Biotechnology Advances xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Essential steps in bioprinting: From pre- to post-bioprinting

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ARTICLE INFO

Keywords:
Bioprinting
Biofabrication
Bioink
Bioprinter
Extrusion-based bioprinting
Droplet-based bioprinting
Laser-based bioprinting

ABSTRACT

An increasing demand for directed assembly of biomaterials has inspired the development of bioprinting, which facilitates the assembling of both cellular and acellular inks into well-arranged three-dimensional (3D) structures for tissue fabrication. Although great advances have been achieved in the recent decade, there still exist issues to be addressed. Herein, a review has been systematically performed to discuss the considerations in the entire procedure of bioprinting. Though bioprinting is advancing at a rapid pace, it is seen that the whole process of obtaining tissue constructs from this technique involves multiple-stages, cutting across various technology domains. These stages can be divided into three broad categories: pre-bioprinting, bioprinting and post-bioprinting. Each stage can influence others and has a bearing on the performance of fabricated constructs. For example, in pre-bioprinting, tissue biopsy and cell expansion techniques are essential to ensure a large number of cells are available for mass organ production. Similarly, medical imaging is needed to provide high resolution designs, which can be faithfully bioprinted. In the bioprinting stage, compatibility of biomaterials is needed to be matched with solidification kinetics to ensure constructs with high cell viability and fidelity are obtained. On the other hand, there is a need to develop bioprinters, which have high degrees of freedom of movement, perform without failure concerns for several hours and are compact, and affordable. Finally, maturation of bioprinted cells are governed by conditions provided during the post-bioprinting process. This review, for the first time, puts all the bioprinting stages in perspective of the whole process of bioprinting, and analyzes their current state-of-the art. It is concluded that bioprinting community will recognize the relative importance and optimize the parameter of each stage to obtain the desired outcomes.

1. Introduction

In the last few years, the applications of tissue engineered constructs have expanded from clinical tissue regeneration to fabrication of tissue models for drug discovery (Knowlton et al., 2016; Peng et al., 2016), pathological understanding (Elson and Genin, 2016; Gomes et al., 2017) and controlled drug delivery systems (Do et al., 2017). The key enablers for these applications are advanced fabrication techniques that allow generation of tissue constructs mimicking the complex native extracellular matrix (ECM) organization (Guo et al., 2016). One of the most emerging techniques is bioprinting, which allows for precise deposition of cells and biomaterial components in pre-defined computer generated designs (Cornelissen et al., 2017). Indeed, experimental evidences on bioprinting have accrued at very rapid pace with increasing number of publications and involved research groups worldwide in recent times (Rodríguez-Salvador et al., 2017). These concoctions of cells and biomaterials (in some cases only cell aggregates) are often referred to as bioinks (Hölzl et al., 2016). The success of bioprinted tissue constructs is invariably determined by the properties of bioink. Usually, the process of generating bioprinted tissue constructs involves several steps. The first step is the creation of a computer-aided design model, suitable for bioprinting, whose resolution is determined by the applied image acquisition techniques, such as three-dimensional (3D) laser scanning, micro-computed tomography (μ-CT) and magnetic resonance imaging (MRI). In the design stage, it is also important to note if the methods used for generating a 3D model could be easily deployed in a surgical setting (e.g. short computational interval, and good compatibility between software and hardware).

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https://doi.org/10.1016/j.biotechadv.2018.06.003
Received 7 January 2018; Received in revised form 15 May 2018; Accepted 10 June 2018

After the design is finalized, the fabrication process commences along with obtaining cells with sufficient quantity and robustness. Bioink can be characterized by different parameters prior to bioprinting. In addition, clinical application becomes more feasible if the cellular and biomaterial components are obtained by minimally-invasive surgical procedures and if the protocols followed for expansion of cells are cost effective and achievable under general good laboratory practice (GLP) conditions. Thereafter, at the bioprinting stage, multiple factors influence the properties of engineered constructs. Cell densities that can be printed along with appropriate physicochemical properties become important determinants of dispensation through printheads. Such physicochemical properties include rheology, surface properties and most importantly, the gelation kinetics of the bioink. One of the most important challenges is to figure out a balance between printability and immediate solidification after bioprinting, so that the desired structure is retained. Alternatively, bioprinting can be performed in a microgel (e.g. carbopol) support bath or using nanoclays (e.g. laponite) to directly print structures in air without the need for instantaneous gelation (Bhattacharjee et al., 2015; Hinton et al., 2015, 2016; Jin et al., 2016, 2017). Bioink formula must also be affordable and biocompatible. In particular, for fabrication of hollow organ structures, it is required to use sacrificial inks and hence, the difference in properties of two types of bioink becomes important. In turn, the use of sacrificial inks is determined by the physical properties of the functional inks and aspect ratio of the vascular network to be fabricated. Subsequently, the applied bioprinting technique makes an important contribution to the mechanical and structural properties of constructs.

