Channels in a porous scaffold: a new player for vascularization

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Vascularization is essential for tissue regeneration. Despite extensive efforts in the past decades, sufficient and rapid vascularization remains a major challenge in tissue engineering. Many studies have shown that the addition of channels in a porous scaffold can provide the ability to promote cell growth and rapid vascularization, thus leading to better outcomes in new tissue formation. Large size scaffolds lack perfusable channel networks and negatively impair the survival of transplanted cells and tissue function development, leading to necrotic core formation and the failure of functional tissue formation. Presently, there are many methods to produce channels in porous scaffolds for vascularization. Here, we review the function of channels in porous scaffolds and the approaches to produce those channels.

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Vascularization in tissue engineering

Synthetic tissue-engineered scaffolds hold great promise for tissue regenerative therapy without the undesirable side effects of autografts or allografts, such as limited supply, donor site morbidity, prolonged recovery and pathogen transfer of the grafts [1–3]. Tissue-engineered scaffolds have been extensively studied in the past decades [4–6]. However, insufficient vascularization in the entire implanted scaffolds hinders the successful transplantation of porous scaffolds for bone, cartilage and cardiac tissue repair [7–11].

Several strategies have been developed for enhancing rapid vascularization and instant blood perfusion in implanted scaffolds [12–14]. These methods include using angiogenic growth factors [7,15] and coculturing pericytes and endothelial cells on scaffolds [16–18]. Although these strategies can initialize angiogenesis and growth of blood vessels, they can only promote blood vessels to grow a short distance from the surface of the implants. New blood vessels grow too slowly to provide instant blood perfusion to the center of the implanted constructs [7]. One major hindrance may be the spatial challenges posed by porous scaffolds, which prevent host vasculature and nutrient transportation from invading and diffusing rapidly into the center zone [19]. Although interconnected pores are essential, and are also required to facilitate nutrient diffusion and cell migration, pores alone cannot provide enough space and a clear path, and are insufficient for the in-growth of host vasculature and nutrient diffusion [19,20]. The vascular system provides most tissues in our body with a supply of nutrients and oxygen and ensures proper waste removal. Considering that the maximum distance between capillaries is around 200 μm [21], oxygen and nutrients cannot be sufficiently supplied to a large-sized construct through pores alone, which leads to necrosis and tissue death in the center of the implanted construct [22–24]. When cells grow beyond the diffusion range of oxygen, hypoxia initiates the invading of new blood vessels. However, blood vessels require several weeks to invade a millimeter-sized implant for complete vascularization. In this case, pores and biological growth factors are not sufficient. Scaffold structures like channels imitating blood vessels or facilitating the invasion of blood vessel become critical [7,25]. In this paper, we review the importance of channels in a porous scaffold for tissue regeneration.

The theoretical support of using channels for vascularization

Tissue engineering strategy utilizes a porous scaffold, growth factor and cells for tissue regeneration [26]. The prerequisite condition for success is the harmonious cooperation of the three components. However, despite the recent significant progress in tissue engineering, many challenges stand between the current porous scaffold and its therapeutic implementation in humans. One of the most critical issues in cell survival and growth is insufficient mass transport, which leads to slow diffusion of oxygen and nutrients in the center of a thick porous scaffold [27,28]. The low diffusion ability of a thick porous scaffold makes cells in the central region suffer from insufficient nutrient and oxygen supply, and waste products accumulate within the scaffolds, which cause cell death or heterogeneous cell distributions in the scaffold [29,30].

