

Bioprinting: An immense potential in Tissue Engineering

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Abstract

The increasing gap in the demand and supply of organs puts the lives of end organ failure patients under extreme risk. Hence any ability to generate organs in the lab would come as a big relief. Bioprinting is a growing technique with promising success in the printing of simple 2D organs as well as initial partial successes in printing of complex tissues like lungs, heart valves etc. It is also useful in many other applications like drug testing, tumor studies etc. However the technique still has to overcome many challenges before it can become a dependable process in the clinics and labs.

Keywords: tissue engineering, regenerative medicine, scaffold

Introduction

Tissue and organ transplantations are indispensable needs in many end stage of organ failures. Science has advanced a lot in this yet the true potential of the organ transplantation is limited by the factors like demand supply gap, immunorejection^[1], infections^[2, 3] etc. The ability to generate tissues and organs at will, hence would go a long way in solving the crisis of organ demand and supply gap^[4]. Bioprinting is an offshoot of 3D printing which involves the layering of the cells one above the other in a controlled format and over a compatible scaffold to generate tissues and organ of desire^[5]. Tissue engineering is almost three decades old and has been used to partial success in the structural functions regeneration and restoration^[6]. However various limitations exist that make generating complex tissues difficult. Hence bioprinting has evolved as the recent technology which employs scaffold to align the cells over to generate the microstructure involved in complex tissues. The ideal scaffold needs to be biocompatible, biodegradable and suitably strong and flexible^[7, 8]. The conventional methods of scaffold synthesis leaves many loopholes with respect to its microstructure and the ability to transport nutrients and oxygen among others^[9, 10]. Additive Manufacturing (AM) offers solution to many of the conventional methods. AM selectively adds materials and is categorized into techniques like stereo lithography, fused deposition manufacturing and selective laser sintering. This technology provides for a better size and shape control at the microscopic levels other than ensuring the right deal of porosity in the tissues. Moreover, AM technology can be used to generate 3D tissues and organs from the Magnetic resonance imaging (MRI) and CT scans using Computer aided design and manufacturing (CAD/CAM)^[11-13].

This paper tries to summarise the present status of bioprinting and the various types of techniques used in the same. It also tries to assess the costs involved and the other applications it has apart from generating functional tissues for the surgical transplantations in future. We review the success reported in the recent times and the challenges it needs to overcome in future to become the technology of dependence.

The Techniques of Bioprinting

- 1. The Ink-Jet Bioprinting:** This is similar to the traditional ink jet paper printing. However here the conventional ink is replaced by the bio-ink that is a liquid suspension having the requisite cells suspended in it. Each of the droplet of the bio-ink that contains around 30000 cells are ejected from fine nozzles. The technique offers a great deal of control over the pattern to be printed^[15-17]. The Inkjet bioprinting further could use various methods like in thermal inkjet printing the ink chamber is supplied with heating elements^[18]. The heat generated by these elements produce bubbles inside the chamber forcing the droplets containing cells out from the nozzle. In another variant the piezoelectric bioprinter piezoelectric crystals are fixed at the back of the ink chamber. Pulse of current on this substance initiates vibrations in the chamber forcing the droplet out of the nozzle^[18]. This method generates very small droplets and hence offers a very high print resolution^[19]. However both the methods are known to put the cells under stress and are responsible for cell damage^[20]. Moreover, owing to the small droplets size that it produces the technique is not efficient with the high viscous bioinks and hence cannot be used to print out tissues like the extracellular matrix^[21].
- 2. Laser Assisted Bioprinting:** This technique employs the laser beams to fall on the substrate having the assembly of cells^[22]. The simplest of all, Laser based direct writing (LBDW) involves laser beam guiding the individual cells from the source to the substrate. In another commonly used laser induced forward transfer (LIFT) method the laser beam is used to vaporise the bioink in a controlled manner. The vapors containing the cells are then made to be collected on the substrate^[18, 23]. This method has reportedly improved the cell viability to upto 95% maintaining the spatial resolution at the same time^[24, 18].
- 3. Extrusion Based Bioprinting:** Also called as Pressure based bioprinting system, this involves extrusion of living cells as a continuous thread from the nozzle under the pressure driven piston or screw driving mechanism^[25]. These threads of cells are then crosslinked

physically or chemically to generate the 3D structure of need. Extrusion based bioprinting offers two big advantages. It is capable of printing tissues with high viscosity and also offers high viability of the living cells. More than 90% of the cells survive through the process. A wide variety of tissues could also be printed using this technique.

Applications of Bioprinting

In the recent times Bioprinting has acquired immense importance for its promise to generate tissues and organs in 2D and 3D in a customised manner. This could revolutionise the tissue engineering in general and regenerative medicine in particular [18]. Varying degrees of success has been achieved in the Bioprinting of various tissues like Bone [26, 27], Skin [28-30], Cartilage [31, 32], blood vessels [33, 34]. Additionally initial success has been achieved in printing lung organoid [35], heart tissue [36] and nerve tissues [37] as well. However it is going to take some time before the technology can be considered as a regular option for the treatment of organ transplantation conditions.

Despite the direct application of bioprinting taking its time to mature, many indirect applications make bioprinting a technology of importance. Drug discovery for instance, is a time consuming and cost prohibitive process. Only one in ten drugs that enter the clinical trials end up entering the market³⁸. Recently acquired abilities in 3D tissue printing will help generating *in vitro* assay system expediting the drug testing process. The bioprinted tissues hence are being increasingly considered in pharmaceutical industry for toxicity testing and high throughput screening³⁹.

In yet another important case of cancer study, so far we have been dependent upon the two dimensional cell cultures to study the cancer pathogenesis and metastasis. However it does not enable the true visualisation of the cell to cell and cell to matrix interaction that happens in a three dimensional environment. Bioprinted cancer tissues would act as good models in this regard to offer a correct microenvironment for the study purpose [40]. Bioprinting in many recent experiments has been used for integrating the stem cells on a given substratum. The individual subpopulations can then be made to develop into specific lineages as per the need by the specific growth factors and the other biomolecules. These developed and terminally differentiated cells could then be used for earlier mentioned applications like high throughput drug screening, disease progression and pathology and genomic analysis [41].

Challenges and Future Prospects

Bioprinting offers a lot of hope to many biomedical applications. So far the technique has been successfully utilised to print out simple tissues. However printing complex three dimensional organs still is a big challenge. Most prominently the tissues printed presently lack in mechanical strength and consistency as the natural one. The low viscosity requirement of the bioink makes the hydrogels generated to have lesser mechanical strength. Moreover complex tissues contain a heterogeneous mix of multiple cell types with the cellular integrity managed upto the micrometer levels. However the resolution of the bioprinting still remains a major issue. Another major challenge in bioprinting of major tissues is the lack of vascularisation. Hence it creates the

problem of lack of nutrients and oxygen to the cells [42, 43]. The technology is still grappling with the issue of print time required for the proper biofabrication. Any increase in the speed leads to the shear stress and hence lowers the cell viability. This issue must be addressed as well⁴⁴. Most of the present day research on bioprinting is based on the murine models. Hence it needs a scale up to porcine model to bring it closer to human trials. This certainly would require the close interaction of vessels and nerve which in itself is a big challenge for the future of this promising technique [45].

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