Engineering 6 (2020) 1244–1257

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Medical Additive Manufacturing—Review

Microfluidics for Medical Additive Manufacturing

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ARTICLE INFO

Article history:
Received 10 June 2019
Revised 23 January 2020
Accepted 5 May 2020
Available online 8 October 2020

Keywords:
Microfluidics
Biomaterial
Additive manufacturing
Droplet
Fiber

ABSTRACT

Additive manufacturing plays a vital role in the food, mechanical, pharmaceutical, and medical fields. Within these fields, medical additive manufacturing has led to especially obvious improvements in medical instruments, prostheses, implants, and so forth, based on the advantages of cost-effectiveness, customizability, and quick manufacturing. With the features of precise structural control, high throughput, and good component manipulation, microfluidic techniques present distinctive benefits in medical additive manufacturing and have been applied in the areas of drug discovery, tissue engineering, and organs on chips. Thus, a comprehensive review of microfluidic techniques for medical additive manufacturing is useful for scientists with various backgrounds. Herein, we review recent progress in the development of microfluidic techniques for medical additive manufacturing. We evaluate the distinctive benefits associated with microfluidic technologies for medical additive manufacturing with respect to the fabrication of droplet/fiber templates with different structures. Extensive applications of microfluidic techniques for medical additive manufacturing are emphasized, such as cell guidance, three-dimensional (3D) cell culture, tissue assembly, and cell-based therapy. Finally, we present challenges in and future perspectives on the development of microfluidics for medical additive manufacturing.

1. Introduction

Additive manufacturing, also known as a solid freeform fabrication technique, is defined as "a process of joining materials to make objects from three-dimensional (3D) model data, usually layer upon layer, as opposed to subtractive manufacturing methodologies" [1,2]. Since the first 3D structure was created using a layer-by-layer technique in the 1980s, additive manufacturing has emerged as a novel and powerful tool in both industry and scientific research [3,4]. Through additive manufacturing, complex structures can be built from a variety of materials, including polymers, metal, ceramic, biomaterial, and organic compounds, with various properties [5–8]. It has been extensively applied in many fields, including the food, mechanical, pharmaceutical, and medical fields [8–11]. Among these areas, medical additive manufacturing has led to especially obvious improvements in medical instruments, prostheses, implants, and so forth based on the advantages of cost-effectiveness, customizability, and quick manufacturing. Many methods have been developed for medical additive manufacturing, such as selective laser sintering, electrohydrodynamic jetting, fused deposition modeling, 3D printing, and microfluidics [12–15].

Among these strategies, microfluidics is the technology of controlling fluids (usually 10^{-9} to 10^{-18} L) in systems with micro-scaled channels (tens to hundreds of micrometers) [16–20]. Because of its ability to control fluids precisely, the microfluidic technique has emerged as a promising and versatile approach for fabricating droplets or fibers with complex structures [21–24]. By assembling or stacking the droplet/fiber templates, various 3D constructions with different structures and material components can be achieved. In addition, biocompatible cell constructions can be fabricated by encapsulating or loading cells in droplet/fiber templates [25–27]. Benefiting from the features of precise structure control, high throughput, and good component manipulation, microfluidic techniques present distinctive advantages in medical additive manufacturing and have been applied in the fields of drug discovery, tissue engineering, and regenerative medicine [28–30]. Thus, a comprehensive review on microfluidic techniques for medical additive manufacturing is helpful for scientists with various backgrounds.

This critical review provides an overview of recent developments in microfluidic techniques for medical additive

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https://doi.org/10.1016/j.eng.2020.10.001

manufacturing. We start by demonstrating the generation of building microcarriers based on single, double, and multiple emulsion droplets from microfluidics. Next, we describe the microfluidic fabrication of fibers with different structures, as well as their weaving, stacking, and winding. We then focus on the diverse applications of the generated microcarriers or microfibers in the field of medical additive manufacturing, such as cell guidance, 3D cell culture, tissue assembly, and cell-based therapy. Finally, challenges and future perspectives on the development of microfluidics for medical additive manufacturing are discussed.

2. Microfluidic droplets for medical additive manufacturing

Microfluidics is a technique for the systematic manipulation and control of fluids in micro-scaled channels. Since the advent of microfluidics in the 1970s, this multidisciplinary technology has developed rapidly and has gradually penetrated into many fields, including biology, microelectronics, medicine, materials, chemistry, and machinery [31–36]. The fluids in microfluidic systems are characterized by highly efficient mass–heat transfer, a relative dominance of viscous over inertial forces, and significant surface effects, making the microfluidic control of fluids and fluid interfaces important and practically useful. In addition, the highly integrated nature of the microfluidic system promotes the coexistence and interactions of multiphase fluids, enabling the construction of complex fluid systems [27,33,37,38]. Thus, the microfluidic technique is considered to be a promising method for achieving constructions with complex morphologies and structures.

2.1. Mechanism of droplet microfluidics

As an important branch of microfluidic technology, droplet microfluidics introduces immiscible multiphase fluids (dispersed phase and continuous phase) in microfluidic channels and breaks off the fluid into discrete droplets [21,39]. These discrete droplets have the advantages of monodispersity, small volume, controllable size, stable generation conditions, and a confined environment without pollution. Therefore, droplets generated from microfluidics can be used as ideal templates or microreactors, and thus have important value in materials fabrication, chemical synthesis, cell culture, and other applications.

