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Current developments in 3D bioprinting for tissue engineering

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Abstract

The field of 3-dimensional (3D) bioprinting has enjoyed rapid development in the past few years for the applications in tissue engineering and regenerative medicine. In this review, we summarize the most updated developments in 3D bioprinting for the applications in tissue engineering with a focus on the printable biomaterials used as bioinks. These developments include 1) novel printing regimes have been enabled by the use of fugitive inks for the creation of intricate structures e.g. vascularized tissue constructs; 2) mechanical strength of printed constructs can be enhanced by co-printing soft and hard biomaterials; 3) bioprinted *in-vitro* models for drug testing applications are closer to reality. We conclude that the research and application of new bioinks will remain the key highlights of the future developments in 3D bioprinting for tissue engineering.

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Introduction

The field of 3-dimensional (3D) bioprinting has enjoyed rapid development in the past few years for the applications in tissue engineering and regenerative medicine. The rapid developments have been fuelled by the new bioprinting technologies, bioprinters, novel printable biomaterials or bioinks and exciting applications for in-vitro models or transplantable tissues. We believe the new developments in the materials have been the key to many recent achievements. Therefore, this article aims to review the most recent developments in the field of 3D bioprinting for the applications in tissue engineering with a focus on printable biomaterials. Figs. 1 and 2.

Novel printing regimes

Fugitive inks

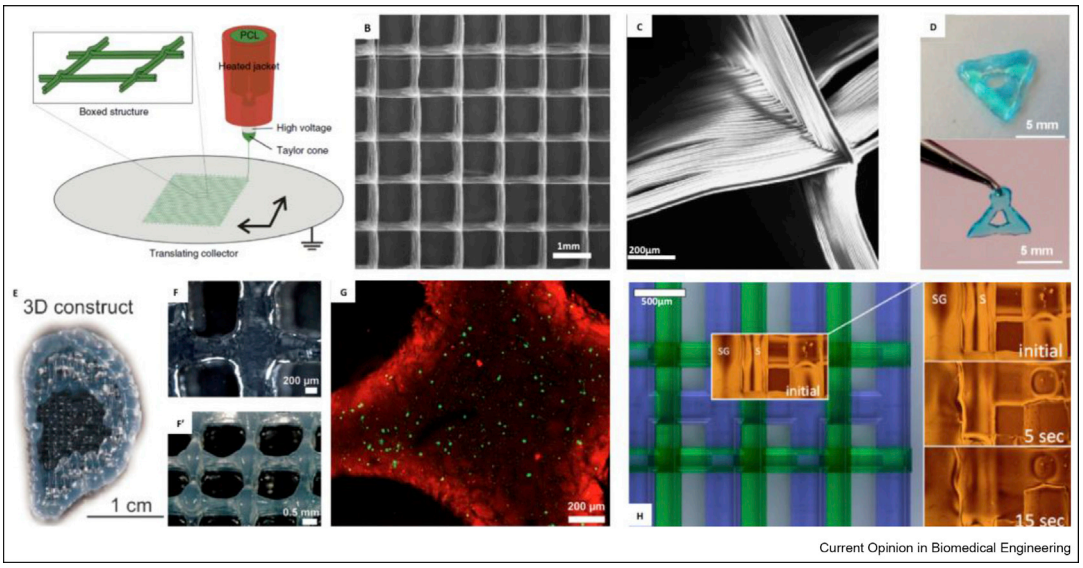
Some research groups are using a different type of hydrogel material "fugitive inks" along with standard bio-inks to support their bioprinted structures. After the printing is completed, the fugitive inks are transformed from a gel to a liquid by simply altering the temperature and then the resulting liquid can be drained away leaving the bioprinted structure behind. Fugitive inks are used in two ways, either around the structure (in the form of a supporting bath) to support its creation in free space [1–3] or inside the structure to enable the creation of internal channels [4].

