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TOPICAL REVIEW

Microfluidic hydrogels for tissue engineering

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Abstract
With advanced properties similar to the native extracellular matrix, hydrogels have found widespread applications in tissue engineering. Hydrogel-based cellular constructs have been successfully developed to engineer different tissues such as skin, cartilage and bladder. Whilst significant advances have been made, it is still challenging to fabricate large and complex functional tissues due mainly to the limited diffusion capability of hydrogels. The integration of microfluidic networks and hydrogels can greatly enhance mass transport in hydrogels and spatiotemporally control the chemical microenvironment of cells, mimicking the function of native microvessels. In this review, we present and discuss recent advances in the fabrication of microfluidic hydrogels from the viewpoint of tissue engineering. Further development of new hydrogels and microengineering technologies will have a great impact on tissue engineering.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Tissue engineering has made great advances for achieving its goal to restore and improve tissue/organ function [1–3]. With the development of tissue engineering, hydrogels have attracted extensive interest because of their advantageous properties similar to those of the native extracellular matrix (ECM), such as high content of water, unique biocompatibility, biodegradability, as well as tunable physical and chemical properties (figure 1) [4, 5]. A large number of hydrogels have been developed to fabricate tissue-engineered scaffolds [6, 7] or directly encapsulate cells [8–10], leading to the successful engineering of thin or avascular tissues, such as skin [11], bladder [12] and cartilage [13]. For example, it was demonstrated that engineered human bladder tissues made from autologous cells seeded collagen–polyglycolic acid scaffolds gained bowel function promptly after surgery [12]. Despite these advances, there are still several challenges ahead in the construction of complex and large functional tissues (e.g., heart, liver, kidney) with high cell densities, great metabolic requirements, and intricate intra-architectures [14]. A major reason is the limited diffusion property of hydrogels, which is often restricted to 200 μm and results in necrotic core [15, 16]. To sustain cell viability and growth, hydrogels must provide effective nutrient transfer, gas exchange (i.e. O_2 and CO_2), and metabolic waste removal.

With advances in microengineering technologies such as particle leaching, soft lithography and bioprinting, enhanced

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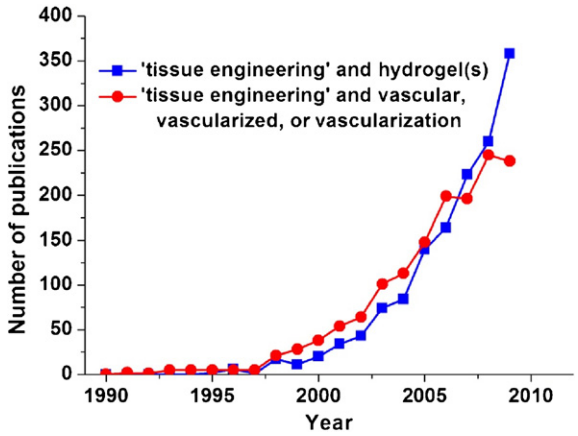

Figure 1. Total number of publications on 'tissue engineering' and hydrogel(s), 'tissue engineering' and vascular, vascularized, or vascularization. (Source: Science Citation Index Expanded [SCI-EXPANDED].)

pores [17–19] and microfluidic channels [20–22] were engineered into hydrogels to improve perfusion for the encapsulated cells. In human tissues, blood vessels are broadly distributed, performing important functions such as delivery of oxygen and nutrients, removal of metabolic waste products, body temperature control, immune protection and damage repairing [23]. Hydrogels that contain microfluidic networks (i.e. microfluidic hydrogels) have the potential to create functional tissues with enhanced vascularization [24, 25] and provide the ability to control three-dimensional (3D) cell behavior by regulating the distribution of chemical cues in hydrogels through microchannels [26]. For example, Ling *et al* [22] demonstrated the efficient delivery of nutrient to encapsulated cells by molding microfluidic channels (50 μm $\times$ 70 μm and 1 mm $\times$ 100 μm) into cell-laden agarose hydrogels. Yoo's group [20] constructed multi-layered collagen structures embedded with microchannels through a bioprinting method and showed that significantly elevated cell viability could be achieved compared to those without any channels. Choi *et al* [25] created microfluidic channels ($\sim$100 μm scale) in calcium alginate by soft lithography and found convective mass transfer and quantitative control of the 3D soluble chemical microenvironment experienced by the encapsulated cells. Kloxin *et al* [27] and Sarig-Nadir *et al* [28] observed guided 3D cell behavior (e.g., cell growth, cell connectivity) along microchannels by photopatterning poly(ethylene glycol) (PEG)-based hydrogels or laser photoablating PEGylated fibrinogen hydrogels in the presence of cells, respectively. Thus, microfluidic hydrogels hold great promise in tissue engineering.

In this review, we present the state of the art of current microengineering methods for the fabrication of hydrogels embedded with pores and microchannels. In section 2, methods for fabricating porous hydrogels and remaining challenges are presented. In section 3, the current progress of microfluidic hydrogels in tissue engineering is discussed. In section 4, efforts in rational design and optimization of microfluidic hydrogels are presented. Finally, a conclusion and future trends in the development of hydrogel-based vascularized tissue constructs are given.

2. Hydrogels with enhanced pores for tissue engineering

2.1. Methods for fabricating porous hydrogels

The introduction of enhanced pores can provide more space and increased surface area-to-volume ratio of hydrogel scaffolds for cell growth, tissue invasion and local angiogenesis, and facilitate nutrient transport [7, 29]. For example, porous alginate showed enhanced cell proliferation and increased permeability by nearly three orders of magnitude as compared to nonporous conditions [30]. Porous collagen prepared with ice particulates improved cell distribution and chondrogenesis [31]. Pore size is important for regulating cell behavior, such as neovascularization [32] and amoeboid-like changes of mammalian cells [33]. The mechanical properties of porous hydrogels are dictated by pore size and density [34–36]; furthermore, porous hydrogels can greatly impact the viability of the encapsulated cells since the diffusion properties are affected by the porosity and pore interconnectivity of the hydrogels [30]. Therefore, it is crucial to control the various pore features (e.g., pore size, porosity, pore distribution and interconnectivity) within hydrogels. Various methods have been developed to fabricate porous hydrogels, including solvent casting/particle leaching [31, 37, 38], freeze drying [39, 40], gas foaming [41, 42], polymer phase separation [43], frontal polymerization [44], and electrospinning [45, 46], as summarized in table 1.

