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3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances

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ABSTRACT

3D printing, an additive manufacturing based technology for precise 3D construction, is currently widely employed to enhance applicability and function of cell laden scaffolds. Research on novel compatible biomaterials for bioprinting exhibiting fast crosslinking properties is an essential prerequisite toward advancing 3D printing applications in tissue engineering. Printability to improve fabrication process and cell encapsulation are two of the main factors to be considered in development of 3D bioprinting. Other important factors include but are not limited to printing fidelity, stability, crosslinking time, biocompatibility, cell encapsulation and proliferation, shear-thinning properties, and mechanical properties such as mechanical strength and elasticity. In this review, we recite recent promising advances in bioink development as well as bioprinting methods. Also, an effort has been made to include studies with diverse types of crosslinking methods such as photo, chemical and ultraviolet (UV). We also propose the challenges and future outlook of 3D bioprinting application in medical sciences and discuss the high performance bioinks.

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1. Introduction

As the main process involved in cell growth and reconstruction of organs, tissue regeneration is currently under extensive study. Organ transplantation, replacement and repair are the options for patients with damaged organs depending on the situation and intensity of the damage. Extensively long waiting lists for organ transplantation exist all around the world. According to U.S. Department of Health & Human Services, as of June 2017, around 120000 patients are in need of lifesaving organ transplant in the United States while only about 5200 donors are available. Also, while the number of transplants performed every year since 2003 has been somehow constant, the number of patients waiting at the year-end has been growing (https://optn.transplant.hrsa.gov). Under these circumstances, scientists are eager to find alternative ways to compensate for this shortage of organ. Tissue engineering, on the other hand, has been considered as an effective method to help save lives and improve the quality of life. Since proposed in 1993 [1], tissue engineering has been intended to develop practical replacements for damaged tissue by means of applying biology and engineering principles. Scaffolds have found their place in tissue engineering as templates for cell interaction, providing physical support to the afresh developed tissue [2]. Also, scaffolds can function as delivery vehicles to incorporate essential growth factors to control and enhance tissue growth [3]. A combination of cells and biomaterials is often employed as the printing precursor in 3D bioprinting of scaffolds. 3D Bioprinting is an actively studied method in tissue engineering since it shows effective control over scaffold fabrication and cell distribution. Printing resolution of 3D bioprinting techniques is 10–10000 μm which is a wide range showing flexibility of bioprinting compared to other assembly methods such as molding and porous scaffolds [4,5].

As an additive manufacturing technique, 3D bioprinting is based on deposition of biomaterials, either encapsulating cells or loaded with cells later on, in micrometer scale to form subtle structures comparable to tissue. In most cases, a three-axis mechanical platform controls the movements of extruders printing the bioink in

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the required algorithm and shape. This platform's movement is governed by coordinates created by the designer and saved in a file format such as g-code that could be easily followed by the printer. Due to vantages such as precise deposition, cost-effectiveness, simplicity, and cell distribution controllability, 3D bioprinting development and application has been increasing constantly over the past few years. As a result, need for new bioinks providing required properties for successful printing, such as printability, printing fidelity, and mechanical properties has been rising leading to extensive work to develop new materials. In the present review, an account of the most recent and functional research studies on bioinks and bioprinting developments is presented. To this end, first outstanding works in major bioprinting methods, including extrusion-based, inkjet, stereolithography-based, and laser-assisted bioprinting methods, are reviewed. Also, a brief review of the above mentioned bioprinting techniques is presented in Table 1 and a short summary of recent outstanding bioprinting studies is tabularized in Table 2. Next, the most fundamental recent studies in bioink development and applications are cited in "High performance bioink" section. Later on, challenges in bioink development and bioprinting, as well as applications and future perspective of bioprinting is discussed. Finally, a short summary of the present article is presented.

