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SHORT PAPER

A perspective on 3D bioprinting in tissue regeneration

Samad Ahadian[1,2,3] · Ali Khademhosseini[1,2,3,4,5]

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Introduction

A rapidly increasing need for organ transplantation and short list of donated organs have led to the development of new materials and technologies for organ manufacturing. Although some simple organs, such as skin and cartilage, have been successfully fabricated and commercialized, it is still difficult to make tissues and organs with high complexity. Engineered tissues have other biomedical applications, such as drug screening models and bio-actuators. Three-dimensional (3D) bioprinting has become a great tool to fabricate tissue constructs on demand for transplantation and other biomedical applications [1, 2]. Three-dimensional bioprinting is a computer-assisted technology allowing for spatial control of cells, biomaterials, and soluble factors down to micrometer resolution in a 3D environment. The concept of 3D bioprinting stems from additive manufacturing field where a 3D object is created using sequential deposition of solid layers. Three-dimensional bioprinting has rapidly evolved to fabricate biomimetic structures with great potential for clinical studies. This paper outlines important advances, major challenges, future directions in 3D bioprinting technologies and materials.

✉ Ali Khademhosseini
khademh@ucla.edu

1 Department of Bioengineering, University of California, Los Angeles, CA 90095-1600, USA

2 Center for Minimally Invasive Therapeutics (C-MIT), University of California, Los Angeles, CA 90095-1600, USA

3 California NanoSystems Institute (CNSI), University of California, Los Angeles, CA 90095-1600, USA

4 Department of Radiological Sciences, University of California, Los Angeles, CA 90095-1600, USA

5 Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA 90095-1600, USA

Bioinks

Proper selection of bioinks, which refer to cell-laden biomaterials as the ink for bioprinters, is a key in fabricating functional tissue structures. A spectrum of cells and natural and synthetic biomaterials have been proposed for 3D bioprinting of different tissues in the body. An ideal bioink should possess physicochemical properties (i.e., rheological, mechanical, chemical, and biological properties) close to those in the native tissues. A variety of naturally derived biomaterials (e.g., alginate, gelatin, collagen, fibrin, silk, hyaluronic acid, and Matrigel®) have been used in bioinks. However, there is a batch-to-batch variability in derivation of natural biomaterials. Therefore, printed tissues using a single natural biomaterial-based bioink may not have similar characteristics in terms of biological and physicochemical properties including cell-binding sites, mechanical stiffness, and biodegradation. On the other hand, synthetic biomaterial-based bioinks (e.g., polyethylene glycol-based bioinks) have distinct and tunable physicochemical properties and offer a better option for reliable production of tissues and organs. However, most synthetic biomaterials are not biologically active. Cells encapsulated in hybrid natural and synthetic biomaterials as the bioinks have been also investigated to take the advantages of both biomaterials.

Biomaterials have been combined with nanomaterials (e.g., gold nanoparticles, carbon nanotubes, and graphene) to make functional bioinks in tissue bioprinting (Fig. 1a) and particularly in bioprinting electro-active tissues, such as skeletal muscle, cardiac, and neural tissues [3]. Biocompatibility and dispersion of nanomaterials in bioinks should be ensured to make bioactive inks in tissue fabrication and regeneration [4]. Bioprinted tissues using nanomaterial-based bioinks have been used only for in vitro applications, such as bio-actuators, bio-robots, or disease models [5] because cytotoxicity or fate of nanomaterials in the human body is still unclear and needs further investigation.

Decellularized extracellular matrix (dECM)-based bioinks have also been used in 3D bioprinting to mimic the natural ECM in the body [6] (Fig. 1b). However, there may