Particle leaching has become a common strategy as it is simple and reproducible, allowing a wide range of pore sizes to be produced. Pores were formed through solvent casting, followed by particle leaching, with pore sizes controlled by pore-forming agent sizes (e.g., salt and sucrose crystals, polymer beads). This method was applied to generate micropores within PEG and fibrin scaffolds [38, 47, 48]. Recently, soft sacrificial pore-forming agents made from hydrogel beads (e.g., calcium alginate, gelatin) were utilized to fabricate porous hydrogels having continuous, open-pore topologies, with pore diameters ranging from 40 to 400 μm [30, 40, 49]. Gas-forming has been used to generate hydrogels (e.g., PEG) with pore sizes in the range of 100 to 600 μm [42]. Polymer phase separation was exploited to generate porous hydrogels where a removable phase acts as the pore-forming agent [43]. For example, porous dextran hydrogels with a broad range of pore sizes from 10 to 120 μm have been created by varying concentrations of methacrylated dextran within PEG [50]. Cheng *et al* [51] produced porous poly(N-isopropylacrylamide) (PNIPAAm) hydrogels through phase separation of PNIPAAm in different concentrations of aqueous sodium chloride solutions. Cellulosic hydrogels with interconnected pores ($\sim$92–120 μm in diameter) and nano-sized structures were also fabricated by temperature-mediated phase separation, lyophilization and then re-hydration [18].

2.2. Challenges ahead of porous hydrogels for tissue engineering

Although the shape and size of pores can be varied, it is difficult to control the pore distribution (which is often random) and

Table 1. Recent development (since 2007) on the fabrication of porous hydrogels.

Method	Hydrogels	Cells	Pore size (μm)	Reference
Particle leaching	Alginate	HepG2 hepatocarcinoma cells encapsulated	~150–300[a]	[30]
	Agarose	Porcine articular chondrocytes encapsulated	200–400	[119]
	PEG	NIH-3T3 fibroblasts seeded	15–86	[38]
		Human mesenchymal stem cells seeded	100–600	[42]
	Cyclic acetal	Human mesenchymal stem cells seeded	72–194	[120]
	Fibrin	NIH-3T3 fibroblasts seeded	30–95	[48]
Freeze drying	Chitosan	NIH-3T3 fibroblasts seeded	50–150	[121]
	Collagen	Bovine articular chondrocytes seeded	35–118	[31]
Gas foaming	Gelatin	–	80–360	[122]
		Human skin fibroblasts seeded	~4–80	[41]
Liquid–liquid immiscibility	Cellulose	NIH3T3 fibroblasts seeded	~92–120	[18]
		Human hepatoblastoma cells seeded		
		Human MCF-7 breast cancer cells seeded		
		Human foreskin fibroblasts seeded		
Frontal polymerization	Polyacrylamide	–	~1–2	[44]
Electrospinning	Gelatin/PCL	Human adipose-derived stem cells seeded	20–80	[123]

PEG = poly(ethylene glycol); PCL = poly(3-caprolactone); hMSCs = human mesenchymal stem cells.
[a] Pore-forming agent size.

pore size within a biologically relevant length scale (i.e. several to hundreds of micrometers) [16]. Experiments demonstrated that pore size is dependent on the kind of cells used. For example, 5 μm pores were needed for neovascularization, 5–15 μm for fibroblast ingrowth, 20–125 μm for dermal repair, 100–350 μm for regeneration of bone, and greater than 500 μm for fibrovascular tissues [52–54]. In addition, encapsulating cells into porous hydrogels is challenging because undesirable procedures and chemicals are often employed during the fabrication process [29], while seeding cells on the surface of the hydrogel scaffold often results in a necrotic core due to the limitations of cell penetration and nutrient exchange [55]. Furthermore, a substantial driving pressure is required to ensure physiologically useful flow rates to deliver enough solutes to the interior of porous hydrogels, which may result in the collapse of hydrogel scaffolds and produce harmful shear stress on encapsulated cells. Recently, it was proposed that microfluidic hydrogels have the potential to provide adequate perfusion to cells in the context of tissue engineering [56].

3. Hydrogels embedded with microfluidic channels

Microfluidic channels in hydrogels offer the potential to maximize the perfusion capacity of the constructs and recreate the spatial complexity of *ex vivo* tissues. Microfluidic hydrogels can greatly promote the development of vascularization tissue engineering and offer 3D *in vitro* tissue models that closely mimic natural conditions for biology and disease studies. For example, microfluidic PEG hydrogels have been introduced to engineer hepatic tissue constructs [21]. Microfluidic PEGylated fibrinogen hydrogels have been fabricated to spatiotemporally direct growth of dorsal root ganglion cells [28]. Microfluidic collagen has been used to study the effect of intracellular second messenger cyclic AMP (cAMP) [57, 58] and mechanical cues [59] on the function and stability of microvessels *in vitro*. To date, several methods have been developed to fabricate microfluidic channels in hydrogels, such as molding [22, 25, 60], bioprinting [20, 61, 62], photopatterning [21, 63] and modular assembly [19, 64], as summarized in table 2. In this section, we discuss recent progress in the fabrication of microfluidic hydrogels for tissue engineering.

3.1. Molding

Molding is a common template strategy that has been used to produce tissue constructs with special architectures. Several types of templates, such as microneedles, glass and polymer fibers, and patterned poly(dimethylsiloxane) (PDMS), obtained by standard lithographic approaches were employed to create microchannels within hydrogels.