Channels have the potential to regulate the distribution of chemicals in porous scaffolds. Mashayekhan and colleagues established a mathematic model to elucidate the theoretical basis of the role of channels in cell growth [31]. The simulation results based on the mathematical model showed that oxygen concentration decreased significantly along with the length of the construct, but with the increase of the channel numbers, the average cell density increased by 30% of the initial seeded cell density. Their model demonstrated that the increase of the channel number within the scaffold significantly increased the cell density. Another simulation model has been established to investigate the effect of a parallel channel array in a porous poly(glycerol sebacate) scaffold on the oxygen distribution in the construct [32]. The parallel channel array mimics the *in vivo* capillary tissue bed. Results showed that the channel was favorable for high cell density. In the channel, the primary modes of mass transfer were axial convection and radial diffusion. In the tissue region, the mass transfer was axial and radial diffusion [32]. In Khademhosseini and colleagues' study, they engineered a 3D model for cell-laden microfluidic channels [33]. Their model system simulated theoretical nutrient diffusion in a cell-laden hydrogel. They compared the spatial distribution of viable cells within the 3D hydrogel without channels and with single- and dual-perfusion microfluidic channels. Results found that, no matter how many channels were in the hydrogel construct, channels increased nutrient and oxygen transport as a way to improve cell viability, compared with the hydrogel construct without channels. This theoretical model demonstrated that there was a close correlation between diffusive nutrient distribution and cell viability throughout the hydrogel construct. The simulation results showed that it is necessary to have channels spaced closely to avoid oxygen transport limitations, as the oxygen concentration was higher in the lumen of the parallel channels than that in the surrounding space [34]. These mathematic models successfully simulated the effect of channels on oxygen distribution in the porous scaffolds, which resulted in a higher cell density in the channel space. Therefore, channels played a promoting role in cell growth and vascularization.

Channel types in different porous scaffolds

Single/multiple channels in hydrogels

To provide sufficient oxygen and nutrients to a tissue-engineered construct, many studies made efforts to mimic the lumen structure of blood vessels by creating microvessel-like channels in the constructs. Takei *et al.* used alginate hydrogel microfibers to assemble vascular-like microchannels [35]. Their study found that the cell culture medium could flow through the channels quickly (within 10 min) after the immobilization of the cells in the assembly gel. The human hepatoblastoma cells proliferated in the gel portions of the microfibers and maintained their specific function during the perfusion culture of 7 days. The novel mimicked vascular-like networks seemed to have the potential to allow the creation of engineered tissues *in vitro*. When implanted *in vivo*, the channeled hydrogel promoted cell infiltration. For the survival of engineered tissues *in vivo*, vascularization is critical, and channels, as a physical structure, provide the entrance for the in-growth of blood vessels. This conclusion was further confirmed in Mao and colleagues' study [36]. They created three microchannels with 1 mm of diameter in poly(ethylene glycol) diacrylate (PEG) hydrogel cylinders. The results from the *in vivo* subcutaneous implantation experiment indicated that there was no obvious infiltrating host tissue observed if PEG hydrogel did not contain microchannels. On the contrary, host tissue infiltration was apparently observed when PEG hydrogel cylinders contained three microchannels. Interestingly, the host tissue infiltration was found primarily in the lumen of the microchannels, but scarcely in the rest of PEG hydrogel. The infiltrating host tissue included vascular structures with erythrocyte-filled blood vessels that were lined by endothelial cells [36]. This study directly showed the importance of microchannels in a hydrogel construct for *in vivo* vascularization. In another study, microchannels in a hydrogel promoted nerve repair. The results of the study showed that the multiple channels in the scaffold-guided axonal regeneration [37]. The tissue regenerative ability may result from the functionality of vascular-like microchannels, which facilitated the mass transport. Khademhosseini and colleagues' study proved that this kind of functionality

Figure 1. Microchannels in a hydrogel construct prepared by using the combination method of a sacrificial agarose material template and 3D bioprinting. Branch channels (A) and lattice channels (B) in a gelatin-modified hydrogel (scale bars = 3 mm). A gelatin template without acetone wash, displaying three dimensionality of the network (C & D). (A & B) Re-edited and reproduced with permission from [38]. (C & D) Re-edited and reproduced with permission from [44].

Figure 2. Channels in a β -tricalcium phosphate scaffold made by the mechanical removal spacer method. Schematic diagrams show the assembly procedures of a microchannel in a type I collagen hydrogel and in a β -TCP graft by using a cell electrochemical detachment technique. (A) A fluorescent image of HUVECs on an oligopeptide-coated rod, (B) a bright-field image of the microchannel in the collagen hydrogel (top view), (C) a fluorescent image of the microchannel in the collagen hydrogel (top view), (D) a fluorescent image of networks sprouting from the microchannel into the surrounding area of the graft (scale bar = 100 μ m). β -TCP: β -Tricalcium phosphate scaffold; CED: Cell electrochemical detachment; HUVEC: Human umbilical vein endothelial cell.