A variety of materials can be used for the construction of microfluidic chips, such as glass, crystalline silicon, quartz, poly (dimethylsiloxane) (PDMS), plastics, and the like [40–43]. Among these materials, the most commonly used are PDMS and glass capillaries, as presented in Figs. 1(a) and (b) [42,43]. The first PDMS microfluidic chip was prepared by Whitesides in 1998 [44]. To fabricate PDMS devices, the following steps are typically required: First, a master containing a positive structure in a photoresist is generated by standard contact photolithography (Figs. 1(a-i–iii)); the replica containing the channels is produced by subsequently pouring mixed PDMS onto the master (Figs. 1(a-iv) and (a-v); finally, the PDMS replica is bonded on a flat substrate (Fig. 1(a-vi)). Due to the advantages of low cost, easy molding, and good biocompatibility and transparency, PDMS devices have been widely promoted. Nevertheless, their two-dimensional (2D) characteristics, swelling and deformation in organic solvents, and high-pressure intolerance limit their applications. Glass capillary-based devices have been introduced with true 3D geometries and superior solvent resistance [42]. These devices consist of coaxially assembled glass capillaries (Fig. 1(b)), with easily and precisely modified wettability of the channel surfaces. Droplets with single, double, and multiple emulsions—or even more complicated structures such as multi-compartments—can be achieved by assembling a series of glass capillaries (Figs. 1(b-ii) and (b-iii)) or using capillary arrays (Fig. 1(b-i)). Even with these advantages, such glass devices are limited in certain situations. For example, the manual fabrication of glass capillary devices restricts their production quantity at a time. Therefore, different microfluidic devices should be chosen to adapt to various situations.

In microfluidic channels, immiscible fluids meet at the junction and droplets are formed under hydrodynamic forces. The size, velocity, and production frequency of the droplets are determined by the flow rates, geometric parameters, and fluid properties. Based on the channel geometries and fluid configurations, different droplet-formation techniques have been developed, which can be classified into terrace-like, cross-flow, co-flow, flow-focusing geometries, and so forth [18,27,45]. Typical microfluidic geometries used in generating droplets are shown in Fig. 1(c) [45]. The terrace-like geometries generate uniform droplets by variations of channel confinement, through which the capillary pressure changes sharply. Driven by the interfacial tension, the dispersed phase fragments into droplets at the end of the channel. In the cross-flow category (frequently implemented as a T-junction),

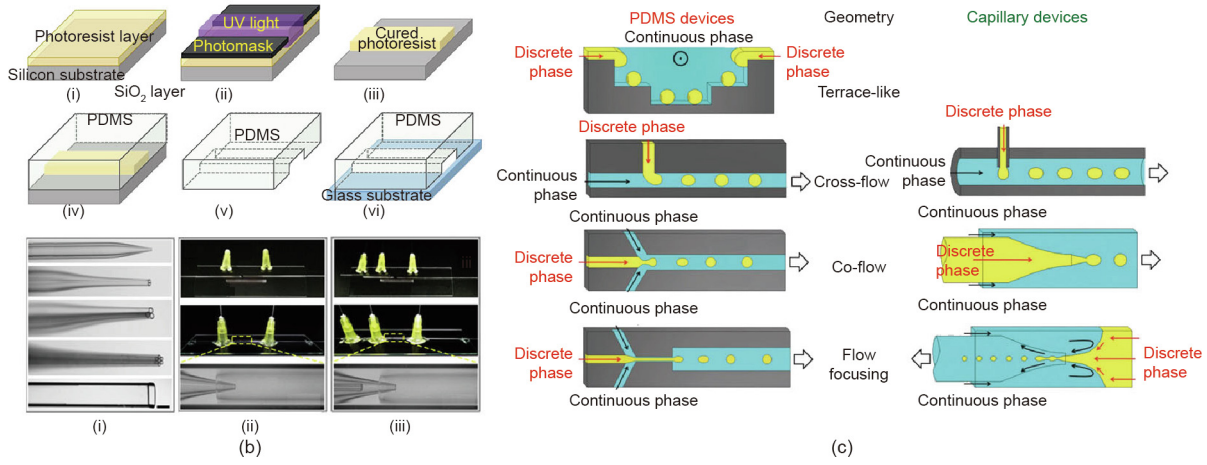

Fig. 1. Microfluidic devices and droplet formation techniques. (a) The fabrication steps of a PDMS microfluidic device; (b) the assembly of glass capillary microfluidic chips; (c) different microfluidic geometries for generating droplets in PDMS and glass capillary chips. UV: ultraviolet. (a) Reproduced from Ref. [43] with permission of Springer Nature Limited, ©2018; (b) reproduced from Ref. [42] with permission of Springer Nature Limited, ©2018; (c) reproduced from Ref. [45] with permission of American Chemical Society, ©1998.

the two fluids meet at an angle ranging from 0° to 180° and form an interface at the junction. Shear forces cause the dispersed phase to enter the continuous fluid channel and subsequently break into droplets due to the pressure gradient. Co-flow streams are achieved with a set of coaxial channels, which can either be quasi-2D planar (PDMS) or 3D (glass capillary) coaxial. Similar to co-flow geometries, the two phases flow coaxially through a narrow region in flow-focusing chips. With the function of shear-focusing in the contraction orifice, the droplets are generated in a smaller size than in the co-flow configuration.

2.2. Single-emulsion and multi-compartmental droplets

Cell microcarriers with designed sizes, structures, and microenvironments have been explored based on different microfluidic droplet templates. In the simplest case, by infusing biocompatible materials into microfluidic channels, monodispersed single emulsions can be generated, which can be subsequently polymerized to achieve microcarriers for cell culture. Liu et al. [46] developed a new type of photonic crystal (PC) microcarrier with controlled size and good biocompatibility by dispersing silica nanoparticles in single-droplet templates. Cells cultured on the surface of the microcarriers have a more 3D morphology in comparison with 2D planar culture (Fig. 2(a-i)), resulting from the features of the cell suspension culture on microcarriers. In addition, Zheng et al. [47] further developed the functions of the PC microcarriers for capturing, detecting, and releasing multiple kinds of circulating tumor cells (CTCs) by decorating the surface with aptamer probes, as shown in Fig. 2(a-ii).