3.1.1. Molding methods based on microneedles and fibers. A simple molding method to fabricate microfluidic hydrogels is to use microneedles located inside a chamber. Hydrogel precursors are then poured into the chamber and gelled, followed by gently removing the needles to create open channels. Using this method, Tien's group [60] fabricated open channels (~100 μm in diameter) through collagen hydrogels. Endothelialized tubes were formed through seeding human endothelial cells on the channel surface. These endothelial tubes displayed cellular organization and function similar to human microvessels and were closely regulated by intracellular second messenger cyclic AMP [57, 58] and mechanical cues (e.g., shear stress and transmural pressure) [59]. Nichol *et al* [65] used microneedles to form endothelialized tubes within gelatin methacrylate (GelMA) hydrogels in the presence of NIH 3T3 fibroblasts, demonstrating the potential for making co-cultured, microvascularized tissue constructs. Recently, Park *et al* [66] produced hepatic cell encapsulated hydrogels with micropores around the microchannel (figures 2(*A*), (*B*)). To do this, the mixture of agarose solution, cells and sucrose crystals was poured into a cylindrical PDMS mold, in which a microneedle was inserted in the middle of the side walls. Hepatic cells viability was analyzed as a function of the distance from the microchannel and showed

Table 2. Fabrication method for microfluidic hydrogels.

Method	Hydrogels	Cells	Advantages	Disadvantages	Reference
Microneedle template	Agarose Gelatin methacrylate Collagen	Hepatic carcinoma cells NIH-3T3 fibroblasts HUVECs HUVECs HDMECs HDLECs Human perivascular cells	Easy to deal with Inexpensive	Limited to simple geometries due to the need of manual handing Difficult to interlink neighboring channels	[66] [65] [57–60]
Fiber template	Collagen	Human oral fibroblasts (viability >80%)	Easy to deal with Inexpensive Scalable	Use of organic solvents	[67]
	pHEMA-co-MAA	Chick cardiomyocytes hESC-CM		Difficult to interlink neighboring channels	[68]
Soft lithography	PEG Collagen Fibrin Gelatin Agarose Alginate Collagen–HA semi-IPNs	NIH-3T3 fibroblasts Human dermal fibroblasts HDMECs AML-12 murine hepatocytes Murine embryonic stem cells (viability >85%) Human hepatocytes Rat lung epithelial cells NIH-3T3 fibroblasts (viability >75–85%)	High repeatability Accurate control at the microscale	Cumbersome for the need of multi-iterative procedures	[71] [72] [22] [25] [73]
Bioprinting	HA-MA:GE-MA Alginate HA crosslinked with tetrahedral PEG tetracrylates Collagen Gelatin Gelatin/alginate/chitosan Gelatin/alginate/fibrinogen Gelatin/chitosan Fibrin	NIH-3T3 fibroblasts Human hepatoma cells Human intestinal epithelial cells HepG2 liver cells Myoblast cells NIH-3T3 fibroblasts HepG2 C3A cells Int 407 cells (viability >79–82%) Human dermal fibroblasts Rat hepatocytes Rat ADSC (viability >95%) Rat hepatocytes HMVECs	Precise cell placement High repeatability Possibility of fabricating arbitrary complex constructs Automated Scalable	Require specific equiment	[86] [10] [82] [20] [84] [83] [61]
Photopatterning	PEG-based PEGylated fibrinogen	Rat hepatocytes NIH 3T3-J2 cells Human dermal fibroblasts Human dermal fibroblasts Fibrosarcoma cells hMSCs Dorsal root ganglion cells	Accurate control at the microscale Bulk patterning available Possibility of fabricating arbitrary complex constructs	Limited to photosensitive hydrogels Time-consuming Expensive	[21] [96] [63] [27] [28]

Table 2. (Continued.)

Method	Hydrogels	Cells	Advantages	Disadvantages	Reference
Modular assembly	Collagen Gelatin PEG	Human hepatoma cells HUVECs (viability >90%) NIH-3T3 fibroblasts	Biomimetic Scalable Uniform	Lack of control over inner-architectures	[64, 101, 103] [19]

HA = hyaluronic acid;
HA-MA = methacrylated hyaluronic acid;
GE-MA = methacrylated ethanolamide derivative of gelatin;
PEG = poly(ethylene glycol);
Poly(HEMA) = poly(2-hydroxyethyl methacrylate);
pHEMA-co-MAA = poly(2-hydroxyethyl methacrylate-co-methacrylic acid);
HUVECs = human umbilical vein endothelial cells;
HDMECs = human dermal microvascular endothelial cells;
HDLECs = human dermal lymphatic microvascular endothelial cells;
HMVECs = human microvascular endothelial cells;
hESC-CM = human embryonic stem cell-derived cardiomyocytes;
ADSC = adipose-derived stromal cells;
hMSCs = human mesenchymal stem cells.

