Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biomedical Engineering

Three-dimensional bioprinting for bone tissue regeneration

Shivakalyani Adepu[a], Nandini Dhiman[b], Anindita Laha[c], Chandra S. Sharma[d], Seeram Ramakrishna[e] and Mudrika Khandelwal[a]

Abstract

Three-dimensional bioprinting can prove to be a promising technology for bone tissue regeneration as it facilitates good spatio-temporal distribution of cells in scaffold. The feed for bioprinting is bioink, which comprises of cells incorporated in the scaffold material. Progress has been made on the incorporation of growth factors in the bioink, which not only enables efficient regeneration but at the same time proves the feasibility of large constructs. Important parameters which determine the suitability of bioink have been discussed here. Lack of vascularization limits the success of this technology in its present form. Advances in inducing vascularization and growth factors have also been discussed. Towards the end, challenges and opinions in the area of bioprinting of bone tissue regeneration have been presented.

Addresses

[a] Natural and Nature Inspired Material for Society Laboratory, Department of Materials Science & Metallurgical Engineering, Indian Institute of Technology, Hyderabad, Kandi, 502285, India

[b] Department of Biomedical Engineering, Indian Institute of Technology, Hyderabad, Kandi, 502285, India

[c] Department of Chemical Engineering, Indian Institute of Technology, Hyderabad, Kandi, 502285, India

[d] Creative & Advanced Research Based on Nanomaterials Laboratory, Department of Chemical Engineering, Indian Institute of Technology, Hyderabad, Kandi, 502285, India

[e] Department of Mechanical Engineering, National University of Singapore, Singapore

Corresponding author: Khandelwal, Mudrika (mudrika@iith.ac.in)

Current Opinion in Biomedical Engineering 2017, **2**:22–28

This review comes from a themed issue on **Additive Manufacturing**

Edited by **Seeram Ramakrishna, Carlijn V. C. Bouten and Roger Narayan**

http://dx.doi.org/10.1016/j.cobme.2017.03.005

Keywords

Bioink, Growth factors, Controlled release, Angiogenesis, Osteogenesis.

Introduction

Three-dimensional (3D) printing has advanced the field of bone regenerative medicine by overcoming engineering challenges of biomimicking biological tissues or organs. The conventional 3D printing approach involves the predefined layered printing of scaffolds followed by cell seeding and perfusing the construct before implantation. However, this method suffers from a lack of uniform spatial and temporal distribution of cells and growth factors in the construct. A new class of 3D printing called bioprinting – printing along with the cells – promises to overcome all these limitations.

In this mini review, we report the most recent advances in the field of 3D bioprinting of bone with respect to methods, bioink properties and growth factors/drug aided vascularization in the constructs. Later, we present our opinion and the challenges needed to overcome, to advance the field of bone bioprinting.

Bioprinting techniques and bioinks

Current focus has been to develop novel bioinks which can be 3D printed in cell-compatible conditions to fabricate a cell-laden 3D structure. Bioink comprises of cells embedded in a printable material which aids proliferation of cells by maintaining a supply of nutrients, oxygen and growth factors (GFs). Bioinks can be in the form of hydrogels, viscous fluids or microcarriers [1]. Polymeric hydrogels are preferred as they mimic native extracellular matrix (ECM) and facilitate cell adhesion and matrix integration [2]. Selection of suitable combination of bioprinting method and bioink is very essential for a successful fabrication of tissues [3]. The widely employed bioprinting techniques include drop based or inkjet, laser-assisted and extrusion bioprinting [4], some of which are summarized in Table 1. The essential properties required for bioink vary with different bioprinting method employed (Table 2).

Natural polymers are preferred over synthetic polymers because of cell affinity and resemblance to the ECM. However natural polymers undergo uncontrollable degradation and possess poor mechanical stability. Crosslinking and polymer blending can control the scaffold degradation kinetics [3,4,12]. Mechanically superior biocompatible synthetic polymers are used with growth factors (to improve cell adhesive properties) provide better control over cell specific stiffness and elastic modulus [3,13]. A tabular presentation of

Table 1

Additive manufacturing techniques for bone tissue scaffold (conventional and bioprinting).

Technique	Resolution (μm)	Pore size (μm)	Porosity (%)	Advantages	Disadvantages	Ref.
1. Photo polymerization based Techniques						
Stereo lithography (SLA)	14–150	20–1000	<90	Complex internal features;	Only for photopolymers;	[5]
Micro-stereolithography (MSLA)	0.5–10	100–300	<90	GFs and cell loading possible	Toxic photoresins; shrinkage issue;	
Digital light processing	40	500	<90		Need of support structure	
2. Powder based techniques						
Selective laser sintering (SLS)	50–1000	30–2500	<40	Solvent free, fast operation; No need of support structure; No post processing required;	Expensive; Surface Powder finishing; Difficult to remove blocked powder; high temperature involved; Resolution depends on diameter of laser beam	[6]
3D Printing	50–300	45–1600	45–60	Mild process conditions	Poor mechanical properties	[7]
3. Extrusion based techniques						
Fused deposition modeling	100–150	100–2000	<80%	Good mechanical integrity; Solvent and support structure not needed;	Limited filament materials; material exposed to high temperature; Scaffold with small pore size is difficult to fabricate.	[8]
Low temperature deposition	300–500	200–500	<80%	Broad range of material usage; Able to incorporate growth factors;	Solvent usage; Freeze drying is required;	
Pressure assisted micro-syringe	10–1000	10–600	70%	Very fine resolution	Printable viscosities are limited in range	
Robocasting/Direct Ink writing	100–450	5–100	<90%	Independent and customized 3D nozzle movement with high resolution; Highly viscous suspensions can be used; Support is not required; 1 mm structures;	Expensive; Optimization of Bioink properties is crucial;	
4. Bioprinting						
Method and material	**Resolution (μm)**	**Droplet size (μm)**	**Cell viability**	**Advantages**	**Disadvantages**	**Ref.**
Droplet based bioprinting For hydrogels	~50 μm	50–300	<85%	Compatible with narrow viscosity range; Compatible with various cells and GFs; Suitable to deposit cells on microarrays or organ-on-a-chip Inexpensive, flexible, and commercially available	Non-uniform droplet size; Nozzle blocking in fibrous bioink and in high cell densities; Cross contamination while printing multiple bioinks simultaneously	[9,10]
Micro extrusion based Bioprinting Liquids, pastes and gels	5 μm–1 mm wide	100 μm–1 mm	40–80%	Compatible with wide range of viscosities; Enables printing of scaffold free bioink of tissue spheroids	Shear stress of highly viscous bioink, tiny nozzle diameter, and large dispensing pressure causes significant cell damage; Not useful for high-throughput bioprinting of tissues; Low resolution limits the microchannel incorporation for vascularization. Limited control on cell–cell and cell– matrix interactions	
Laser Assisted Bioprinting Cell suspension;	100–600 μm	>20 μm	>95%	Nozzle free technology enables less cell damage; High precision (1cell/droplet); Supports vascular channels	Laborious; Expensive; difficult to print hetero cellular scaffolds; Limited commercial viability	