Besides silica, hydrogel materials are widely used to fabricate cell microcarriers, such as alginate [48], gelatin [28], collagen [49], poly(ethylene glycol) [50], and chitosan [51]. These kinds of microcarriers can be cured through various methods including ionic crosslinking, temperature-induced gelation and freezing, and ultraviolet (UV) irradiation. The size, shape, porosity, and mechanical properties of the microcarriers can be tuned by adjusting the microfluidic channel size, flow rate, precursor concentration, and crosslinking density. Segura et al. [52] demonstrated a microfluidic approach to fabricate injectable microcarriers that were mainly composed of multi-armed poly(ethylene) glycol-vinyl sulphone (PEG-VS) backbones and cell-adhesive peptide (Fig. 2(b)). The cells adhered to the surface of the microcarriers, acquiring high cell viability and fast cell proliferation. It is worth noting that microcarrier building blocks can be assembled to create microporous annealed particle scaffolds with desired shapes.

Apart from being seeded on the surfaces of microcarriers, cells can be encapsulated in hydrogel precursors before the latter are emulsified and polymerized. García et al. [53] fabricated microcarriers with controlled size, permeability, and cellular microenvironment from microfluidics (Fig. 2(c)). They demonstrated the capacity of the microcarriers to support high viability of clinically relevant cells, such as human pancreatic islets and human mesenchymal stem cells (hMSCs). Increasing attention has recently been aroused in the area of responsive hydrogels for cell microcarriers. For example, Zhao et al. [54] developed a new

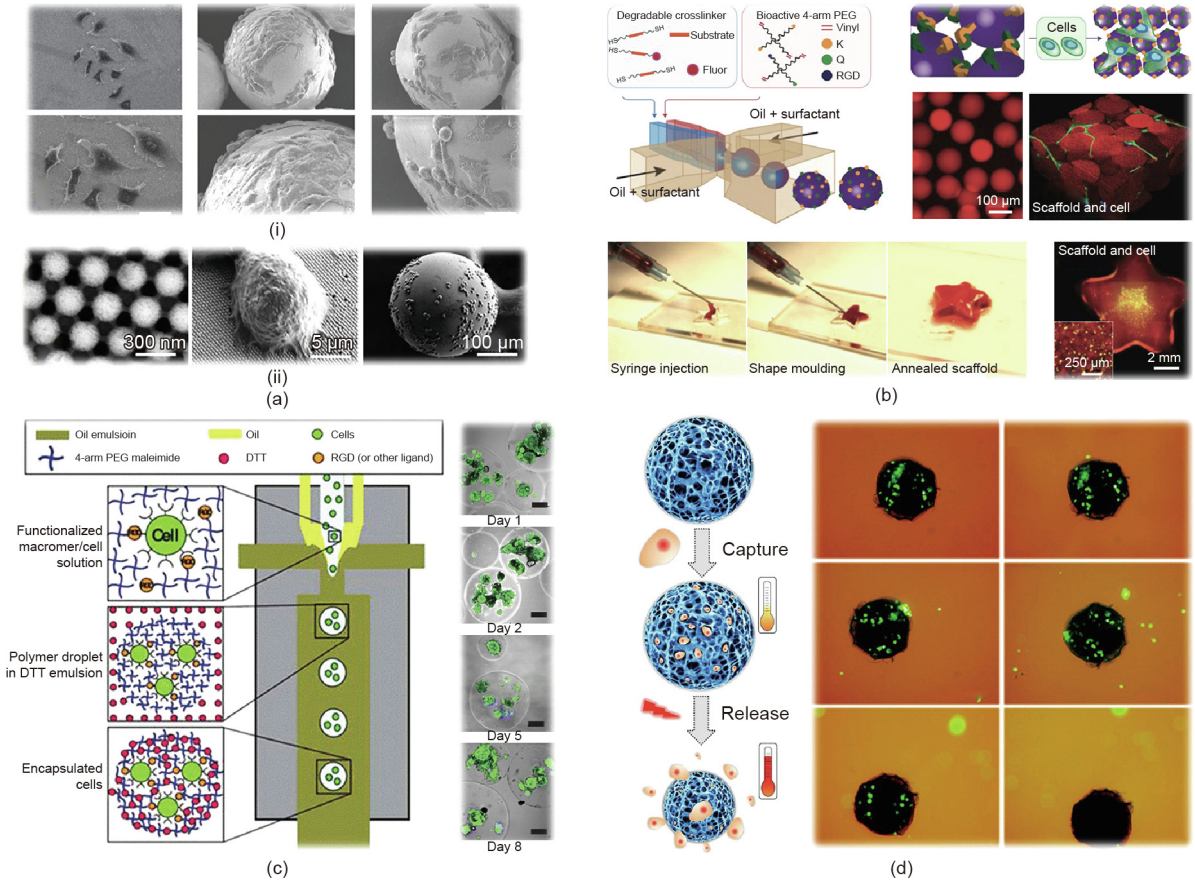

Fig. 2. Microcarriers generated from single-emulsion templates. (a) PC microcarriers; (b) PEG-VS microcarriers; (c) microcarriers with a controlled cellular microenvironment; (d) NIR-responsive hydrogel microcarriers. DTT: dithiothreitol; RGD: alginate-arginine-glycine-aspartic acid. (a) Reproduced from Ref. [46] with permission of Wiley-VCH, ©2013 and Ref. [47] with permission of John Wiley & Sons, Inc., ©2014; (b) reproduced from Ref. [52] with permission of Springer Nature Limited, ©2015; (c) reproduced from Ref. [53] with permission of John Wiley & Sons, Inc., ©2014; (d) reproduced from Ref. [54] with permission of China Science Publishing & Media Ltd., ©2018.

near-infrared (NIR) light-responsive hydrogel microcarrier system for controllable cell culture, as shown in Fig. 2(d). The graphene oxide in the hydrogel precursor can absorb NIR light and transform it into heat, which contributes to the shrinkage of the temperature-responsive hydrogel and the release of captured cells in the microcarrier pores. The cells released from the microcarriers were demonstrated to have similar viability as conventionally digested cells, while the microcarrier scaffold could protect the cells from the immune system before releasing. These features promote the application of microcarriers in building tumor models in mice.

