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Review

A comprehensive review on droplet-based bioprinting: Past, present and future

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ABSTRACT

Droplet-based bioprinting (DBB) offers greater advantages due to its simplicity and agility with precise control on deposition of biologics including cells, growth factors, genes, drugs and biomaterials, and has been a prominent technology in the bioprinting community. Due to its immense versatility, DBB technology has been adopted by various application areas, including but not limited to, tissue engineering and regenerative medicine, transplantation and clinics, pharmaceutics and high-throughput screening, and cancer research. Despite the great benefits, the technology currently faces several challenges such as a narrow range of available bioink materials, bioprinting-induced cell damage at substantial levels, limited mechanical and structural integrity of bioprinted constructs, and restrictions on the size of constructs due to lack of vascularization and porosity. This paper presents a first-time review of DBB and comprehensively covers the existing DBB modalities including inkjet, electrohydrodynamic, acoustic, and micro-valve bioprinting. The recent notable studies are highlighted, the relevant bioink biomaterials and bioprinters are expounded, the application areas are presented, and the future prospects are provided to the reader.

1. Introduction

Three-dimensional (3D) bioprinting aims to fabricate tissue and organ constructs by selectively depositing biologics, such as living cells, biomaterials, drugs, growth factors and genes, in a layer-by-layer fashion [1–4]. It currently enables fabrication of scaffold-based or scaffold-free tissue and organ constructs [5], mini-tissues [3] and organ-on-a-chip models [6–9], and is envisioned to facilitate fabrication of functional replacement human organs such as heart, liver and kidney in the future [10]. However, bioprinting of such organs at present is impractical because of the challenges such as the need for built-in vascularization at the single-cell level and complex-heterocellular tissue patterning, and the development of biodegradable as well as biomimetic materials which are bioprintable while enabling rapid cell growth and proliferation [2,3,11]. Despite these challenges, 3D bioprinting serves in several other application areas. For example, 3D tissue models [1,12] can improve in-vitro drug testing by replacing two-dimensional (2D) cell culture and animal models as animal models are not effective at predicting human toxicological and pathophysiological responses [13] and 2D culture models do not closely mimic complex 3D micro-tissue environment [10,12,14]. Bioprinted tissues have also been used in tissue engineering and regenerative medicine such as bioprinted bone and cartilage which can help in musculoskeletal injury healing and rehabilitation [2,3,15]. Furthermore, in-situ bioprinting, technology enabling bioprinting directly into lesion sites in surgery settings, can regenerate complex large tissues through neo-vascularization driven by nature in human body [4,16,17]. Overall, 3D bioprinting provides an opportunity to envision radical solutions to existing medical and healthcare problems.

Bioprinting offers three main types of modalities including laser- [18–20], droplet- [21–24] and extrusion-based bioprinting [25]. Despite the commonly used extrusion-based bioprinting and the high-precision laser-based bioprinting, droplet-based bioprinting (DBB) offers several advantages due to its simplicity, agility, versatility and the great control over the deposition pattern. It enables bioprinting with controlled volumes of bioink deposition

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at predefined locations [3] facilitating spatially heterocellular constructs with well-defined positioning of cells [11].

Droplet-based bioprinting has its roots in inkjet printing technology, which has its beginnings in the 1950s when Elmqvist of Siemens patented the first practical inkjet device in 1951 [26]. Later, Sweet from Stanford University spearheaded the development of continuous-inkjet (CIJ) printing system in 1960s. Later, Zoltan, Kyser, and Sears pioneered the development of drop-on-demand (DOD) inkjet printing system in 1970s. Their invention was licensed in the first commercial DOD inkjet printer, the Siemens PT-80, in 1977. The idea of printing biologics was first introduced by Klebe in 1987 when he used a commercially-available Hewlett-Packard (HP) thermal DOD inkjet printer to deposit a bioink solution comprising collagen and fibronectin [27]. Afterwards, the first inkjet-based 3D printer was developed by Objet Geometries in 2000 [28]. In 2003, Boland demonstrated the feasibility of using a modified thermal DOD inkjet printer to deposit living cells in a viable form [29] and introduced the concept of inkjet bioprinting [30]. Subsequently, Nakamura's group successfully fabricated viable 3D tubular tissue constructs using a commercially available electrostatic DOD inkjet printer [31]. Later, several research groups have successfully adopted DBB technologies for bioprinting of a wide array of cells for various purposes, including but not limited to, bioprinting for stem cell research [17,32–34], tissue engineering [17,24,35,36], controlled release [37], transplantation [24,35], drug screening [38], high-throughput arrays [39], and cancer research [36,40].

In this paper, we present a first-time and thorough review of DBB technology including the modalities used with a comprehensive discussion on their working mechanisms, a detailed comparison of DBB with other bioprinting modalities, recent achievements in DBB, and bioink materials and bioprinters used in DBB. The application areas are discussed and future prospects with highly exciting directions are provided to the reader.