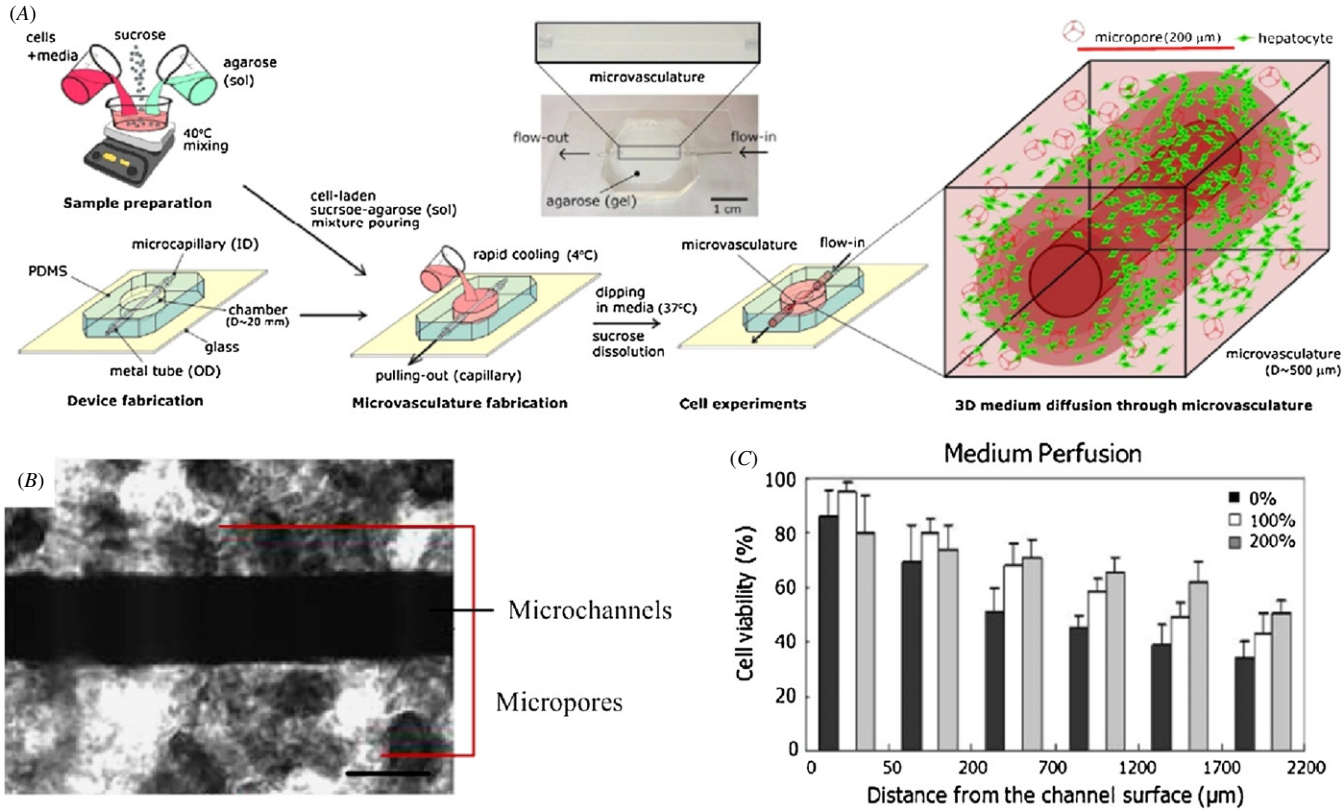

Figure 2. Fabrication of microfluidic porous cell-laden agarose hydrogels [66]: (*A*) schematic of the fabrication process, (*B*) phase contrast images of a dried agarose construct with micropores around a microchannel, scale bar: 500 μm, (*C*) viability of hepatic cells from microchannel within agarose gels after 5 days' culture with medium perfusion at different sucrose concentrations, i.e. 0 wt%, 100 wt%, 200 wt%, respectively. Copyright © 2010 John Wiley and Sons (www.interscience.wiley.com).

that the microchannel significantly affected the diffusion of biomolecules and cell viability (figure 2(*C*)).

Microneedles used during the molding process need manual handling and this method is limited to fabricate simple architectures with straight microchannels. In contrast, glass and polymer fibers can be used as template and removed by self-degradation or dissolved in a special solvent without manual handling. For example, Nazhat *et al* [67] introduced aligned soluble phosphate glass fibers into compacted collagen scaffold. The degradation of these glass fibers formed microchannels with diameters ranging from 10 to 50 μm [67]. These microchannels could play an important role in hypoxia/perfusion limitations. In a recent work, polycarbonate core/poly(methyl methacrylate) (PMMA) optical fibers were incorporated into poly(2-hydroxyethyl methacrylate-co-methacrylic acid) hydrogels

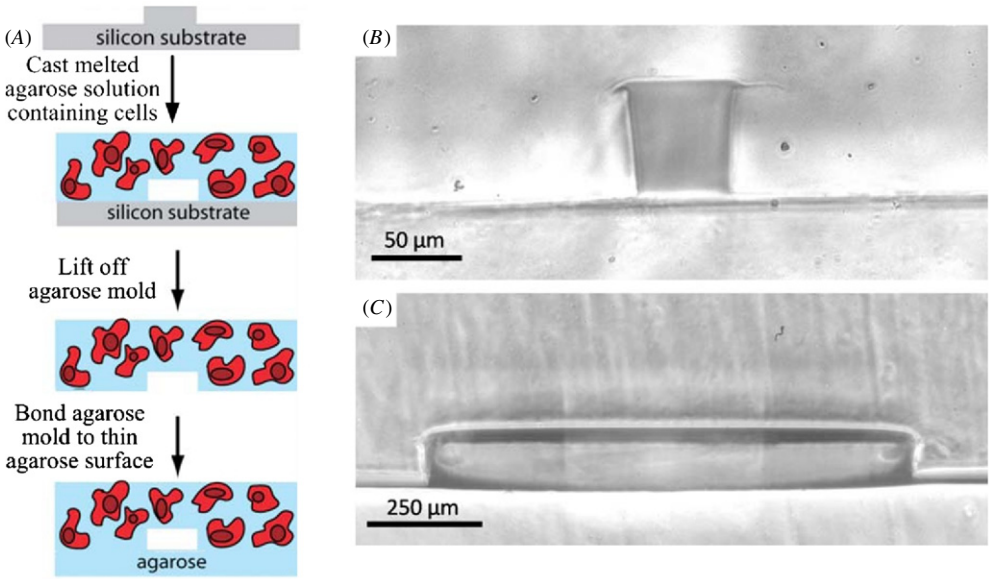

Figure 3. Fabrication of microfluidic cell-laden agarose hydrogels by soft lithography [22]: (*A*) schematic of the fabrication process. (*B*), (*C*) Cross-sectional images of agarose constructs with channel sizes: (*B*) 50 μm × 70 μm and (*C*) 1 mm × 150 μm. Copyright © 2007, reproduced by permission of The Royal Society of Chemistry.

and dissolved in dichloromethane to create microchannels with controlled size and spacing [68]. The channel diameter was optimized (60 μm) for cell seeding and mass transfer, and, channel spacing of 60 μm was chosen to introduce sphere-templated, interconnected pores (20–40 μm) surrounded microchannels to maximize angiogenesis while reducing fibrosis. Although creating microchannels using fibers have many advantages, such as providing the ability to control size and spacing, the undesirable organic solvents (e.g., dichloromethane) used to remove sacrificial fibers make this method less desirable when cell encapsulation is necessary. In addition, it is difficult to interconnect neighboring channels using the above molding methods based on either microneedles or fibers.