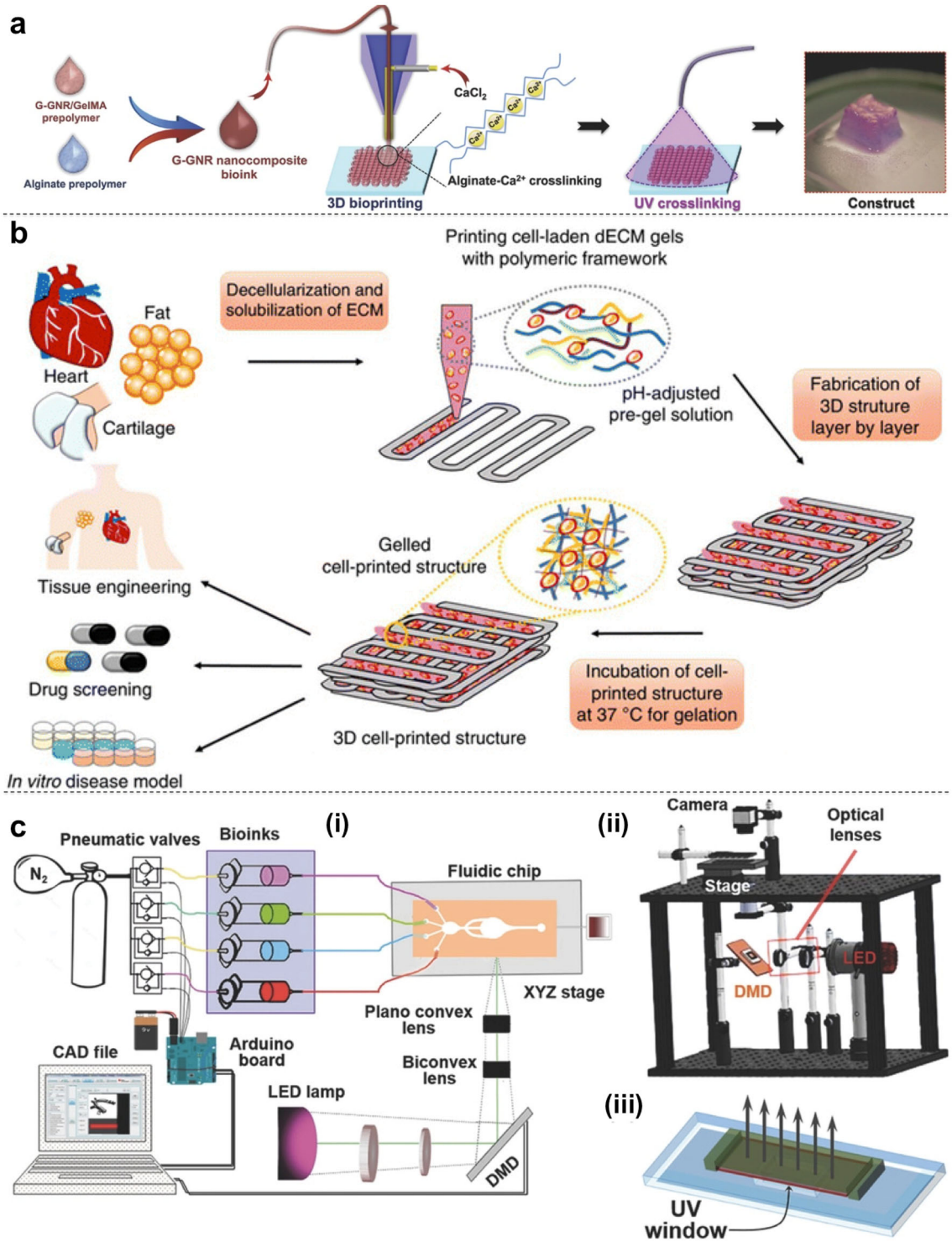

Fig. 1 Bioinks and technologies in 3D bioprinting for tissue regeneration. **a** Schematic of bioprinting using gold nanorod (GNR)/GelMA/alginate composite bioink. The printed construct on the right is composed of 30 layers. Reproduced with permission [17]. **b** dECM was obtained from different tissues and used as the bioink to make tissue analogues. Reproduced with permission [7]. **c** (i) Schematic of bioprinter setup including optical lenses and objectives, UV lamp, microfluidic device, and digital micromirror device (DMD) chip. (ii) Schematic of entire printing platform. (iii) Schematic of open-chamber microfluidic device to make printouts. Reproduced with permission [12]

be batch-to-batch variability between the dECM derived from different resources. In another study, we used platelet-rich plasma (as a rich source of soluble factors) to increase the biological activity of bioinks [7]. Such bioinks can be derived from the patient's body and pave the way toward fabrication of personalized tissues and organs. In general, adding more biological features (e.g., soluble factors for tissue vascularization and macrophage polarization) to bioinks will extend the application of 3D bioprinting to fabricate functional tissues and organs.