With a method that involves replacing the dispersed channel of the single-emulsion microfluidic device with several parallel channels, multi-compartmental droplets can be achieved. The immiscible dispersed phases come into contact with each other and are simultaneously emulsified by the continuous phase. The fluids remain laminar due to the low value of the Reynolds number (*Re*) in microfluidics. Thus, convective flows during droplet formation will not cause mixing of the dispersed phases, which is dominated by fluid inter-diffusion. Therefore, sharp interfaces and distinct compartments appear when the fluid inter-diffusion is rather slow. Based on these theories, Weitz et al. [55] fabricated multi-compartmental microcarriers by applying multiple injection channels (Fig. 3(a)). Rapid calcium-alginate (Ca-Alg) gelation contributed to the sharp interface between the distinct compartments of the achieved microcarriers. The researchers suggested that this technique could offer a new method of investigating cellular interactions at the single-cell level. Multi-compartmental microcarriers with complicated structures can be fabricated in microfluidics. Zhao et al. [56] applied a capillary array microfluidic technology to generate cell microcarriers with heterogeneous structures and macropores in the center, as shown in Fig. 3(b). With the help of a glass capillary array, microfibers coated with multi-compartmental droplets were first generated. By removing the microfibers from the polymerized droplets, multi-compartmental microcarriers with macropores were developed correspondingly. The macropores guaranteed a more sufficient supply of nutrients during cell culture, in comparison with microcarriers without central macropores.

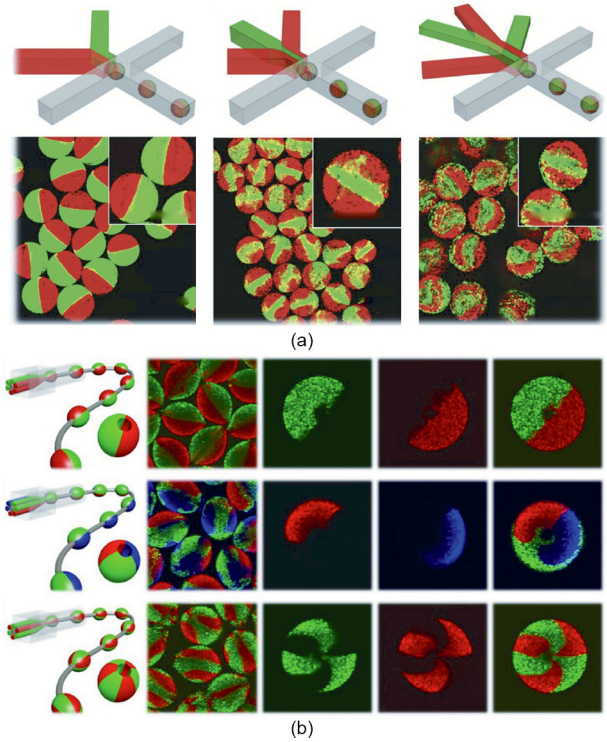

Fig. 3. Multi-compartmental microcarriers generated from different microfluidic devices. (a) PDMS chips; (b) glass capillary chips. (a) Reproduced from Ref. [55] with permission of Wiley-VCH, ©2017; (b) reproduced from Ref. [56] with permission of China Science Publishing & Media Ltd., ©2017.

2.3. Double- and multiple-emulsion droplets

Although cell microcarriers derived from the template of single-emulsion droplets have many advantages, their over-simplified structures limit their applications under some circumstances. Therefore, it is important to be able to fabricate microcarriers with more complicated structures. By implementing multilevel emulsification, double- or multiple-emulsion droplets can be achieved on demand.

On the basis of single-emulsion droplet formation, double-emulsion droplets can be fabricated by secondary emulsification of the mixed fluid. Double emulsions are achieved with a two-step co-flow capillary device, as shown in Fig. 4(a) [57]. First, water-in-oil emulsions are produced at the end of the inner capillary; the mixed fluids are subsequently emulsified into water-in-oil-in-water double emulsions. Based on the double-emulsion droplets, porous microcarriers with external–internal connected structures and biopolymer matrix fillers can be fabricated for 3D cell culture. The porous structure can not only protect the cells from shear forces during culture, but also provide a confined environment for the formation of multiple cell spheroids. In addition to being seeded on microcarriers, cells can be encapsulated in hydrogel microcarriers with a core–shell structure, which are prepared based on double-emulsion droplets. As illustrated in Fig. 4(b) [58], cancer cells have been encapsulated into the core of microcarriers to obtain avascular microtumors. The microcarriers were assembled with stromal cells to fabricate a 3D vascularized tumor for use in cancer research. Compared with ordinary hydrogel microcarriers, core–shell microcarriers feature better cell encapsulation. Fig. 4(c) [59] shows a comparison between islets cultured in solid hydrogel microcarriers and those cultured in core–shell microcarriers, revealing improved cell encapsulation and the immuno-protection of core–shell microcarriers in the latter. Aside from hydrogel cores, the cores of core–shell microcarriers can be liquid, transforming from double-emulsion droplets. Kang et al. [60] injected a cell suspension along with culture medium into the core while an alginate solution was flowed to form the shell of the microcarrier. The researchers found that the cells cultured in the liquid core of the core–shell microcarriers aggregated more easily than those in the solid hydrogel microcarriers (Fig. 4(d)), which was effective in single embryoid body formation.