3.1.2. Soft lithography. With advantages in accuracy and reproducibility, soft lithographic approaches were often used to fabricate templates (e.g., PDMS) for cell patterning and microfluidic systems. For example, biodegradable thermoplastic poly(lactide-co-glycolide) [69] and elastic poly(glycerol sebacate) [70] with microchannels have been prepared through patterned PDMS molds. Recently, this method was extended to produce synthetic (e.g., PEG) [71] and natural (e.g., agarose, alginate, collagen) [22, 25, 72] microfluidic hydrogels for tissue engineering.

Ling *et al* [22] fabricated microfluidic cell-laden agarose hydrogels using soft lithography. Agarose solution suspended with hepatic cells was poured and gelled against templates made from the SU-8 patterned silicon wafer; the surfaces of the molded agarose and another agarose slab were subsequently heated and sealed together to generate microchannels (figure 3(*A*)). Two different sizes (50 μm × 70 μm and 1 mm × 150 μm) of microchannels were constructed (figures 3(*B*), (*C*)). Media pumped through the channels ensured effective delivery of nutrients and removal of waste products. It was shown that hepatic cells were homogeneously distributed in the molded agarose and only those cells in close proximity to the channels remained viable after 3 days' culture, demonstrating the importance of a perfused network in large hydrogel constructs. Microfluidic alginate hydrogels were also fabricated in the presence of human hepatocytes and rat lung epithelial cells with quantitative control of the 3D soluble chemical microenvironment [25]. This approach is promising for spatiotemporal directing proliferation, migration and differentiation of the encapsulated cells in hydrogels. Soft lithography was also employed to generate gelatin networks as a sacrificial element to create microchannels within a second hydrogel (e.g., collagen, fibrin, Matrigel) [72]. In this approach, sacrificial gelatin networks were embedded in the second hydrogel and subsequently removed by melting and flushing, leaving behind interconnected microchannels. These channels replicated the features of the original gelatin networks and preserved the patency of the channels as narrow as 6 μm, which is approximately the size of capillary, and may find great applications in vascularization tissue engineering.

Due to the inherent weak mechanical properties of hydrogels, microfluidic networks embedded within hydrogels are likely to collapse, especially in complex multilayer systems. Cuchiara *et al* [71] proposed to use PDMS housings as mechanical support surrounding PEG, so that robust multilayer microfluidic hydrogels can be constructed, whilst composite collagen–HA semi-interpenetrating network hydrogels with improved mechanical strength have also been developed to produce microfluidic constructs [73].

Soft lithography presently is only limited to fabricating 2D networks which are then stacked to create 3D constructs [74]. It is cumbersome to produce 3D thick tissues due to the need of iterative procedures, such as the production of templates, stamping steps for patterning, and bonding processes between individual layers. In contrast, other methods, such as

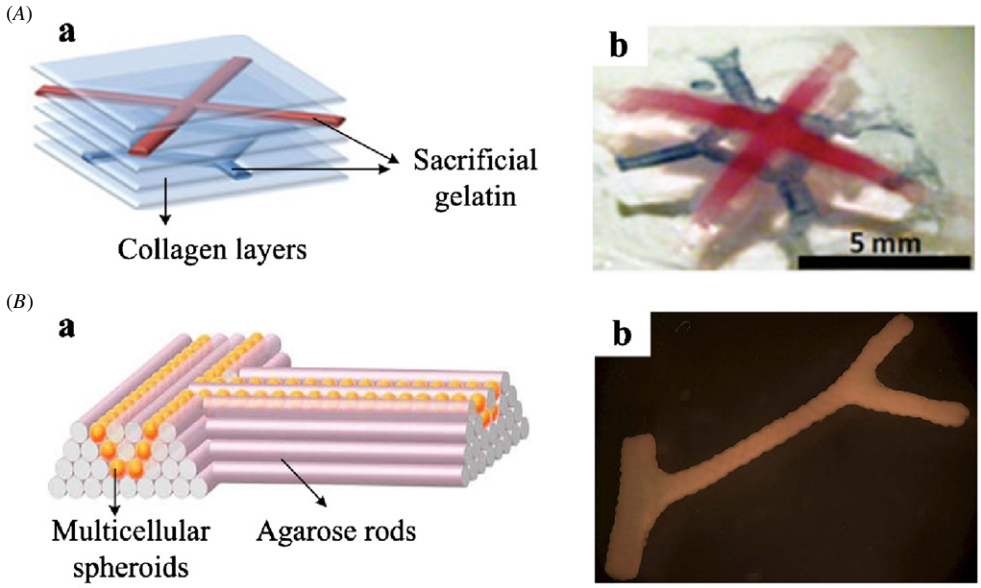

Figure 4. Fabrication of microfluidic hydrogels by bioprinting: (*A*) design template (a) and multi-layered collagen scaffold with microfluidic channels (b) using printed gelatin networks as a sacrificial element [20], copyright © 2010 John Wiley and Sons (www.interscience.wiley.com). (*B*) design template (a) and post fusion pattern (b) of branched constructs using agarose rods as filling material [85]. Copyright © 2009, reprinted with permission from Elsevier.

bioprinting and photopatterning, may provide the much needed 3D versatility to form complex microfluidic hydrogels [24], as discussed below.

3.2. Bioprinting

Bioprinting is a rapid prototyping strategy that aims to produce functional living tissues and organs through computer-aided, layer-by-layer deposition of multiple types of cell and biomaterial (usually hydrogels) [75–78]. Previously, we have demonstrated the feasibility of bioprinting in the fabrication of 3D cell-laden hydrogel tissue constructs with defined reproducible patterns [8, 10, 79–81]. The possibility of using the method of bioprinting to fabricate vascularized hydrogel tissue constructs has also been explored [5, 61, 82].