Fugitive support baths

There have been some interesting improvements made to the technique of printing complex structures within a hydrogel reservoir to provide support. Previously when printing within a hydrogel, filler material is required to restore the resulting voids and crevasses left by the nozzle as it travels through the hydrogel material [1]. However, a new technique called Freeform Reversible Embedding of Suspended Hydrogels (FRESH) [2], or more simply Freeform Reversible Embedding (FRE) [3], has been developed which uses a supporting bath composed of a material that exhibits a Bingham plastic rheology, flowing as a viscous fluid at high shear stresses but behaving as a rigid body at lower shear stresses. Due to this property of the bath, the syringe nozzle can travel through the supporting material with negligible resistance while the extruded material is supported and the geometry of the printed structure is maintained. In FRESH the supporting material is composed of a slurry of gelatin microparticles which is melted and washed away by simply raising the temperature to 37 °C when the structure is completed [2]. While FRE utilised a hydrophilic Carbopol gel to support the 3D printing of hydrophobic PDMS prepolymer resins; after the PDMS print is cured, it can be released by liquefying the Carbopol in the presence of ionic solutions such as phosphate buffered saline solution [3].

Conventionally, supporting materials have special properties to allow them to support structures during printing i.e. Bingham plastic [2], high-density hydrophobic fluorocarbons [5] or more rigid biodegradable materials are used. A novel bioprinting scheme has been developed by Ghanizadeh Tabriz et al. [6] wherein 3D cell-laden alginate structures are built up using a three-stage cross-linking process. Partially cross-linked

Figure 1



Biomaterials for use in 3D bioprinting. (A–C) [20] Highly organized microfibers of PCL are used to reinforce hydrogels for hard tissue engineering. (A) Thin PCL fibres are deposited by using melt-electrospinning in a direct writing mode. (B) Fibres were stacked in a 0–90 orientation at a 1 mm interval. (C) Detailed image of fibres that fused at cross-sections. (D) [41] 3D printed construct made from DNA-peptide hydrogel, strong enough to be picked up (E) [34] Silk peptide hydrogels are mechanically strong enough to create large, intricate structures. (F) [28] Close up of cell encapsulated in silk peptide hydrogels, in 2 (F) or 8 (F′) layers. A life dead staining of human fibroblasts in the gel (G) shows good cell survival. (H) [33] Demonstration of solubility modification of silk by the addition of polyols. Blue strands were printed with insoluble Silk:Glycerol (SG) and green strands with silk (S). After the addition of water, the silk strands solve in roughly 15 s, leaving only the Silk:Glycerol strands. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alginate is extruded onto a porous PMMA platform which is lowered into a bath of 100 mM calcium chloride solution as each layer is completed thus further cross-linking the structure before finally treating the completed structure with barium chloride in order to extend the degradation time of the hydrogel. This technique allows for the creation of cell-laden structures with overhangs that would normally not be possible using conventional extrusion and the encapsulated cells exhibit a high survival rate.

Internal channel creation via fugitive inks

Another use for fugitive inks is the creation of perfusable channels within other structures, these materials have the mechanical stability to maintain shape while the entire tissue is printed, but can be washed away at a later time, leaving perfusable channels in whatever configuration is needed [4,7,8]. The standard process creates a regular geometric network structure first via bioprinting before cast moulding the desired bulk material around it – epoxy resin for microfluidic devices or cells suspended within a hydrogel – finally the sacrificial structure is removed and the resulting perfusable channels are lined with endothelial cells [4,9].

While the use of fugitive inks to create internal channels is not entirely novel – the Lewis group has been using this technology to create microvascular-like networks in microfluidic devices since 2003 [10] – the technology is gradually developing and the complexity of these tissue-like structures is increasing from simple blocks to more complex multi-cellular structures.

Kolesky et al. [4] showed that by using this technique, they can create a thick, perfusable tissue construct. This helps to keep cells alive all throughout the construct, and more importantly, to perfuse it with growth factors that differentiate the printed Mesenchymal Stem Cells (MSCs) toward the osteogenic lineage. Lee et al. [8] showed that by not only lining the channels with endothelial cells, but also incorporating endothelial cells within the printed construct, micro-vascularisation is created between the larger channels.

New developments in biomaterials for 3D bioprinting

Biomaterials traditionally have been defined as materials used in biomedical devices, made specifically not to harm organs or tissues. However, over time, biomaterials have evolved to include a variety of materials. From rigid materials, like metals and ceramics for implants, to hydrogels for drug delivery and cell encapsulation, to nanodots and quantum particles for imaging and drug delivery. The classifications of different biomaterials is ever expanding with the vast amount of research being done in the field of tissue engineering [11,12]. In the field of 3D bioprinting, two major groups of biomaterials