Similar to the formation of double emulsions, multiple-emulsion droplets can be produced through multiple droplet generators or by one-step emulsification methods. Fig. 4(e) [60] presents the microfluidic fabrication of triple-layered microcarriers with three flow junctions. Kang et al. [60] aimed to use this device to design microcarriers with stem cells in the core and differentiated cells in the surrounding layer for directed differentiation; the outer shell would contain functional beads or drugs for fluorescence tagging, magnetic manipulation, or drug delivery.

3. Microfluidic fibers for medical additive manufacturing

3.1. Microfluidic spinning technology

Microfluidic spinning technology is used for fabricating fibrous constructions with diverse morphologies, components, and structures; it is analogous to droplet microfluidics in terms of chip design and manipulation principles [19,42,61–64]. Different flow

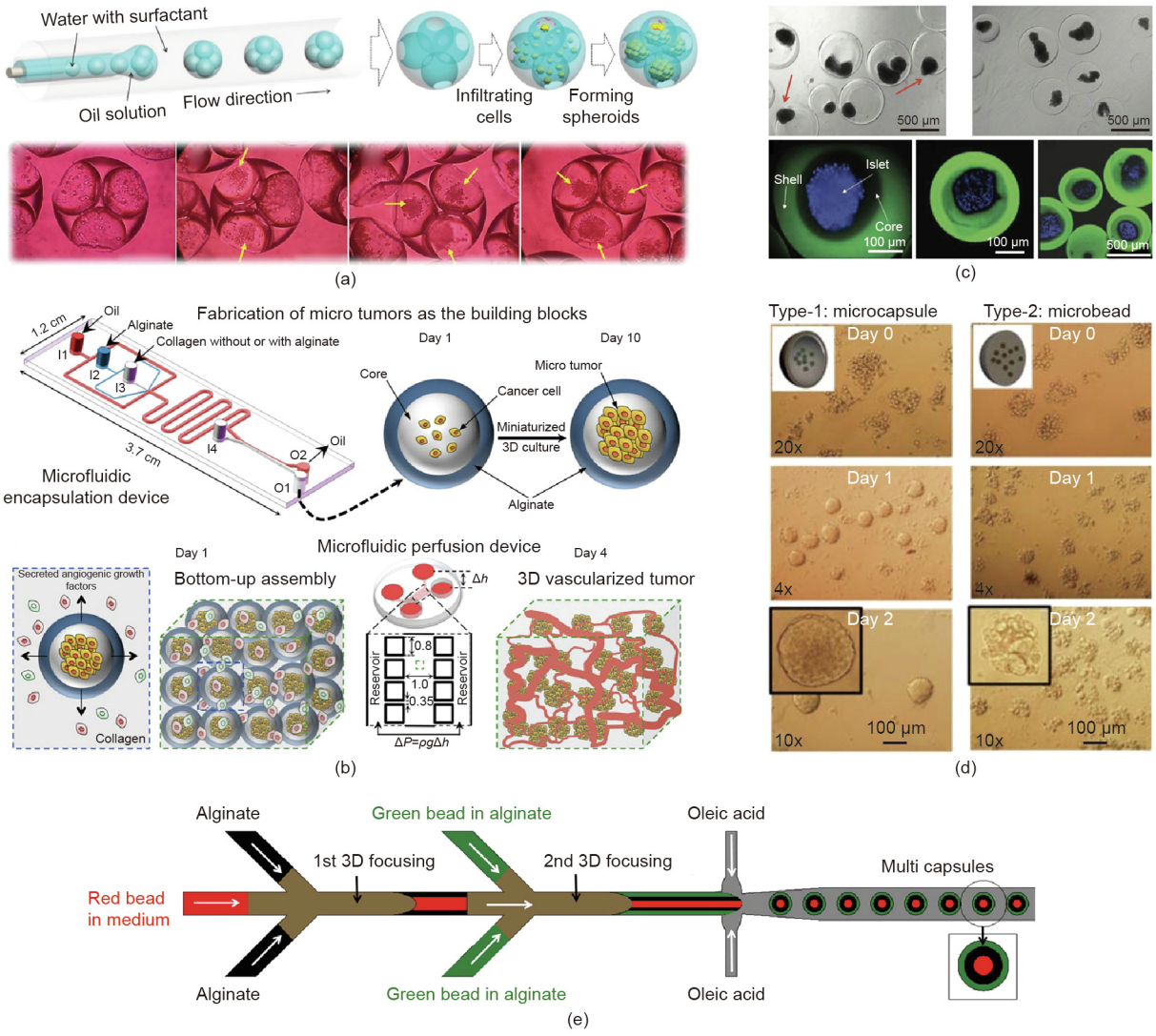

Fig. 4. Microcarriers generated from double- and multiple-emulsion droplets. (a) Porous microcarriers; (b) hydrogel microcarriers with a core–shell structure; (c) core–shell microcarriers with improved cell encapsulation; (d) microcarriers with liquid cores; (e) triple-layered microcarriers. (a) Reproduced from Ref. [57] with permission of ACS Publications, ©2015; (b) reproduced from Ref. [58] with permission of ACS Publications, ©2017; (c) reproduced from Ref. [59] with permission of John Wiley & Sons, Inc., ©2013; (d, e) reproduced from Ref. [60] with permission of Royal Society of Chemistry, ©2011.

regimes of the microfluidic chips, such as cross-flow, flow focusing, and co-flow, are applied for the generation of microfibers, as shown in Fig. 5(a) [62]. Among them, co-flow geometries are widely used for the stable and mild fabrication of fibrous materials. The core and sheath fluids meet at the junction of the chip device via two concentric channels. Numerous parameters such as the viscosity and interfacial tension of the fluids, as well as the device geometry, affect the fluid flow behavior in the channels. The fluids remain laminar without turbulence due to the low *Re* in the channel, which is mainly dominated by the micro-scaled dimensions of the fluids. Thus, the mixing between the core and shell phases is limited to the slow diffusion at the interface. The core fluid is hydrodynamically focused by the shell fluid to form a coaxial configuration of the two fluids. With the *in situ* solidification of the spinning core fluids, fibers are formed and extruded through an outlet channel.