2. Extrusion-based bioprinting

Extrusion-based methods have been widely employed in recent years to provide researchers with alternative methods for scaffold fabrication. The extensive popularity of extrusion-based methods mostly relies on clear-cut processing method leading to simplicity, diversity and predictability of this technique. Bioinks having viscosity in the range of 30–6×10^7 mPa s are reported to be printable via extrusion printing [13]. In comparison with inkjet bioprinting, extrusion-based bioprinting offers higher cell densities but lower speed and resolution [13]. Wide range of printable biomaterials and inexpensive equipment are among extrusion bioprinting advantages. Many researchers have simply modified conventional commercial 3D printers to print biomaterials or developed their printing machines in-house to reduce the costs [2,24,29,31,33–35,38,41–43,49,55,56]. On the other hand, due to the need for development of bioprinters, commercial bioprinters have become widely available and employed by researchers [5,23,27,37,44–46,51–54], focusing on enhancing the printing quality and suitability for printing wider range of biomaterials. A review of the outstanding research works using extrusion-based techniques is presented in this section. Moreover, Fig. 1 illustrates common extrusion-based printing methods categorized into pneumatic, piston-driven, and screw-driven dispensing. In pneumatic dispensing, air pressure provides the required driving force, while in piston and screw-driven dispensing, vertical and rotational mechanical forces initiate printing, respectively.

There are three main factors to take into account toward printability via extrusion bioprinting: 1) adjustability of the viscosity, 2) bioink phase prior to extrusion, and 3) material-specific biofabrication window [11]. Viscosity can be a function of temperature or shear thinning and therefore, needs to be adjusted for different printing methods. Also, bioink needs to be in liquid phase to avoid nozzle clogging. Finally, not all biomaterials are printable and those which are printable may not be printable in a wide range of processing parameters. To illustrate the current state of the art, the most recent extrusion bioprinting studies are cited in the following paragraphs.

To begin with, Rees et al. considered two types of oxidized nanocellulose 3D printed structures as wound dressings [23]. First type was prepared by (2,2,6,6-tetramethylpiperidin-1-yl) oxidanyl (TEMPO) mediated oxidation and the second type was prepared by carboxymethylation and periodate oxidation combined. The produced nanocellulose bioink was then used to print 3D porous structures, studied for bacterial growth support, and shown to have the potential to carry and release antimicrobial components while not supporting bacterial growth. Yu and Ozbolat utilized a coaxial nozzle system to print tissue strands as a bioink for organ printing [24]. Alginate-based bioink developed in this work showed mouse TC3 cell viability close to 90%. Also, human umbilical vein smooth muscle cells were incorporated in the bioink to fabricate structures similar to pancreatic tissue to further demonstrate the applicability of their method. In another study, a hydrogel based on gelatin, alginate, and collagen was used for cell-laden 3D printed tissue constructs [2]. One integral part of this work was to control the degradation rate of the hydrogel by changing the mole ratio of sodium citrate present in the medium to the sodium alginate present in the hydrogel. High cell proliferation rate indicated the possibility to improve the alginate bioink by utilizing the method used in this work.

Although bioprinting has been developing extensively in recent years, but the current technologies implemented in bioprinting are mostly incapable of printing functional solid organs. Researches have approached this issue by developing templates that could be used in vivo to support the development of vascularized solid organs such as bones [4]. Stem cells were encapsulated in a gamma-irritated alginate-based bioink that was further reinforced by adding PCL fibers. RGD peptides were also incorporated to improve

Table 1
A brief review of common bioprinting techniques.

	Extrusion	Inkjet	Stereolithography	Laser-assisted
Advantages	Simple, capable of printing various biomaterials, ability to print high cell densities	Ability to print low viscosity biomaterials, fast fabrication speed, low cost, high resolution	Nozzle-free technique, printing time independent of complexity [6,7], high accuracy and cell viability	High resolution, deposition of biomaterials in solid or liquid phase
Drawbacks	Only applicable for viscous liquids	Inherent inability to provide a continuous flow [8], poor functionality for vertical structures, low cell densities	UV light source and near-UV blue light's toxicity to cells [9,10], lack of printing multi-cells, and damage to cells during photo curing [11]	High cost, thermal damage due to nanosecond/femtosecond laser irritation [12]
Speed	Slow [13,14]	Fast [13,14]	Fast [14]	Medium [14]
Cost	Moderate [8,15]	Low [8,15]	Low [8,15]	High [8,15]
Vertical printing ability	Good [6]	Poor [6]	Good [6]	Medium [6]
Cell viability	89.46 ± 2.51% [16]	80-95% [17,18]	>90% [19,20]	<85% [12]
Cell density	High [21]	Low [21]	Medium [21]	Medium [21]
Resolution	100 μm [8]	50 μm [8]	100 μm [19,20]	10 μm [22]
Viscosity	30–6×10^7 mPa s [13]	<10 mPa s [13]	No limitation [7]	1-300 mPa s [13]

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