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of fabricated vascular networks embedded in hydrogels. In Khademhosseini and colleagues' study, the vascular-like microchannels were fabricated by 3D-bioprinted sacrificial materials and embedded in a hydrogel (Figure 1A–D). This kind of microchannel promoted mass transport, cellular viability and differentiation [38].

Microchannels in a hydrogel construct provide not only a tubular structure to facilitate nutrition transport but also a luminal tube for endothelial cells to align on the wall surface to form an endothelium (Figure 2) [39–41]. Thus, a microchannel becomes a functional vascular network, not just a physical support. The cells attached to the wall of the microchannels in a hydrogel construct grew and invaded into the hydrogel to form new networks for vascularization.

Besides parallel microchannels array or single/dual straight channels in hydrogels, branched microchannels and complex microchannels networks had been developed to mimic vascular system (Figure 1C & D) [42–44]. A complex 3D vascular-like multiscale system that was composed of larger vascular perfusion channels promoted the growth of a capillary network and provided a feasible and promising approach to address the vascularization issue [45]. The multiscale vascular system fabricated by using a 3D bioprinting technique significantly promoted the formation of a capillary network [45]. Additionally, the bioprinting technology allowed cells to be easily encapsulated into the 3D matrix within a scaffold at the desired location [46].

Single/multiple channels in polymer/ceramic porous scaffolds

Many studies focused on embedding microchannels in cross-linked hydrogels, as hydrogels are biocompatible and shapeable to encapsulate cells. Based on these advantages of a hydrogel, the addition of channels further enhances the function of the hydrogel in vascularization. For polymers or bioceramic porous scaffolds, hollow channels also brought a promising platform for integrating vascularization strategies in a single construct. For example, in a calcium phosphate cement material, interconnected hollow channels exhibited favorable characteristics for vascularization [47]. The degree of vascularization *in vivo* was different based on different channel sizes. Interconnected multiscale channels promoted the ingrowth of blood vessels from host to scaffold through channels [47]. Apparently, the scaffold with micropatterned channels favored the penetration of blood vessels throughout the newly formed tissue, as well as in the surrounding host tissue [48]. Kaplan and colleagues' studies found that multiple channels in porous silk scaffolds promoted the survival of transplanted cells *in vivo* [49]. For acellular scaffolds, hollow channels with 254 μm diameter not only improved endothelial cell survival and *in vitro* prevascularization but also promoted *in vivo* initial integration and tissue ingrowth. Coculturing endothelial cells in the hollow channels additively promoted rapid vascularization and perfusion *in vivo* and synergistically promoted tissue ingrowth [49]. These results proved that silk scaffolds with multiple channels of 254 μm had the functionality to promote tissue regeneration. These channels also provided conduits for medium perfusion and efficient transport of oxygen to the cells, by a combination of perfusable flow and molecular diffusion over short distances between the channels. In another study [50], a porous elastomeric scaffold with an array of channels promoted high efficiency ($77.2 \pm 23.7\%$) of cell seeding when seeded with neonatal rat cardiac myocytes. Uniform spatial cell distributions were obtained when the scaffold had channels for perfusion seeding.

Channels with cells can be utilized to engineer thick tissue-engineered constructs. As indicated in our previous published studies [39], we used our template-casting method to create a single macrochannel (3 mm) in a porous β -tricalcium phosphate (β -TCP) scaffold, and integrated a cell-lined collagen channel inside (Figure 2). The channeled β -TCP scaffold integrated with cell-lined collagen microchannel was implanted in the subcutaneous pocket of a mouse. Interestingly, we found that host vasculature first grew into the channel to form blood flow and then into the other surrounding porous areas of the scaffold (8 mm diameter). Interestingly, it took much longer for a porous scaffold without a channel to form blood flow [39]. These results suggest that a channel efficiently improved the nutrition diffusion and blood flow to the central zone of the porous scaffold and facilitated sufficient vascularization [39,51]. Based on these results, it is plausible to conceive that an array of hollow macrochannels in a porous scaffold could robustly facilitate nutrient transportation and instant blood perfusion. In our new study [52], we implanted porous β -TCP scaffolds with multiple channels (1 mm diameter each channel) into mandibular bone defects of beagle dogs. Results showed that the vertical hollow channels accelerated the degradation of the β -TCP scaffolds. More volume of newly formed bone tissues with more blood vessels were augmented in the channeled scaffolds. Channeled scaffolds significantly promoted the new bone formation and increased the height of the mandible. These findings indicated that channeled scaffolds facilitated vascularization and bone formation [52].

