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#### **Review Article**

# **3D Bio Printing: Classification of Bio-Inks and Bioprinting Technique**

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#### ABSTRACT

Three-dimensional (3D) bioprinting is a revolutionary technique in biomedical engineering, enabling the fabrication of tissue and organ-like structures using bio-inks. This review focuses on the classification of bio-inks and the major bioprinting techniques used in tissue engineering. Bio-inks, the essential component of bioprinting, are categorized into natural and synthetic types. Natural bio-inks such as collagen, gelatin, alginate, and agarose offer excellent biocompatibility, while synthetic polymers like polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVP) provide greater mechanical strength and tunability. Critical properties of bio-inks-printability, biocompatibility, and viscosity-are discussed with respect to their influence on the fidelity and stability of printed structures. Furthermore, this review elaborates on three major bioprinting methods: extrusion-based, inkjet-based, and laser-assisted bioprinting. Each technique is analyzed based on its working principles, resolution, cell viability, and applications. Extrusion-based printing is favored for high-viscosity inks and cell density, inkjet-based printing for cost-effectiveness and precision, and laserassisted printing for its non-contact, high-resolution output. This review concludes that the selection of appropriate bio-ink and printing technology is crucial for the successful development of engineered tissues, which holds significant potential in regenerative medicine and pharmaceutical research.

### **INTRODUCTION**

The Three-dimensional (3D) printing is also known as additive manufacturing or rapid prototyping <sup>[1]</sup>. Additive manufacturing is one of the 3D scaffold fabrication method. It is a process of fabricating 3D solid objects from a digital file. The fabrication of 3D printed object is achieved using additive process. An object is created layering or built down successive layer by layer of material until the entire object is created. Each of these layer can be seen as a thinly sliced horizontal cross-section <sup>[2]</sup>. The main objective of tissues/organ engineering is to reconstruction of

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the damaged or diseased tissue or organ with cells and bio active molecule <sup>[3]</sup>. In addition 3D bio printing taking image of the damaged or targeted tissue/organ obtained by computed tomography (CT) or magnetic resonance imaging (MRI) scan <sup>[4]</sup>. Three dimensional (3D) printing was first described by Charles W. Hull in 1986, He named his method 'Stereolithography'. Thin layer of material that can be cured with ultra-violet (UV) light were sequentially printed in layer to form a solid 3D structure <sup>[5]</sup>. Later application of this process made it possible to create a sacrificial resin molds for fabrication of 3D scaffolds using biological material <sup>[6]</sup>. bio-ink is the main component used in the 3D printing. these bio ink varies with the printing. And the selection of bio ink is based on their properties like compatibility, printability, viscosity etc., <sup>[7]</sup>. There are several techniques for 3D bioprinting. They are Laserextrusion, assisted printing, Inkjet, and stereolithography. Among this extrusion-based bio-printing is the most common method for the bio printing technique<sup>[8]</sup>. In this review we discuss about the classification of bio-ink and also extrusion-based, inkjet-based and laser-assisted method 3D bioprinting. First we consider the bioink classification. Then next, extrusion-based, inkjet-based and laser-assisted method.

# 2.BIO-INK

Bio-ink is one of the most important requirement for 3D printing, It is composed of cells with biomaterials like hydrogel or cell aggregates and it plays a crucial role to fabricate 3D structure in 3D bio-printing <sup>[9]</sup>. Bio ink are classified into two types Natural and Synthetic bio-inks.

# 2.1 NATURAL BIO-INKS

Natural hydrogels or bio-inks are important role in bio inks for 3D bio printing of tissues and organs because, it is highly biocompatible with the other tissues and it is adaptable for the structural and functional organization of cells. Natural polymers are Collagen, Gelatin, Alginate, Agarose, Fibrin etc.,

# 2.1.1 COLLAGEN

Collagen type I is the main structural protein component of various connective tissues in the extra cellular matrix (ECM) of body and it is the most abundant protein in mammals <sup>[10]</sup>. Collagen maintain the cell adhesion, proliferation, differentiation and migration <sup>[11]</sup>. Collagen type I hydrogels shows a greater bio-compatibility and highly bio active presenting cell-adhesion sites. Which have been widely used in many bio medical application <sup>[12,13]</sup>. Collagen hydrogel is too weak to fabricate the scaffolds so, it is used with other polymers <sup>[14]</sup>.

# 2.1.2 GELATIN

Gelatin is a protein material. The main origin of gelatin is animals. It is obtained from the bones, cartilage, tendons, ligaments and skin of animals such as cattle, pigs, and also obtained from fishes or chickens. Gelatin is derived from the partial hydrolysis of collagen obtained from the animals which mentioned in the previous or above sentence. Gelatin is commonly used for increasing emulsification, thickness and elasticity <sup>[15,16]</sup>. In tissue engineering field it is widely used due to its excellent, bio-compatibility, bio-degradability, non-immunogenicity and also cell-interactivity <sup>[17]</sup>. Gelatin that undergoes changes in response to external temperature. Gelatin undergoes reversible sol-gel transition by cooling below 35°C <sup>[18]</sup>.