2. Droplet-based bioprinting

2.1. Modalities of droplet-based bioprinting

Droplet-based bioprinting, as shown in Fig. 1, comprises inkjet [3,11,21,22,27,41–43], acoustic-droplet-ejection (or simply acoustic) [44] and micro-valve bioprinting [32,33,40,45,46]. Inkjet bioprinting is classified into three: (i) CIJ, (ii) DOD and (iii) EHD jetting. Continuous-inkjet bioprinting leverages Rayleigh-Plateau instability to break bioink jets into droplets. Drop-on-demand bioprinting, on the other hand, uses thermal or piezoelectric actuators, or electrostatic forces to generate droplets. In contrast, electrohydrodynamic jet (EHD) bioprinting uses high-ranges of electric voltage to eject droplets. Whereas, acoustic bioprinting uses acoustic waves to produce droplets and micro-valve bioprinting uses a solenoid pump to eject droplets.

2.1.1. Inkjet bioprinting

Inkjet bioprinting physically manipulates a bioink solution to generate droplets. It leverages gravity, atmospheric pressure and the fluid mechanics of the bioink solution to eject droplets onto a receiving substrate.

2.1.1.1. Continuous-inkjet bioprinting. In CIJ bioprinting, the bioink solution is forced under pressure through a nozzle, which subsequently breaks up into a stream of droplets owing to Rayleigh-Plateau instability [47] as illustrated in Fig. 2A1. The phenomenon of Rayleigh-Plateau instability has been described elsewhere in details [48] but briefly, a cylindrical volume of liquid jet is perturbed by several factors including but not limited to the potential energy owing to surface energy of the jet and the kinetic energy due to motion of the jet. When the wavelength of the perturbed jet exceeds its initial radius by a certain limit (such that the product of the wave number (k) (the number of waves per unit length) and the initial jet radius (R_0) is less than 1 ($kR_0 < 1$)), the perturbation grows exponentially and eventually the jet distorts itself to minimize its potential energy and breaks up into a stream of droplets.

2.1.1.2. Drop-on-demand inkjet bioprinting. Drop-on-demand inkjet bioprinting is preferred over CIJ bioprinting for tissue bioprinting purposes. Drop-on-demand inkjet bioprinters generate droplets when required, which makes them more economical, handy to control and easy to pattern biologics [49]. Drop-on-demand bioprinters consist of a single or multiple printheads. Each printhead contains a fluid chamber and a single or multiple nozzles. The bioink stored in the fluid chamber is held in place by the surface tension at the nozzle orifice [49]. Pressure pulses are introduced in the fluid chamber through means of a thermal or a piezoelectric or an electrostatic actuator such that a droplet is ejected when the bioink overcomes the surface tension. Some printhead assemblies may require back pressure (pneumatic pressure (static pressure through means of pressurized-air) and/or vacuum) to supplement the pressure pulses for droplet generation. Drop-on-demand inkjet bioprinters rely on three different mechanisms to generate droplets including (i) thermal inkjet (TIJ), (ii)

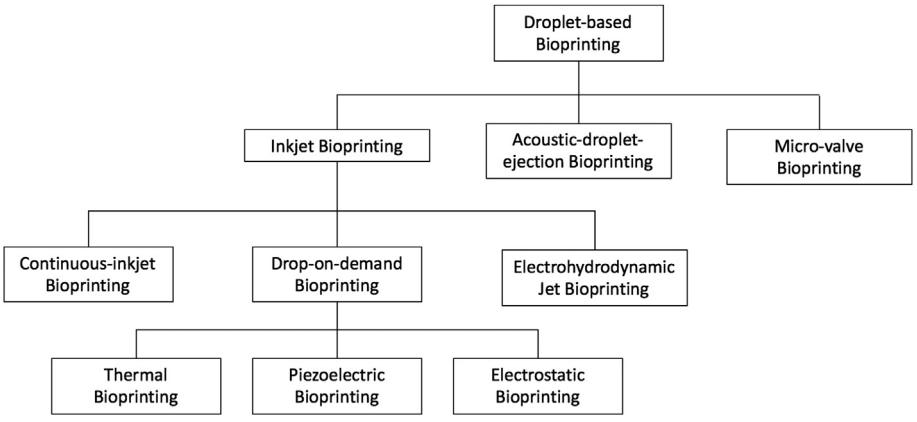

Fig. 1. Classification of droplet-based bioprinting into inkjet, acoustic, and micro-valve bioprinting modalities. Inkjet bioprinting is further classified into continuous inkjet, drop-on-demand and electrohydrodynamic jetting modalities. Drop-on-demand inkjet bioprinting comprises thermal, piezoelectric and electrostatic techniques.