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A Review of Hydrogels in Droplet-based Bio-Fabrication Techniques

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ABSTRACT

The Rapid Prototyping (RP) technologies with hydrogels as biomaterials have gained tremendous popularity in Tissue Engineering applications for scaffold development, especially for the soft scaffold developments. Droplet-based RP technologies which use hydrogels as printing materials have seen growing acceptance in past decade, as they facilitate the encapsulation of living cells and improvement of cell seeding efficiency. In this review different droplet-based RP techniques have been briefly reviewed along with various natural hydrogels used for the fabricating the scaffolds.

INTRODUCTION

Tissue Engineering (TE) which aims to repair, replace or improve the functionality of ill or damaged organs using cell seeding *in vitro* or *in vivo* environment is finding as a very promising technology for the realization of the same with the help of rapid prototyping techniques [1]. While the traditional RP techniques use the thermoplastic polymers, metals and ceramics are not suitable for the living cells and bio-active proteins due to high fabricating temperatures [2]. The hydrogels containing high water content can be processed under cell friendly conditions and looks more fascinating biomaterials for incorporating the living cells and bioactive component as they can furnish an instructive, aqueous environment, simulating natural extracellular matrix [3], [4]. Most of the hydrogels like alginate, fibrin, gelatin, chitosan, collagen and hyaluronic acid have been used for the TE applications are derived from the natural polymers. These naturally derived hydrogels provide the highest cell viability and proliferation rates because of the presence of the abundant chemical signals [5]. But because of their different printability, construction of the scaffolds with these materials become the challenging. In contrast to natural hydrogels, the synthetically prepared hydrogels like poly (ethylene glycol) and poloxamer are having the better printability [6]. However, they are providing the inert environment to the living cells and hence resulting into the low viability and proliferation rates [3]. In order to deal with these difficulties bio-active compounds like peptide sequences and growth factors are added into synthetic hydrogels to use them in bio-fabrication [7].

Although considerable progress has been made in the bio-fabrication with both natural as well as synthetic hydrogels, they still have significant difficulties in fulfilling the biological and physical requirements, like complex architecture, mechanical integrity and variational degradation rates with respect to time, in order to facilitate cell migration, exit to degradation byproducts, differentiation among original cells and embedded cells and proliferation [8], [9] .

RP TECHNIQUES FOR DROPLET-BASED BIO-PRINTING.

There are many RP techniques in the area of TE which use hydrogels as a printing material and can be broadly classified as (i) Laser-induced forward transfer, (ii) Inkjet printing & (iii) Robotic dispensing. **Fig. 1.** Shows the broad classification of hydrogel based RP techniques [4], [9]. In the laser-based system, focused laser pulses induced on donor slide causes local evaporation of absorbing layer resulting in high gas pressure which propels bioink from another side. These systems provide better resolutions with high gelation rate but fabrication speeds are low [10]. The inkjet printing, in which the bioinks dispense through micro dispensing tips and small droplets are positioned precisely can be further subdivided into the three types based on the actuation methods [9] viz., (i) Electromechanical in which biomaterials are actuated with a piezoelectric material (ii) Electrothermal in which bioinks are thermally actuated. (iii) Electrostatic spraying in which bioinks actuated with voltage difference [11]. The Robotic dispensing approach to is also subdivided into the following three subcategories: (i) Pneumatic (ii) Piston & (iii) screw actuated. Generally in the robotic dispensing system, to maintain the construct shape, more hydrogel is yielded instead of positioning single droplet [4]. Each of these methods is having advantages & disadvantages.

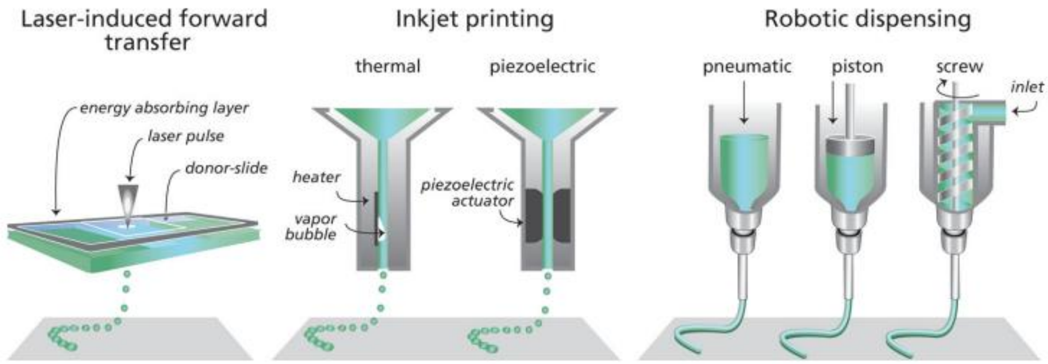

Fig. 1. Classification & working methodology of the hydrogel based RP techniques [4]

Hydrogel	Fabrication technique	Gelation method	Remark	References
Alginate	Laser-induced	ionic	High viability, Medium to high printability	[10], [12]
	Electromechanical Electrothermal Electrostatic Piston-driven Pneumatic-driven	ionic	Good printability, High viability	[13] [14] [11] [15]
Gelatin	Electromechanical Electrostatic Piston-drive Pneumatic-driven	ionic chemical thermal+chemical pH+themal	Low printability with less viscosities, poor cell differentiation	[16] [11] [16] [2]
Collagen type1	Electrothermal Pneumatic-driven	ionic+enzymatic thermal	Migrating cells	[4], [17]
Agar	Piston-drive Pneumatic-driven	thermal thermal	Good printability	[4]
Agarose	Piston-drive Pneumatic-driven	thermal thermal	Good viability	[3], [18]
Alginate+fibrin	Pneumatic-driven	ionic+enzymatic	Poor printability	[4]
Alginate+gelatin	Piston-drive	ionic+thermal	High printability, good viability	[19]
Alginate+gelatin +chitosan	Piston-driven	ionic+chemical + enzymatic	Proliferating cells	[18]

Table 1.Hydrogels used in droplet based tissue construction techniques.

HYDROGELS FOR DROPLET-BASED FABRICATION

The selection of the hydrogel for any specific fabrication method mainly relies on the physicochemical properties of the hydrogels under the conditions imposed by that method. The major properties which decide the printability of the bioink for the specific process are rheological properties such as viscosity, pseudo-plasticity, yield stress as well as the crosslinking mechanisms like ionic, chemical, thermal, enzymatic, photo-polymerization crosslinking [4]. These properties also affect the cell viabilities & proliferations. As concentration (viscosity) of hydrogel increases, the solution becomes less aqueous and reduces viability [2], [4]. **Table 1** shows the different hydrogels used for different droplet-based construction techniques. With mixing different bioinks, droplet-based as well as continuous hydrogel printings were carried out and has been reviewed.

CONCLUSION:

Currently available hydrogels with droplet-based fabrication techniques allow to design & build better architecture for bio-fabrication application. But a lot of biological and physiochemical demands of the tissues needs to be overcome by the researchers. With the new mixing of hydrogels, the new bioinks are providing better printability and in satisfying the complex requirements. But still there is a lot of scope for the optimization of both techniques as well as the properties of hydrogel and future research on the same will help in the evolution of TE.

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