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Research review paper

3D bioprinting for engineering complex tissues

Christian Mandrycky [a,1], Zongjie Wang [b,1], Keekyoung Kim [b,*], Deok-Ho Kim [a,**]

[a] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA
[b] School of Engineering, The University of British Columbia, Kelowna, BC V1V 1V7, Canada

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ABSTRACT

Bioprinting is a 3D fabrication technology used to precisely dispense cell-laden biomaterials for the construction of complex 3D functional living tissues or artificial organs. While still in its early stages, bioprinting strategies have demonstrated their potential use in regenerative medicine to generate a variety of transplantable tissues, including skin, cartilage, and bone. However, current bioprinting approaches still have technical challenges in terms of high-resolution cell deposition, controlled cell distributions, vascularization, and innervation within complex 3D tissues. While no one-size-fits-all approach to bioprinting has emerged, it remains an on-demand, versatile fabrication technique that may address the growing organ shortage as well as provide a high-throughput method for cell patterning at the micrometer scale for broad biomedical engineering applications. In this review, we introduce the basic principles, materials, integration strategies and applications of bioprinting. We also discuss the recent developments, current challenges and future prospects of 3D bioprinting for engineering complex tissues. Combined with recent advances in human pluripotent stem cell technologies, 3D-bioprinted tissue models could serve as an enabling platform for high-throughput predictive drug screening and more effective regenerative therapies.

Contents

* Correspondence to: K. Kim, School of Engineering, University of British Columbia, EME 4263, 1137 Alumni Ave., Kelowna, BC V1V1V7, Canada.
** Correspondence to: D.-H. Kim, Department of Bioengineering, University of Washington, N410G William H Foege Building, 3720 15th Ave NE, Box 355061, Seattle, WA 98195.
E-mail addresses: keekyoung.kim@ubc.ca (K. Kim), deokho@uw.edu (D.-H. Kim).
[1] These authors contributed equally to this work.

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1. Introduction

In the United States, one name is added to the organ transplant waiting list every 15 min (Abouna, 2008). While this list grows rapidly, less than one-third of waiting patients can receive matched organs from donors (Ozbolat and Yu, 2013). This growing deficit, however, is unlikely to be met by a supply of transplantable organs that has stagnated over the last decade (Bajaj et al., 2014). One of the most promising techniques to alleviate this organ shortage crisis is tissue engineering, the use of a combination of cell, engineering, and material methods to generate artificial tissues and organs (Langer and Vacanti, 1993). In tissue engineering, three strategies are used to replace or induce targeted tissues: (1) the use of cells alone, (2) the use of biocompatible biomaterials, (3) the use of a combination of both cells and biomaterials (Khademhosseini et al., 2006). These cells and biomaterials are combined into scaffolds through a variety of processes, which can generally be classified as either top-down, or bottom-up. In top-down approaches cells are often seeded sparsely and homogenously in biomaterials shaped to resemble biological geometries. On the other hand, in bottom-up approaches modular units of cells and biomaterials are combined to form macro tissues. Top-down methods have been in wide use for years, however, these methods often cannot accurately control the distribution of cells, and fail to generate the appropriate extracellular matrix (ECM) (Khademhosseini et al., 2006). Without a proper ECM microenvironment, cells cannot function as tissues properly. This limitation is addressed in bottom-up approaches that build up tissues brick by brick *via* micro- and nano-technologies. As a result, cell distribution can be defined at the micrometer scale, which significantly improves the controllability of scaffold fabrication (Jiao et al., 2014). Motivated by developments in nanotechnology, techniques like self-assembly and soft-lithography have been applied to bottom-up tissue engineering (Kim et al., 2013, 2014a; Shapira et al., 2014). Among the micro-scale bottom-up techniques recently applied to tissue engineering, bioprinting, a form of additive manufacturing, has become one of the most promising and advanced fabrication methods (Table 1).

In bioprinting, small units of cells and biomaterials are dispensed with micrometer precision to form tissue-like structures (Fig. 1). Unlike conventional 3D printing techniques that have been used to print temporary cell-free scaffolds for use in surgery (Bracci et al., 2013), bioprinting requires a different technical approach that is compatible with depositing living cells. The advantages of bioprinting include accurate control of cell distribution, high-resolution cell deposition, scalability, and cost-effectiveness. For those reasons, the development and subsequent applications of bioprinting have greatly increased during the last five years. In this review, we discuss the basic principles of bioprinting, including bioprinter device design, workflow, biomaterial options, and current and potential applications.

2. Bioprinting techniques

To date, no single bioprinting technique enables the production of all scales and complexities of synthetic tissues. The three major bioprinting techniques of inkjet, laser-assisted, and extrusion bioprinting each have specific strengths, weaknesses, and limitations. A concise comparison of these approaches is also provided in Table 2.

2.1. Inkjet printing

Inkjet bioprinting was the first bioprinting technology (Tuan et al., 2003) and is very similar to conventional 2D inkjet printing (Singh et al., 2010). A hydrogel pre-polymer solution with encapsulated cells (called a bioink) is stored in the ink cartridge. The cartridge is then connected to a printer head and acts as the bioink source during the electronically controlled printing process. During printing, the printer heads are deformed by a thermal or piezoelectric actuator and squeezed to generate droplets of a controllable size, as shown in Fig. 1B. The advantages of inkjet printing include: (1) low cost due to similar structure with commercial printers, (2) high printing speed conferred by the ability of the printer heads to support parallel work mode, and (3) relatively high cell viability (usually from 80% to 90%), as determined by many experimental results (Cui et al., 2012a, 2012b, 2013).

Table 1
Comparison of tissue engineering methods.

	Assembly method			
	Bioprinting	Molding	Porous scaffolds	References
Materials	Natural and synthetic polymers High concentration cell solutions	Natural and synthetic polymers High concentration cell solutions Cell sheets	Natural and synthetic polymers Ceramics Metals	Agarwal et al. (2013) and Skardal and Atala (2014)
Resolution	10–1000 μm	>500 nm	100 nm–1000 μm	Kim et al. (2010), Lu et al. (2013) and Bajaj et al. (2014)
Advantages	Control of tissue geometry across a wide range of scales; rapid production of scaffolds; precise cell and material patterning	Accurate control of small (<100 μm) features; scaffold fabrication is rapid and molds are often reusable; gentle on encapsulated cells	Controllable material properties (*e.g.* porosity, modulus); wide range of materials available for use	Lu et al. (2013), Bajaj et al. (2014), Jiao et al. (2014) and Murphy and Atala (2014)
Disadvantages	Printing techniques may reduce cell viability or have unknown consequences; limited material selection due to crosslinking speed	Scaffolds are generally homogenous or require combination of multiple scaffolds to create patterns	Scaffold geometry is less controllable; technique may damage encapsulated cells or require seeding after assembly; less control of cell patterning	
Techniques	Extrusion Laser-assisted Inkjet Stereolithography	Cell sheet stacking Lithography Injection molding	Electrospinning Phase separation Freeze drying Self-assembly	Ballyns et al. (2008), Zheng et al. (2012), Lu et al. (2013) and Jiao et al. (2014)