Different biomaterials are brought into the generation of the microfibers, which can be classified into naturally extracted materials and synthetic materials [65–71]. Naturally extracted materials are typically composed of polysaccharide materials such as alginate, hyaluronic acid, chitosan, and agarose, as well as protein-based materials such as collagen, gelatin, and fibrin. By means of physical/chemical crosslinking of these materials, biocompatible microfibers can be formed with hydrogel network structures, which can promote cell growth. Although naturally extracted materials have superior biocompatibility, they are limited by their inherent properties such as poor mechanical strength and difficult modification. In contrast, synthetic materials are highly engineered and can be modified in order to systematically research the relationship between material structure, function, and properties. Typical synthetic materials such as poly (ethylene glycol) dimethacrylate (PEGDA), polyl(actic-*co*-glycolic acid) (PLGA), and poly(*N*-isopropylacrylamide) (pNIPAM) can be applied in microfluidic spinning technology to fabricate functional microfibers after curing.

A variety of polymerization methods are required based on different fiber materials, and can be categorized into three groups: ① photopolymerization, ② ionic crosslinking, and ③ solvent exchange (Fig. 5(b)) [72]. In a photopolymerization process (Fig. 5(b-i)), photopolymerizable polymers and the photoinitiator

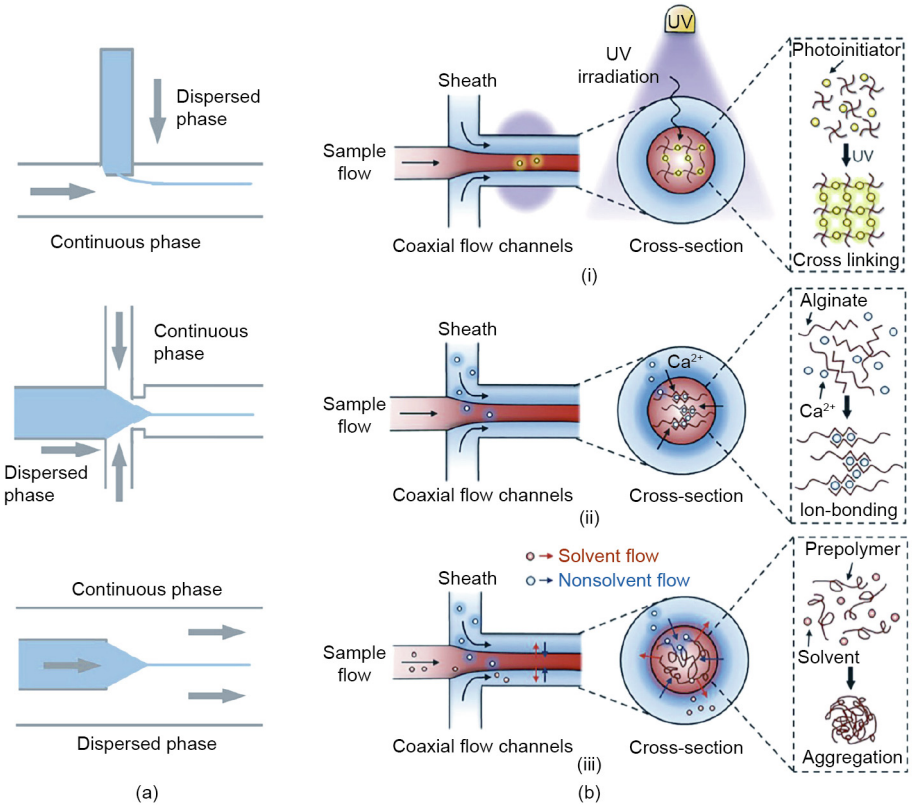

Fig. 5. Microfluidic spinning techniques and polymerization of microfibers. (a) Different flow regimes of the microfluidic spinning chip; (b) three kind of polymerization methods. (a) Reproduced from Ref. [62] with permission of MDPI, ©2017; (b) reproduced from Ref. [72] with permission of Royal Society of Chemistry, ©2014.

are injected as the core fluids; these are generally used as the backbone materials and are irradiated by UV light to initiate polymerization. The sheath fluid acts as the lubricant to accelerate the extrusion of the solidified fiber. Although they have the advantages of easy and fast polymerization, the microfibers fabricated with this method are limited by the potential harm to cells and non-biodegradability of the materials. For ionic crosslinking, the most typical example is the pair of alginate and calcium ions (Ca^{2+}) (Fig. 5(b-ii)). The alginate and Ca^{2+} solutions are introduced into the chip channels as the core and sheath fluids, respectively. The alginate is cross-linked immediately once the two fluids meet. Similar to ionic crosslinking polymerization, the solvent and non-solvent solutions are separately infused as the core and shell fluids in the solvent-exchange method (Fig. 5(b-iii)). Microfibers are achieved by the precipitation of the core fluid, which is induced by the diffusion-based exchange. Typically, PLGA microfibers can be fabricated via this method. Microfibers with different shapes, components, and structures can be produced continuously using microfluidics. Their porous hydrogel structure, small diameter, high area-to-volume ratio, and good biocompatibility make these fibers a favorable cell culture platform.