Current bioprinting technologies are based on one amongst extrusion-based bioprinting (EBB), droplet-based bioprinting (DBB) or laser-based bioprinting (LBB), as depicted in Fig. 1. EBB exploits automated three-axis robotic system for continuous extrusion of bioinks in filament forms. Herein, pneumatic or mechanical driven dispensing systems are mostly employed. In EBB, high extrusion pressure and resulting shear stress are the cause of concern for cell survival, but the modality usually produces most mechanically-robust constructs amongst all bioprinting techniques. In DBB, the bioink made up of living cells and other biological materials (e.g. hydrogels) in culture media is deposited in droplets form with precise noncontact positioning. The droplets are generated by one of thermal-, piezoelectric- or electrostatic- drop-on-demand technologies. DBB generally provides appreciable cell viability, though electrohydrodynamic jetting or micro-valve bioprinting can facilitate 80-90% viability (Gudapati et al., 2016; Ng et al., 2017a). DBB is a relatively rapid technique with a low cost and high resolution, but it can result in non-homogenous droplet size and cause nozzle-clogging while printing high density bioinks (Gudapati et al., 2016). LBB operates on the principle of a laser energy beam utilized for precise patterning of cells. Laser energy can be used in two different modalities, one of which involves photopolymerization (e.g., stereolithography or two-photon polymerization), and the other modality is based on cell transfer (e.g. laser guided direct writing and laser induced forward transfer). LBB is advantageous over the other modalities as it causes minimal clogging and damage to cell survival. Several advances in digital projection stereolithography techniques have been applied for bioprinting applications (Gauvin et al., 2012; Gou et al., 2014). Though LBB also provides high resolution, it is an expensive and time consuming modality (Datta et al., 2017a; Peng et al., 2017). Each bioprinting technique has advantages and disadvantages with respect to cell survival against the process. Apart from the basic processes associated with each bioprinting modality, several innovations like aerosol assisted crosslinking, use of electric fields to reduce shear stresses, hybrid electrospinning-bioprinting and core/shell bioprinting has been developed (Lee et al., 2017a, 2017b). In all cases, it is important that cellular biomaterials are printed in a manner that allows intricate cell-material interactions, which are crucial for the tissue development. During this phase, it is essential that cells are printed with sufficient resolution to facilitate proper cell-cell interactions. Finally, bioprinted tissue constructs are required to become mature in suitable bioreactors before they can be implanted. Degradation of the biomaterial support, if present, also demonstrates significance during the post-bioprinting stage.

In this review, we critically present the current literature on abovementioned aspects of bioprinting technology from the design phase to post-bioprinting steps. The complete bioprinting process, including pre-bioprinting, bioprinting and post-bioprinting along with their components, is schematically illustrated in Fig. 2. As shown in the Fig. 2, bioprinting is multi-disciplinary area, and the successful fabrication of tissue constructs requires understanding the dynamic interaction between different disciplines. However, most reviews available till date only concentrate on single domain specific analysis of current literatures, and a comprehensive presentation on all aspects has not been demonstrated yet. Therefore, we provide all the stages involved in this process for the first time in literature and thoroughly discuss these stages including pre-bioprinting, bioprinting and post-bioprinting with their essential components.

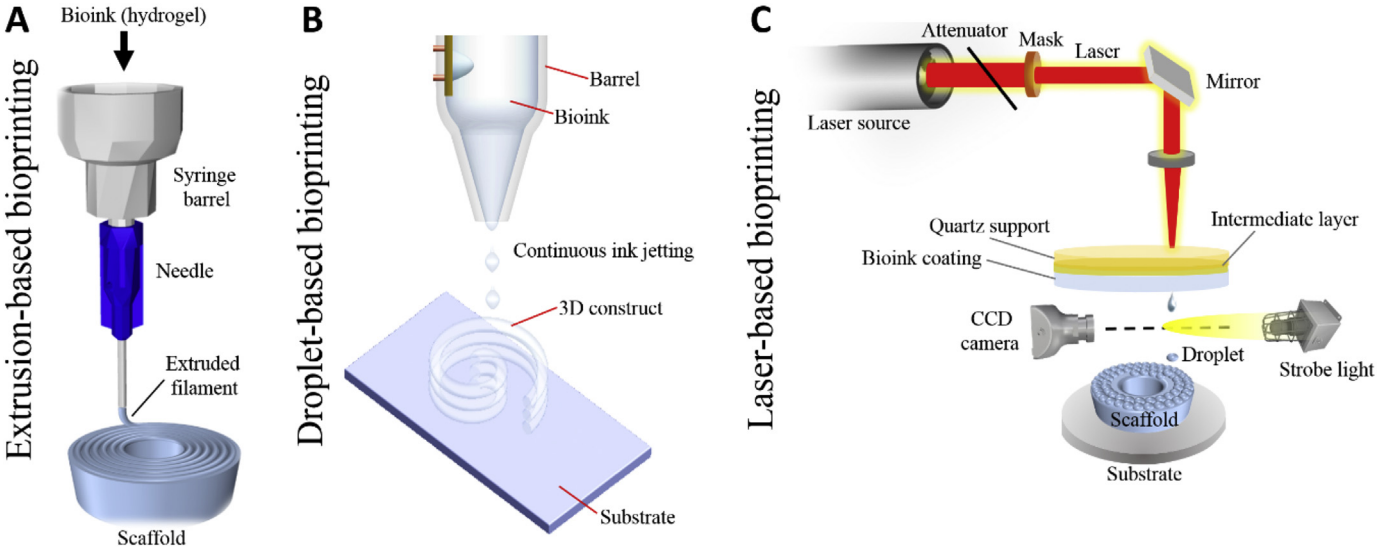

Fig. 1. Mechanisms of bioprinting techniques: A) extrusion-based bioprinting, B) droplet-based bioprinting, C) laser-based bioprinting.