Although the size and number of the channels in different scaffolds were different, all these studies showed that channel played a critical role in vascularization. Channel types related details are listed in Table 1.

Approaches of channel fabrication

To create perfusable channels in hydrogels, polymers or ceramic scaffolds, many strategies have been developed.

Mechanically removable templates

Using templates or removable rods can generate defined channels. The channel diameter and space in hydrogels, polymers and ceramic porous scaffolds can be controlled by the dimensions of the templates [39,49,53]. Figure 3 shows the schematic procedures for the preparation of channels in a scaffold using a template of spacers. Kaplan and colleagues used linear wire arrays to make porous silk scaffolds with 254 or 508 μm diameter of vertical channels [53]. Kang and colleagues used templating rods to make β -TCP scaffolds with five channels of 1 mm diameter [52]. The template spacer strategy showed the potential to not only create channels in the porous scaffold but also provide the structure for cells to align in the formatted channels [54]. To vascularize a tissue-engineered construct, endothelial cells are commonly used. To align endothelial cells on the wall of the channels, cell suspension was injected into the formed channels, and endothelial cells then attach on the inner wall surface of channels. The attached cells in the channels can grow and sprout out into the surrounding area of hydrogels for the vascularization of the entire construct. Fukuda and colleagues developed a unique technique to realize the creation of channels and

Table 1. Channel types in different scaffold types and their functions.

Channel type (number, size)	Scaffold type (porous size)	Function results	Refs.
200, 50 μm	Alginate hollow microfibers	Microfibers maintained their specific function of HepG2	[35]
3, 1 mm	PEG hydrogel	Host cell infiltrated into the lumen of the channel not in the other part	[36]
7, 250 μm or 14, 500 μm	Agarose/collagen fibers	Microchannels promoted fascicular-like nerve repair	[37]
6, 500 μm	Methacrylated gelatin hydrogel	Form an endothelium aligned in the wall surface of the channel	[38]
1, 600 μm	Collagen hydrogel	Form functional vascular networks	[39–41]
Branches, 500 μm	Alginate hydrogel	Perfusable channel for vascularization	[42–44]
2, 1 mm	Fibrin hydrogel	Multiscale vascular network and capillary formed within a fibrin	[45]
130 tetrahedrons, 1–2 mm	Polycaprolactone (100–200 μm)	Support liver cells' growth and function	[46]
2, (255 and 507 μm)	Calcium phosphate cement	Pre-established vascular networks in CPC material anastomose with host vessels	[47]
500 μm	Chitosan scaffold (600–900 μm)	Micropatterned scaffolds enhanced neovascularization	[48]
Array, 254 μm	Silk scaffold	Promoted vasculature in-growth	[49]
Array, 250 μm	Poly(glycerol sebacate) scaffold (75–150 μm)	Preferential cell attachment at the channel surfaces	[50]
5, 1 mm	Tricalcium phosphate scaffold (500 μm)	Promoted blood vessels and bone formation	[51,52]

CPC: Calcium phosphate cement; PEG: Poly(ethylene glycol).

Figure 3. A schematic graph shows the experimental protocol for the vascularization of hollow channeled porous silk scaffolds. Host tissue rapidly infiltrated into the prefabricated arrayed channels. The *in vitro* incubated vascular networks rapidly anastomosed with the infiltrated host blood vessels. Re-edited and reproduced with permission from [49].

cell alignment of the channels in a hydrogel construct in one step (Figure 4) [55]. Their approach involves using a glass rod to create a microchannel in a hydrogel [40,56]. A glass rod with a defined diameter was coated by a gold layer, then immersed in a peptide solution. The sequence of the peptide was particularly designed. Endothelial cells were seeded onto the surface of the peptide-coated rod. The cell-rod was then inserted into a mold and a collagen solution was casted into the mold. After the collagen solution was solidified, an electric current was applied onto the rod. The electric current cleaved the peptide, resulting in cell detachment. The detached cell layer was transferred to the surrounding wall of the channel in the hydrogel at the same time. The rod was then removed, leaving endothelial cell-lined vascular-like channels structure that was precisely aligned in the channel wall within the hydrogel [40,57].