3.2. Microfibers with distinctive morphologies and structures

Microfibers are produced continuously in a mild manner in microfluidic systems, carrying many advantages in terms of flexible and reproducible fabrication, tailorable geometric and chemical complexity, and high surface area-to-volume conditions. These features make cell-laden microfibers quite promising and pivotal in the fields of cell research and tissue engineering [73,74]. Increasing attention and effort have been dedicated to fabricating cell-laden microfibers with special morphologies and structures. Zhao et al. [75] used a glass capillary microfluidic device for the scalable formation of alginate microfibers (Fig. 6(a)). The Ca-Alg microfiber was generated *in situ* based on hydrodynamic focusing of the steam and fast diffusion-controlled ion crosslinking. The researchers also demonstrated that by changing the flow rates of the streams, microfibers with four kinds of flow conditions (clogging, wavy, straight, and spiral) could be achieved in the collection channel [76]. This theory was further applied to fabricate helical microfibers by increasing the ratio of the inner flow rate to the outer flow rate, as shown in Fig. 6(b) [76]. The phenomenon of fiber transformation was ascribed to the velocity difference between the two phases, which induced a coiling instability in the channel. The novel helical microfibers were used as mechanical sensors for the contraction of cardiomyocytes, which played a crucial role in the fabrication of a novel heart-on-a-chip.

In addition to a helical structure, microfibers with more complicated morphologies and structures can be obtained through improvements and modifications of the microfluidic channels. Seki et al. [77] fabricated a microfluidic device with three inlet-channel networks combined with a flat micronozzle. Sodium alginate solutions with or without cells were first injected into multiple channels, and then combined together before extruding out through the special nozzle. Microfiber sheets were eventually generated with this device, as shown in Fig. 6(c) [77]. The researchers further embedded hepatoma cells and fibroblasts inside the patterned fiber sheets to mimic hepatic microstructures. Lee et al. [78] used a computer-controlled pneumatic valve to control the inlets individually in order to produce alginate microfibers coded in a serial, parallel, or mixed manner, as illustrated in Fig. 6(d). In addition, by applying a grooved round channel, the researchers generated tubular fibers with grooved surface (Fig. 6(e)) [78]. The grooves