In one study, a modified thermal inkjet printer was developed to deposit human microvascular endothelial cells in conjunction with fibrin (bio-ink) into fibrinogen solution (bio-paper) to form tubular structures at the microscale [61]. In another work, Yan *et al* [83] employed a pressure-assisted cell assembly technique to fabricate 3D gelatin/chitosan constructs with designed arrays of channels (∼100–300 μm in diameter). Recently, the same group [84] developed a new method of double-nozzle assembling to produce hybrid cell/hydrogel constructs with special intrinsic/extrinsic architectures. Inspired by Golden's work on soft lithography [72], Lee *et al* [20] printed simultaneously sacrificial gelatin networks as a sacrificial element (which were later thermally removed) and cell-collagen to fabricate multi-layered (up to 17 layers) microfluidic constructs (figure 4(*A*)). Compared to soft lithography, in which the sacrificial gelatin networks were fabricated by molding, this method allowed more rapid formation of microfluidic channels in hydrogels [10]. Recently, agarose rods were used in bioprinting as the filling material to control the shape and size of vascular tubes (figure 4(*B*)) [85]. With this method, blood vessels of desired geometry (e.g., branching, double layer) were successfully produced. However, the agarose filler had to be manually removed, limiting its scalability and the achievement of more complex structures.

To date, only a few hydrogels have been explored in bioprinting for the fabrication of microfluidic cell-laden tissue constructs, partially due to the lack of printable hydrogels. Skardal *et al* [86] reported the use of photocrosslinkable HA–gelatin hydrogels in a two-step bioprinting strategy to produce cellular tubular constructs. Extrudable hydrogels made from tetra-acrylate derivatives co-crosslinked thiolated HA and gelatin derivatives were also ultilized for bioprinting vessel-like constructs with improved stiffness and equivalent or superior ability in supporting cell growth and proliferation [82]. Recently, Stupp *et al* [87] developed noodle-shaped peptide amphiphile hydrogels that may be employed in bioprinting for fabricating complex scaffolds with nano-scale features to direct cell growth and cell–cell interconnections.

To obtain functional vascularized tissue constructs, many factors need to be considered and optimized, including process parameters (nozzle tip size, shear at nozzle, heat in ejection reservoirs and impact on the surface, etc) and material parameters (polymer viscosity, crosslink density, etc) [10, 88, 89]. Currently, whilst bioprinting is still at the primary stage of development, it is believed that this technology holds great promise in tissue engineering.

3.3. Photopatterning-based method

Methods based on photopatterning utilize light to selectively crosslink polymeric precursor solution or degrade bulk materials have been employed to fabricate customized structures. This approach has been applied to produce hydrogel tissue constructs with predesigned microfluidic