‘Bottom-up’ modular technique

Besides microchannels alone in a hydrogel construct, cells can be encapsulated into the hydrogel to vascularize the entire construct. In this case, the channels in the hydrogel facilitate the nutrient transport to the encapsulated cells and the infiltration of host cells. In Khademhosseini and colleagues’ study [58], a ‘bottom-up’ tissue engineering approach was used to build cell-laden microengineered hydrogel (microgel) constructs embedded with vascular-like microchannels. Endothelial cells and smooth muscle cells were encapsulated into the microgel units with spatial arrangement mimicking the vascular physiology, which were then assembled into a blood vessel-like microchannel structure. Such a ‘bottom-up’ micromodular technique was often used to fabricate assembly microchannels within cell-laden hydrogels [38].

Figure 4. A schematic graph shows the fabrication procedure of a thick microchanneled fibrin hydrogel in a custom-made aluminum chamber. Gold-coated rods ($\varnothing$ 600 μm) attached with HUVECs were placed in a Teflon mold (8 mm $\times$ 6.35 mm) and hydrogel was casted. After gelation, HUVECs were electrically (-1.0 V) transferred to the microchannels within the hydrogel. A three-hole PDMS cap was placed on top of the construct and rods were removed.

HUVEC: Human umbilical vein endothelial cell; MSC: Mesenchymal stem cell; PDMS: Polydimethylsiloxane. Re-edited and reproduced with permission from [41].

Figure 5. A schematic diagram shows the fabrication of microfluidic gels. Sealing a PDMS stamp to a substrate (glass or preoxidized PDMS) created a microfluidic network. Sequential introduction of a pluronic (1–6% in PBS) and a liquid gelatin into the channels, and gelation at 4°C for 15 min and at 23°C for 2 h, yielded a gelatin mesh that was easily separated from the channels. Encapsulation of the mesh in a liquid hydrogel precursor (type I collagen, fibrinogen, Matrigel), followed by polymerization of the precursor and flushing at 37°C, yielded a hydrogel with open microchannels.

PBS: Phosphate-buffered saline; PDMS: Polydimethylsiloxane.

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Sacrificial template

Although removable template spacers can be involved in producing channels in a porous scaffold or a hydrogel construct, this method can create only unbranched channels, which do not resemble the *in vivo* vasculatures. To mimic vascular-like branched channels, a sacrifice template method has been developed and widely used in the field [59]. This technique utilizes a sacrifice vascular-like model to form perfusable-branched microchannels. The structure of the template mimics the complex network of blood vessels. After the template was made, the precursor solution of a hydrogel was cast and then cross-linked. The template was then melted or dissolved, leaving branched microchannels inside the hydrogel [38,60]. For example, alginate serves as a sacrificial material and then collagen hydrogel was cast into the mold. Alginate material was then dissolved, leaving a complex channel network in the cross-linked collagen hydrogel [61]. Besides alginate materials [62], gelatin often serves as a sacrificial material for channel production, as gelatin material is a solid state at low temperature and becomes liquid at body temperature [47,63]. Utilizing this advantage of a gelatin material, gelatin can be made into all kinds of vascular-like branches for perfusable channels. For example, gelatin can be made into mesh network for channeled collagen constructs (Figure 5) [63]. Gelatin can be made into gelatin fibers and embedded in calcium phosphate cement and incubated [47]. Hollow-channeled calcium phosphate cement was then produced *in vivo* after the gelatin fibers dissolved. This channel fabrication methodology was reproducible and straightforward. These studies showed that sacrificial template method has been widely used to build perfusable vascular-like architectures in

Figure 6. 3D-printed microchannels in hydrogel and ceramic scaffolds. A fabrication process of a 3D alginate structure with built-in microchannels (A); A procedure shows the fabrication of channeled bioceramic scaffolds: mixing bioceramic powders with F-127 solution (concentration: 20 wt%) and alginate, printing the bioceramic inks via the coaxial nozzle (B).

(A) Re-edited with permission from [66].

(B) Re-edited and reproduced with permission from [69] © American Chemical Society (2015).

many different types of hydrogels [43,64]. However, most of these sacrificial templates had a 2D plane-connected network, not a 3D network (Figure 5). A new fused-deposit extrusion 3D filament printing system was developed to fabricate a hydrogel construct with a 3D perfusable-connected network. The perfusable channel network was molded into tree branches [42,65]. Once a complex-branched sacrificial template is printed, hydrogels were casted and then the sacrificial templates were melted or dissolved to form branched vascular-like channeled constructs for vascularization.