### 2.1.3 ALGINATE

Alginate is a polysaccharides naturally occuring anionic polymer derived from sea weeds which is macroscopic algae growing in the marine and



shallow coastal waters and on rocky shores <sup>[19]</sup>. Alginate is an anionic block co-polymer containing 1,4-linked beta-D-mannuronic (M block) and alpha-L-guluronic acid (G blocks) <sup>[20]</sup>. Alginate hydrogels are widely used as biomaterials in the tissue engineering, drug delivery system and wound healing <sup>[21,22]</sup>. When multivalent cation calcium added into the aqueous solution of alginate forms a ionic inter-chain bridges that produce fast sol-gel transition. Cations preferably binding to the alpha-L-guluronic acid (G block) of the chains but in recent studies the M blocks has also has an active role in cross-linking the polymer chain <sup>[23]</sup>.

# 2.1.4 AGAROSE

Agarose is a hydrophilic polysaccharide, it is extracted from red algae. The main structure of agarose consist of altering the units of beta-Dgalactopyranose and 3,6-anhydro-alpha-Lgalactopyranosyl units <sup>[24]</sup>. In nerve regeneration, the agarose is taken as optimal material for scaffold, due to its biocompatibility and stability in spinal cord <sup>[25]</sup>. It is not biodegradable by the mammals but, degraded in vitro by agarases. They are classified into 3 types based on their cleavage pattern  $\alpha$ -agarase,  $\beta$ -agarase and  $\beta$ -porphyranase <sup>[26,18]</sup>.

# **2.2 SYNTHETIC BIO-INKS**

Synthetic hydrogels are used in the 3D bio printing application, including polyethylene glycol (PEG), Polyvinyl pyrrolidine (PVP), poly (L- Lactic) acid (PLA), poly (Latic-co-glycolic) acid (PLGA) etc.,

# 2.2.1 POLY ETHYLENE GLYCOL(PEG)

Polyethylene glycol is a synthetic polymer. It has both hydrophilic and hydrophobic properties, it is soluble in organic solvents or aqueous solvent and has high biocompatibility<sup>[27]</sup>. It is applied for wound dressing and drug delivery system <sup>[28]</sup>. Polyethylene glycol is one of the most widely used hydrogels in the scaffold, drug delivery and cell research <sup>[29]</sup>.

# 2.2.2 POLYVINYL PYRROLIDINE(PVP)

Polyvinyl pyrrolidine is also known as povidone. It is soluble in water and also soluble in organic solvents. The complex formation of Iodine with polyvinyl pyrrolidine gives effective disinfectant having low toxicity <sup>[30]</sup>. It is a synthetic polymer which undergoes crosslinking and form hydrogel. These hydrogels synthesized by using many methods such as gamma-radiation, UV-photo crosslinking, electron beam radiation etc., <sup>[31]</sup>.

# **3.PROPERTIES OF BIO-INKS**

Bio-ink required some properties, they are bioprintability, biocompatibility, viscosity etc.,

# **3.1 BIO PRINTABILITY**

Printability is the capacity of bio-ink to form and maintain 3D scaffolds <sup>[32]</sup>. Bio printability of bio ink was accessed by using various factors like viscosity, surface tension, cross linking ability and ink consistency <sup>[33]</sup>. Printability is important properties for bio printing, It should be imitate both the shape and cellular architecture [34]. Storage modulus, solid-liquid transition stress and the flow transition index are the three rheological parameters, to predict the printability <sup>[35]</sup>. Viscosity is playing major role in printability. If the viscosity is low then the deformation and collapse will occur. On the other hand, nozzles will be jammed when the viscosity is high. It must be overcome by increasing temperature(T1) in the nozzles with the mixture and then eject under the cooled temperature (T2). The T1 should be greater than the melting temperature to avoid jamming



nozzle. T2 should be lower than the solidification temperature to fix the printed structure <sup>[36]</sup>.

# **3.2 BIO COMPATABILITY**

Bio compatibility is the major properties for preparing hydrogels. Because, the 3D printed structure using hydrogels is transplanted into the human or animal body. So, we must be consider the compatibility <sup>[37]</sup>. Various experiments is used to determined the bio compatibility like invitro cytotoxicity screening, direct cell culture, agar diffusion testing etc., The bio compatibility is defined as the ability of bio ink for 3D bioprinting to provide its desired activity that will enhance the cell adhesion, proliferation, viability, activity and tissue regeneration without producing toxic or unwanted effect <sup>[38]</sup>.

# **3.3 VISCOSITY**

This is also a important factor for choosing bio ink for3D printing. The stability of the 3D structure may be increased by enhance the viscosity of bio ink but it also leads to clogging the nozzle while, less viscous ink provide compatible with other cells but resist the printability due to poor flow. It should be overcome by regulate the concentration of polymer, molecular weight and temperature <sup>[39]</sup>.