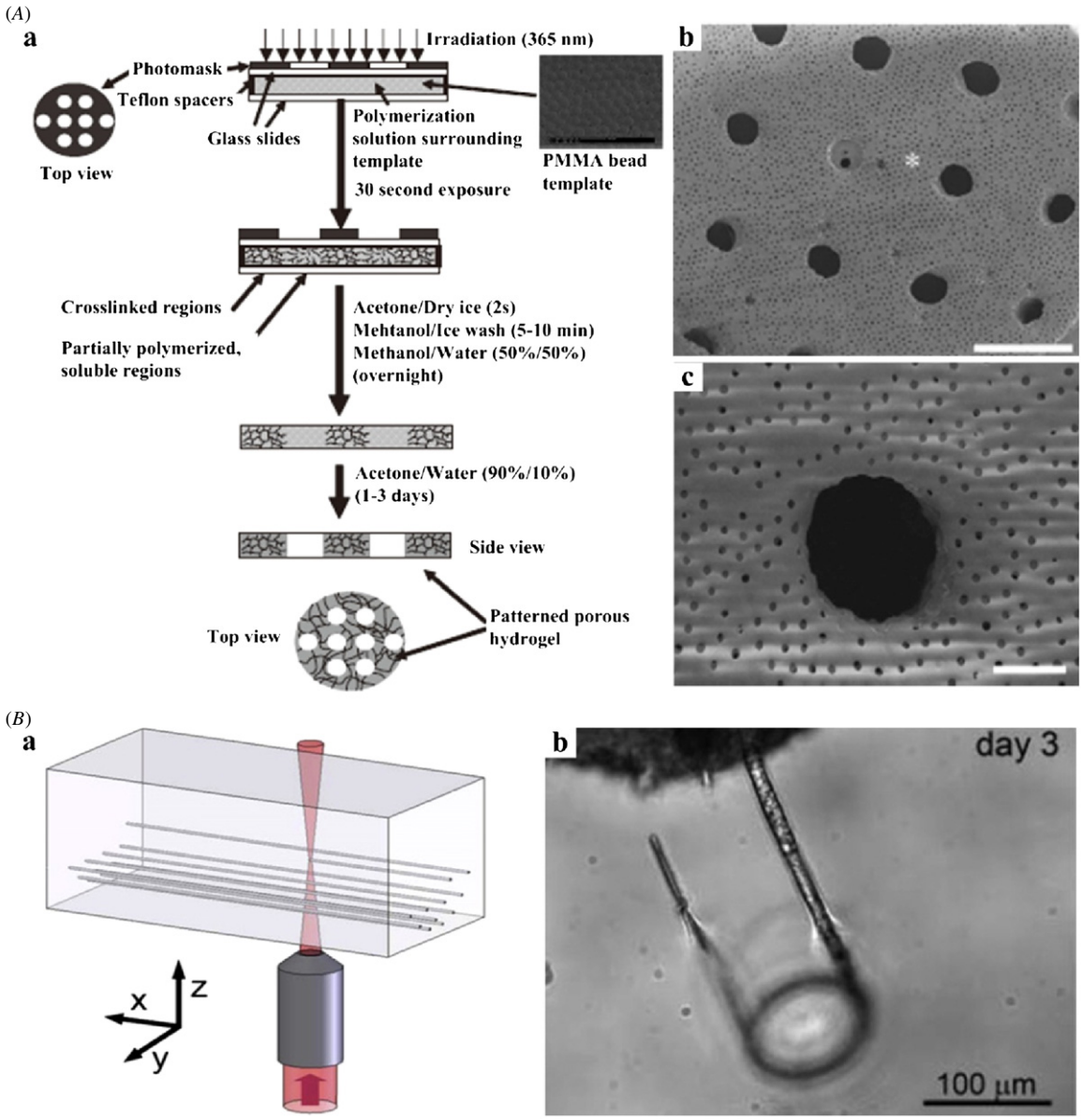

Figure 5. Fabrication of microfluidic hydrogels by photopatterning: (*A*) fabrication of microfluidic porous hydrogels by photopatterning porous poly(2-hydroxyethyl methacrylate) [93], (a) schematic of the fabrication process, (b, c) microfluidic porous hydrogels with pores (62 μm) and channels using photomask with channel diameter 200 μm. Scale bars are 1 mm (b) and 200 μm (c), respectively. (*B*) Fabrication of microfluidic hydrogels with guidance microchannels by laser ablation [28], (a) an illustration for creating microchannels in 3D hydrogels using laser ablation, (b) directed DRG cell growth in 3D microchannels created in PEGylated fibrinogen hydrogels.

channels [63, 90–92]. For example, Bryant *et al* [93] introduced parallel channels with different sizes (360–730 μm in diameter) into ~700 μm thick porous poly(2-hydroxyethyl methacrylate) by photopatterning (figure 5(*A*)). Given the limited penetration depth of light in hydrogels, the feasibility of this method in fabricating thicker microfluidic hydrogels needs to be further explored. Alternatively, using a layered photopatterning approach, Tsang *et al* [21] created polygonal channels in hepatocyte-laden PEG hydrogels. Nonetheless, this method may not be flexible for producing complex large tissues due to the need for photomask and multi-iterative procedures. Another promising photopatterning approach is stereolithography (SL) that enables rapid fabrication of 3D construct having complex architectures [29, 94, 95]. An example is the SL manufacture of cell-laden PEG hydrogels with internal bifurcating channels [96].

With soft lithography, bioprinting and SL methods, 3D microfluidic hydrogels are achieved through layer-by-layer assembly of 2D structures or on-demand printing of droplets. In contrast, photopatterning method based on the laser enables direct 3D patterning in bulk cell/hydrogel constructs via laser degradation or ablation of hydrogels [27, 97]; see table 2. Kloxin *et al* [27] created 3D channels via the laser degradation of cell-laden PEG hydrogels. Sarig-Nadir *et al* [28] demonstrated direct growth of dorsal root ganglion cells in PEGylated fibrinogen hydrogels, which contained

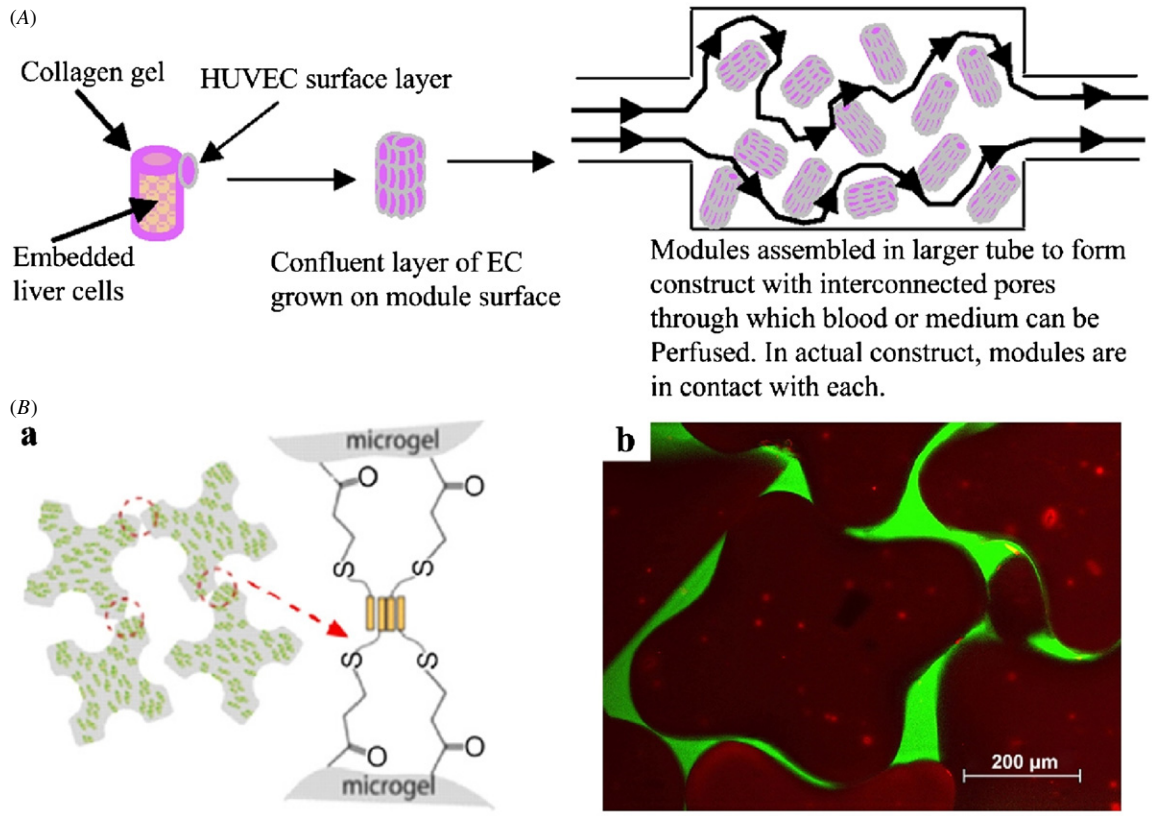

Figure 6. Modular assembly of cell-laden hydrogels: (*A*) schematic for assembling cell-collagen modulars into perfusable constructs with tortuous interconnected channels [64], copyright © 2006 by the National Academy of Sciences, (*B*) assembly of star-shaped cell-laden PEG microgels into porous constructs through a Michael-type addition reaction [19], copyright © 2010, reprinted with permission from Elsevier, (a) illustration of assembly of star-shaped microgels and (b) fluorescence image of assembled construct under perfusion. HUVECs, human umbilical-vein endothelial cells; EC, endothelial cells.

channels of different sizes that were created by *in situ* laser ablation (figure 5(*B*)). The versatility and efficiency of these laser-based approaches need to be further verified. In addition, the method based on photopatterning is limited to photosensitive hydrogels and extended exposures to UV or laser light may be deleterious to encapsulated cells.