3D bioprinting

Although sacrificial template can create microchannels in hydrogels, several steps are required to build up the templates and then make channels. New 3D bioprinting technology has been developed to directly produce channeled scaffolds for tissue engineering. For example, using coaxial nozzle-assisted 3D bioprinting to print hollow alginate fibers for built-in microchanneled scaffolds has been developed (Figure 6A) [66]. Using 3D printing technique to produce porous hydroxyapatite and TCP blocks with a central channel had also been successfully developed for vessel pedicle or nerve integration [67]. A rapid prototyping technology, fused deposition modeling, was designed to produce porous poly(epsilon-caprolactone) scaffolds. The scaffold had a honeycomb-like pattern, fully interconnected channel network and controllable porosity and channel size [68]. In a recent study, Wu and colleagues presented a facile method using coaxial 3D printing strategy to prepare hollow strut-packed bioceramic scaffolds with designed macropores and multioriented hollow channels (Figure 6B) [69]. The hollow channels were not in the bulk of the porous scaffolds but in the struts. The unique hollow-struts structure of the bioceramic scaffold significantly improved cell attachment and proliferation and further promoted the formation of new bone tissue in the center of the scaffolds for large bone defects. Therefore, 3D bioprinting, as a new channel producing method, has become a very promising method to produce new channeled porous scaffolds.

From these studies, it can be seen that different approaches have their advantages and drawbacks (Table 2). According to different properties of the materials, an appropriate approach need to be considered to produce a channeled scaffold for a particular tissue regeneration application.

Table 2. Fabrication approaches of channels.

Methods	Channel size	Advantages/drawbacks	Ref.
Removable template	254, 508 μm	Controllable size/only straight, unbranched	[53]
Templating rods	1 mm	Changeable rods/defined size	[52,54]
Micromold	600 μm	Cell embedded/complicated procedures	[55–57]
Bottom-up	900 μm	Assemble cells into vascular-like shape/difficult to handle	[58]
Sacrificial template	1.16, 0.60 and 0.30 mm	Can make different channels with sizes and shapes/multiple steps	[59–62]
Gel networks	Variable sizes	Vascular-like branched networks/uncompleted removal of the sacrifice gels	[63–65]
3D printing	160–800 μm	Rapidly print built-in channels/need customized printers	[66–69]

Conclusion & future perspective

Sufficient and rapid vascularization of synthetic porous scaffolds is still the key challenge in tissue engineering. Numerous approaches have been developed in the last decade to overcome this problem. Although new technologies and remarkable intermediate aims have been recently achieved, the generation of a functional vascularized tissue is still an unreached vision. The development of the channels in a porous scaffold represents a breakthrough and holds a great promise for vascularization as a new player in vascularization of a scaffold. Channel as a biophysical cue promotes the nutrition transport and rapid removal of wastes, which accelerates cell growth and vascularization. Although many efforts on the development of new reproducible and facile technologies are still needed, new 3D bioprinting technology or the combination of other approaches would bring new promising potentials to create porous scaffolds with vascular-like biomimetic channel structure for functional vascularization.

Executive summary

- Many strategies had been developed to address the key challenge.
- Channels in porous scaffolds provide a physical cue for vascularization.
- Channels promoted cell migration, proliferation and infiltration.
- Channels opened unblocked paths and provided spatial paths to promote nutrients transportation and blood supply, thus facilitating the vascularization of a porous scaffold.
- Numerous approaches have been developed to fabricate channels or microchannels in a porous scaffold.
- Mechanically removable template is the most extensively used approach to make micro-/macrochannels in a porous scaffold, but it is challenging to make branched channels.
- Sacrifice template is a facile method to make branched microchannels.
- 3D bioprinting technologies have been developed to print complex microchannels in scaffolds. 3D printing techniques not only produce branched, complex sacrifice templates for channels but also print channeled scaffolds directly.
- Channels can promote vascularization, which will bring a promising potential for tissue regeneration.
- With the combination of other players in vascularization, micro-/macrochannels become a new player in vascularization of tissue-engineered scaffolds.

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