# 4. METHODS

There are various methods for bioprinting like, Inkjet-based, extrusion-based, laser-assisted, stereolithography, Fused deposition modeling, Vat polymerization <sup>[40]</sup>. Among these extrusion-based, inkjet-based and laser-assisted are the main bioprinting technique used for 3D bioprinting <sup>[41]</sup> is discussed below.

# 4.1 EXTRUSION-BASED BIOPRINTING

Extrusion-based methods are most commonly employed for bioprinting in recent years. It print

high densities cell <sup>[42]</sup>. Bio ink have low viscosity in the range of 30-6 x 107 mpa.s are used in the extrusion based bio printers <sup>[43]</sup>. While high viscosity can leads to clogging the nozzle tip and it can be overcome by adjusting the nozzle tip diameter <sup>[44]</sup>. Compared to the laser or inkjet-based system the resolution is low in extrusion-based printing that is 200µm<sup>[45]</sup>. The distribution of material is achieved by either piston-driven, pneumatic system or screw driven [46]. The principle involved in the extrusion-based bioprinting is the bio ink extrudate from the syringe through nozzle by a continuous force which is driven by pneumatic, piston or screw pressure. The micro filament (extruded material) after solidifying on the substrate it act as an support structure. The substrate may be culture dish or growth medium. It can be influenced by temperature, nozzle diameter, pressure, speed etc., <sup>[47]</sup>. Among this, pneumatic system show great result with high viscous materials with the help of compressed air as the driving force and it can also have limitation due to the presence of compressed gas it delays the distribution of material <sup>[48]</sup>. On the other hand piston driven system shows more control over the fluid flow whereas screw based printing provide the more spatial control <sup>[49]</sup>. Adjustability of viscous, bio ink phase and material-specific bio-fabrication window are the three main factors to print through extrusion printers <sup>[50]</sup>.

# 4.2 INJLET-BASED BIOPRINTING

Inkjet-based bioprinters are cheap and it also work in the mild conditions <sup>[51]</sup>. The natural bio-ink is used in the inkjet based bioprinting that is collagen, fibrin etc., <sup>[52]</sup>. Inkjet based printing is classified into two major categories: Continuous inkjet printing (CIJ) and Drop-on-demand (DOD) inkjet printing <sup>[53]</sup>. In continuous inkjet printing the liquid ink is directed by a high pressure pump to



form droplets continuously through a microscopic nozzle. Drop-on-demand are two types: Thermal DOD and Piezoelectric DOD. In bioprinting applications, Piezoelectric DOD is recommended because thermal DOD may cause damage or death of living cells and also it have some advantage over CIJ printing because it may have chances of contamination. In DOD the inkjet dispenser eject the bio-ink to form a microspheres droplets further it formed into 2D or 3D pattern by deposition on substrate <sup>[54]</sup>. When the droplet deposit on the substrate the impact will occur which leads to affect the repeatability and the dimensional accuracy. The impact is based on the droplet velocity and volume [55]. Different physical and chemical crosslinking mechanism such as crosslinking agents, PH and UV-radiation are used to solidify the deposited droplets on the substrate to form 3D structure <sup>[56]</sup>. The driving force for the ejection onto the substrate is thermal or sound <sup>[57]</sup>. The droplet size is influenced by temperature thus increase in temperature lead to decrease in the droplet size <sup>[58]</sup>.

### 4.3 LASER-ASSISTED BIOPRINTING

Laser-assisted bioprinting (LAB) is a Laserguided direct writing based on the principle of Laser-Induced Forward Transfer (LIFT)<sup>[59]</sup>. It is a non-contact printing device [60]. It consist of two horizontal co-planar glass slide, The upper slide is referred as "Donor slide" and the lower slide is referred as "Collector slide". The upper Donor slide is coated with a two different laser absorbing material that is light absorbing gold layer and a cell layer or biological material. The laser is focused through the upper donor slide into the absorbing gold layer, which locally evaporated. The collector slide provide a suitable environment to the bio-ink (usually a solution embedded with cells) to prevent from the dehydration <sup>[61,62]</sup>. The donor glass slide in the size range of 26 x 26 x 1 mm and it was

cleaned with acetone <sup>[63]</sup>. The laser source is a solid Nd:YAG crystal laser which is driven by a scanning system consist of two galvanometric mirrors <sup>[64]</sup>. The resolution of Laser-assisted bioprinting varies depending upon size from picometer to micrometer. The bio-ink viscosity, thickness, surface tension and wettability of substate influence the resolution <sup>[65]</sup>. It has higher printing accuracy and resolution than nozzle-based printing like extrusion-based and inkjet-based bioprinting. Bio ink having viscosity range of 1-8000 mPa.s used in the Laser-assisted bioprinting. It has a high cell viability than other bioprinting techniques <sup>[66]</sup>.

### CONCLUSION

In this review article, the classification of bio ink used in bioprinting and the types of bioprinting techniques are discussed. The 3D bioprinting is useful for the future research related to the pharmaceutical science and medicine. It also a powerful technology used in tissue and organ printing.

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