During bioprinting procedure or just after that, it is often required to support the stability of printed structure. Bioinks use the advantage of UV or ionic crosslinkable biomaterials to mechanically enforce printed structures in a rapid and facile way. However, such biomaterials may not be fully cross-linked within thick or complex structures upon UV or ion exposure. The UV exposure to the structure in a layer-by-layer mode may also cause cell death or damage as the cells are exposed to the UV light repeatedly. Here, visible light crosslinkable biomaterials can solve this problem by providing a safe cross-linking procedure for cells [8]. However, the injectability of these biomaterials should be evaluated prior to their use in bioinks.

Bioprinting technologies

Commonly used bioprinting technologies are extrusion printing, inkjet printing, stereolithography, and laser-assisted forward transfer. Among these technologies, extrusion printing is the most efficient modality due to its compatibility with different bioinks, high-throughput ability, and relatively low cost [9]. We recently reported a multimaterial extrusion printing, which was capable of printing multiple bioinks in a fast and continuous manner [10]. This technology will enable us to fabricate heterogeneous and complex tissue structures with precise control over tissue gradient. Only one nozzle was used in creating structures using our developed technology. The technology has advantages over conventional multimaterial extrusion printers in which multimaterial architectures are constructed using multiple nozzles in a sequential manner. The difficulties of such bioprinting are the need for careful alignment of each nozzle and introducing defects because of start-and-stop ink flow during the printing procedure [11].

Three-dimensional bioprinting can also be coupled with microfluidic systems to control the deposition of bioinks in a desired arrangement [11, 12] (Fig. 1c). The latter technologies use microfluidic flow to deposit bioinks in droplets or fibers containing cell-laden biomaterials. The printing technologies are particularly useful in creating structures with bioinks having low viscosity and sensitive to shear stress. Moreover, the speed of bioprinting is largely enhanced by which cell viability does not affect during the printing process. The development of bioprinting technologies is still an active area of research to enhance the fabrication resolution of bioprinting and make it rapid and automated.

Conclusion and future perspectives

To date, many natural and synthetic bioinks have been proposed. It is now important to standardize characterization methods of bioinks. Therefore, it would be possible to assess and compare different bioinks. Bioinks should be further used to print large tissue structures for preclinical and clinical studies and be ready to get required regulatory approval for large-scale production.

Three-dimensional bioprinting can be done with stimuli-responsive bioinks by which the printed structures would be able to change their behavior or shape in response to various stimuli [13]. This concept has shaped the field of four-dimensional (4D) bioprinting in tissue regeneration [14]. Four-dimensional bioprinted tissue constructs would enable us to incorporate the tissue morphogenesis mimicking the natural tissue function and development. However, 4D bioprinting is at an early stage of development. It is still difficult to precisely control the transformation of printed objects. Mathematical modeling would be useful in predicting the structure of printed materials as exposed to external or internal stimuli. Functional and stimuli-responsive bioinks should be also developed for 4D bioprinting. In particular, cell viability and function should not be affected by the conformational changes in the printed materials.

There have been great advances on bioprinting technologies. Future work would be the development of facile and portable bioprinting devices that can be used in clinical settings. For example, a handheld printer was recently developed for the fabrication of skin tissues [15]. The device was easy to operate and capable of printing multimaterial bioinks. Bioprinting technologies can also be adapted to do in situ bioprinting. A recent example showed a laser-assisted bioprinting for in situ bioprinting of mesenchymal stromal cells in collagen and hydroxyapatite for bone tissue regeneration [16]. In situ bioprinting would be helpful for computer-assisted medical interventions and development of medical bio-robotics. In addition, the natural body's environment can be used for tissues fabricated using in situ bioprinting and thereby there may not need any bioreactor for tissue maturation in vitro.

In summary, we envision that 3D bioprinting would play a crucial role in fabricating functional tissues and organs, which can be transplanted or used as the models in drug screening procedures or physiological studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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