3.4. Modular assembly

The method of modular assembly utilizes pre-fabricated building units to generate large constructs through manual, physical or chemical assembly procedures [98–100]. McGuigan *et al* [64, 101] fabricated microscale cylindrical collagen modules, which were encapsulated with human hepatoma cells and seeded with endothelial cells on the surface. These modules were then randomly packed into a large tubular chamber. The interstitial void space amongst the modules formed tortuous interconnected channels (figure 6(*A*)). The same group recently designed a microfluidic chamber to study construct remodeling and endothelium quiescence under perfusion through the tortuous interconnected channels [102]. A limiting factor of these collagen modules is that they have weak mechanical properties. To address this, gelatin modules were developed with improved mechanical properties to sustain high pressure and flow rate under perfusion [103]. In this method, glutaraldehyde crosslinking was used to prevent dissolution of gelatin, so that a significant loss of cell viability within the modules could be avoided [103]. Liu *et al* [19] introduced a Michale-type addition reaction to assemble cell-laden PEG microgels into integrated large constructs with empty space between neighboring modules (figure 6(*B*)). Amongst different shapes of microgels tested (e.g., circle, square, star), star-shaped PEG microgels offered comparatively higher perfusion for cells. Although the modular assembly method has the scalable potential, it is difficult to recreate the inner-architecture of complex tissues.

Other methods for fabricating microfluidic hydrogels include punch [104], crystal templating [105] and cell/hydrogel sheeting [106]. For instance, Bian *et al* [104] created 1 mm diameter channels by directly punching chondrocyte-seeded agarose disks of different diameters and thicknesses. However, this method is limited to fabricate only straight channels. Moreover, the mechanical forces during the punching process, such as shear stress, may induce cell death and cracking of hydrogels around the channel. Zawko *et al* [105] developed a 'crystal templating' technique to fabricate fibrillar HA scaffolds with dendritic networks. In this method, dendritic urea crystals grew up in HA solution films. After photocrosslinking HA, the crystal networks were dissolved in water to obtain dendritic channels. The main drawback of this method is that the crystal growth needs to be better controlled

and further optimized. Cell/hydrogel membranes/sheets have also been developed and rolled or stacked to form tubular or laminated tissue constructs for cell culture and vascular tissue engineering [106, 107]. In another interesting work, electrostatic discharge was used to instantaneously construct polymeric scaffold with complex 3D branched microvascular networks [108]. However, the feasibility of using this method to fabricate microfluidic hydrogels for tissue engineering still needs to be verified with further testing.

4. Computer modeling for microfluidic hydrogels

To maximize the metabolic density of constructs and best control the chemical microenvironment of cells, the parameters of microchannel networks (e.g., channel size, channel distribution) and culture conditions should be optimized [109–111]. Mathematical models offer an effective tool in the design and optimization of experimental systems. Predictive modeling can accelerate the development of experimental studies while reducing the cost.

A multitude of mathematical models have been proposed to characterize the diffusion of solute in hydrogels [112]. One typical approach is to build upon the porous media theory, considering the similarity of cell-laden hydrogels and porous media [113, 114]. For example, Song *et al* [115] developed a microfluidic cell-laden hydrogel model to study the influence of the nutrient concentration distribution on the viability of cells encapsulated in hydrogels. Recently, we investigated the optimized conditions regarding the number, size and distribution of open channels, as well as hydrogel properties for enhanced nutrient delivery [116]. This can provide a referenced framework for the design of microfluidic networks in cell-laden hydrogels.

It is important to control the culture conditions, such as concentration of perfused nutrient and perfusion velocity, to obtain maturated tissues *in vitro*. Computational fluid dynamic (CFD) simulation has been developed to model nutrient transport and analyze the effect of the flow rate [117]. For example, the asymptotic homogenization approach developed by Shipley *et al* can predict the nutrient/waste distribution in printed hydrogel tissue constructs and specify the design criteria, such as cell density, initial concentration of nutrient, scaffold geometry and mean flow velocity [118]. During tissue culture, clogging or collapsing of the channels along with tissue remolding is a noticeable phenomenon, which is likely caused by hydrogel swelling or cell-induced contraction [60, 104]. Computational modeling before experimental research may be useful to avoid channel clogging or collapsing so that desirable tissue constructs may be obtained.

5. Concluding remarks and perspectives

Microfluidic networks embedded within hydrogels can greatly enhance mass transport and enable controlled cellular chemical microenviroment in hydrogels. However, there are still several challenges to be addressed before hydrogel-based tissue constructs can be clinically applied. First, the ability to create hierarchically branched vascular networks and intra-complex architectures in hydrogels needs to be greatly extended. Second, the mechanical properties of hydrogels should be improved to sustain 3D microfluidics networks in tissue constructs having biologically and clinically relevant sizes. Current methods are limited to fabricate microfluidic hydrogels with either simple channel architectures or limited tissue sizes. Third, perfusable, interconnected channel networks do not sufficiently capture the function of human vascular systems. The functionalization of microfluidic hydrogels for tissue engineering applications not only requires the recreation of native tissue structures, but also the recapitulation of cellular responses under different stimulations (e.g., mechanical, chemical). Finally, it is challenging to integrate microfluidic networks in engineered tissue constructs with the host vascular system *in vivo*. Despite all these challenges, the emergence and optimization of new hydrogels and microengineering technologies will greatly promote the development of tissue engineering.

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