such as collagen and soy agar gel have been used as a substrate material (John *et al.*, 1977; Lee and Yeong, 2016; Savas and Utkan, 2013). The traditional method of scaffold fabrication uses "mold and fill" method, which is replete with limitations. Controlling the placement of cells on scaffolds precisely, having controlled architecture and degradation of material with time are some problems that the traditional method is unable to address (Yeong *et al.*, 2004). Bioprinting may overcome some of these limitations.

4D Printing

A recent advancement in 3D printing has led to evolution of 4D printing. 4D printing is loosely defined when another dimension time is added to 3D printing. 4D bioprinting can be considered a promising direction in the fabrication of living cells in a shorter period of in vitro culture time (Gao *et al.*, 2016). It enables the user to design almost any arbitrary shape, transformable to another shape using wide variety of materials. The arena of 4D printing has opened up a new platform where different properties can be combined to add functionalities in existing systems and thus have dynamic microstructures that can have wide applications (Jamal *et al.*, 2013). For bio-medical field, 4D printing can enable capabilities where objects constructed using bio-inks can fuse, fold and re-model to generate tissues and constructs in fourth dimension of time. This can help mimic real life body functions and mechanisms. The degree of recovery, folding angle and tenability will depend on the properties of the materials, cell behavior, growth media and other parameters.

Challenges

The choice of printing technique to be used will depend on lot of factors like complexity of design, desired geometry, nature and volume of bio-ink. The field of bioprinting is still in its infancy and requires a lot of challenges to be answered. There is lot of room for improvement in printers where nozzles and print heads can be designed for finer resolution, multi-material printing, higher degree of freedom and more automated control.

Vascularization of engineered tissues is a key issue that needs to be fully addressed for the bio-medical field to be successful. This is especially true for 3D tissue constructs requiring adequate supply of oxygen and nutrients. To take a step further, it would be great if bio-inks can incorporate well-connected capillaries to realize functional tissues and printed organs. This will also require bio-inks and biomaterials to be developed in a special way to have multicellular aggregates for self-vascularization. Material innovation is also needed to impart sufficient mechanical strength after printing, so that structures are able to retain their shape. Extrusion and micro-valve based printing suffers from a common problem-lack of mechanical integrity in the printed hydrogel constructs. This has not only limited the scale and fidelity of the constructs but has also posed challenges for implantation of the printed structure in vivo. Some research groups have worked towards solving the issue. Recently, Schuurman *et al.* (2011) employed extrusion printing of soft, cell-laden hydrogel with another stiffer synthetic polymer. The group demonstrated sequential printing of Polycaprolactone (PCL) and cell-laden alginate, where alginate component was crosslinked with calcium chloride following deposition.

Scalability, reliability and repeatability are others concerns that need to be addressed for 3D printing technologies. With the world talking about production of organs, the above features become even more important.

Conclusion

This review paper provides a general overview of the current state of the art of bioprinting technology. A distinction between laser-, extrusion- and inkjet-based bioprinting technologies is made. Various types of bio-inks are also discussed. Even though the biofabrication of whole organs has not yet been achieved, bioprinting is already changing the way tissues and organs are studied by providing reliable constructs with increasing complexity and resemblance to native tissues. However, despite the great progress and breakthroughs, bioprinting technology is still in its infancy and needs to overcome several challenges and limitations.

Ethics

This article is original. Authors declare no ethical issues that may arise after the publication of this manuscript.

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