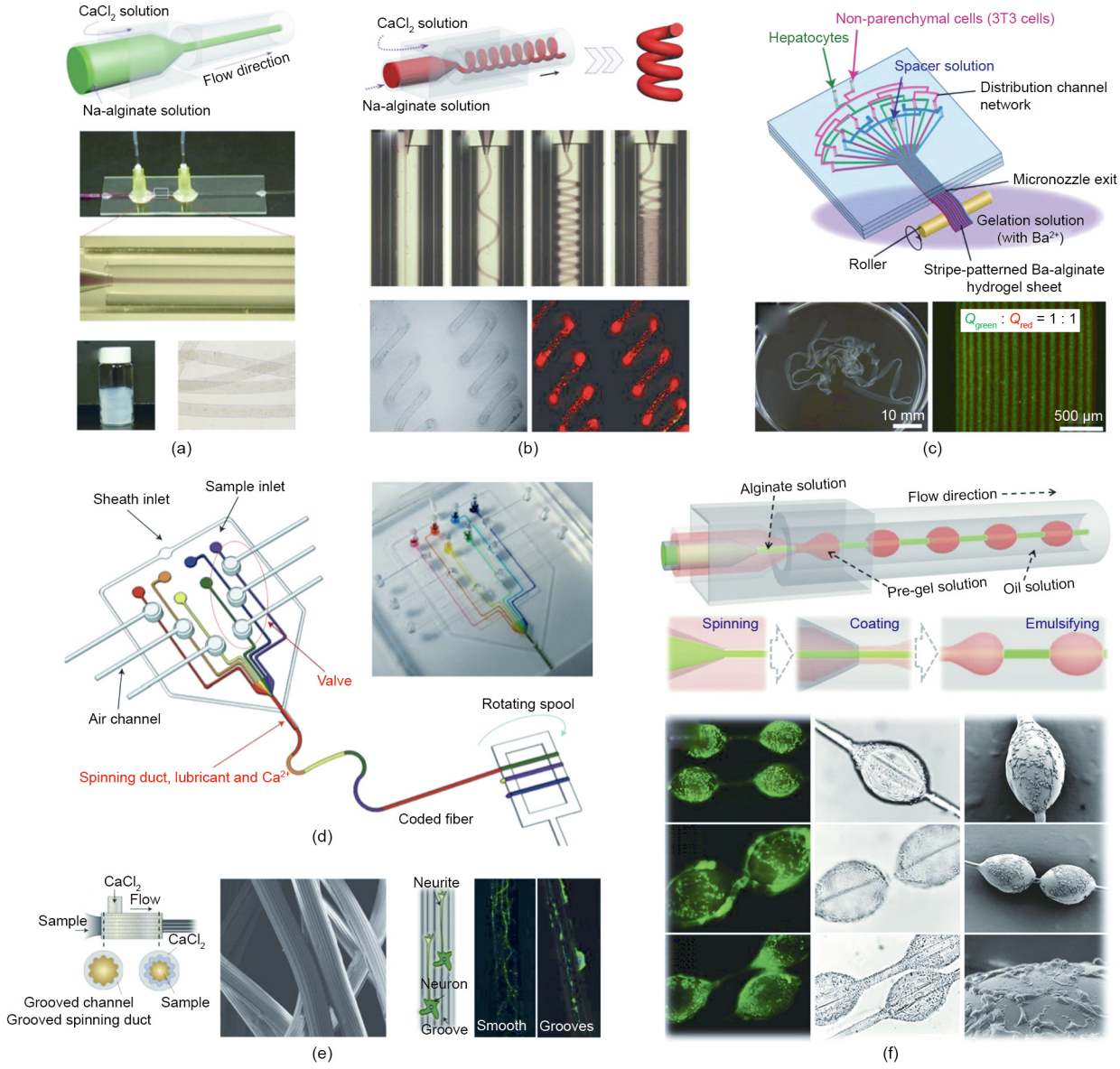

Fig. 6. Microfibers with distinctive morphologies and structures. (a) Solid alginate microfibers; (b) helical microfibers; (c) stripe-patterned fiber sheets; (d) coded alginate microfibers; (e) grooved microfibers; (f) spindle-knotted microfibers. (a) Reproduced from Ref. [75] with permission of John Wiley & Sons, Inc., ©2014; (b) reproduced from Ref. [76] with permission of John Wiley & Sons, Inc., ©2017; (c) reproduced from Ref. [77] with permission of Elsevier B.V, ©2013; (d, e) reproduced from Ref. [78] with permission of Springer Nature Limited, ©2011; (f) reproduced from Ref. [79] with permission of Wiley-VCH, ©2017.

were highly adjustable by changing the channel geometries and the fluid velocities. Such a grooved surface was demonstrated to facilitate the controlled alignment of neurons. Aside from the above two kinds of microfibers with different morphological, structural, and chemical features, spindle-knot fibers can be achieved by applying two alternating fluid streams in this microfluidic system. Similarly, Zhao et al. [79] combined spinning with emulsification to form droplets hanging on the microfibers, which were used as a template for fabricating spindle-knotted microfibers (Fig. 6(f)). The researchers generated gelatin methacrylate (GelMA) spindle-knots on alginate microfibers for 3D cell culture. Other microfluidic strategies have also been developed to produce spindle-knotted microfibers, such as encapsulating air bubbles and droplets in fibers [78,80].

In addition to the solid hydrogel microfibers discussed above, microfibers with core–shell, hollow, and multi-compartment structures can be generated. Zhao et al. [75,81] upgraded the basic microfiber spinning capillary device with multiple inlet channels and hierarchical injection channels to achieve microfibers with more complicated structures, as shown in Fig. 7(a). Hollow alginate microfibers were fabricated by inserting one more calcium chloride ($CaCl_2$) inlet channel in the previous alginate core channel (Fig. 7(a-i)). The alginate was cross-linked immediately at the inner and outer interfaces once the three streams came into contact with each other. As a result, a hollow microfiber was continuously achieved in the channel. Similarly, microfibers with multiple hollows were fabricated by inserting several $CaCl_2$ inlet channels inside the alginate channel, while hollow microfibers with multiple shell layers were achieved by increasing the number of hierarchical injection channels in the hollow microfiber spinning capillary device. As for multi-compartmental microfiber spinning, multi-barrel capillaries should be used as the injection channel instead of the single-channel injection capillary of the original microfiber spinning device (Fig. 7(a-ii)). Multiple fluids in an alginate solution

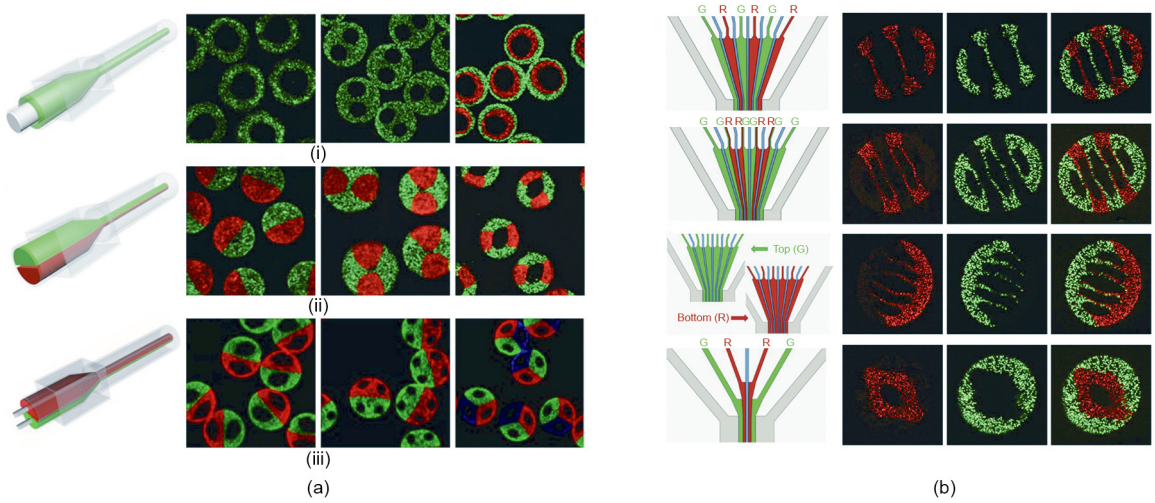

Fig. 7. Multi-hollow or multi-compartmental microfibers from different microfluidic devices. (a) Glass capillary devices; (b) PDMS devices. (a) Reproduced from Ref. [75] with permission of John Wiley & Sons, Inc., ©2014 and Ref. [81] with permission of ACS Publications, ©2016; (b) reproduced from Ref. [82] with permission of John Wiley & Sons, Inc., ©2016.

were pumped into the injection capillaries simultaneously and solidified *in situ* to form multi-compartmental microfibers. Laminar flow formed in the channels due to the low *Re* of the stream, and no obvious diffusive mixing happened between different alginate streams, resulting in sharp and obvious interfaces between the different compartments of the microfibers. Microfibers with multi-compartmental core–shell structures can also be achieved by combining the features of the hollow fiber device and the multi-compartmental fiber device, as presented in Fig. 7(a-iii). In addition to capillary devices, PDMS microfluidic chips can be applied to produce microfibers with special morphologies and structures. Qin et al. [82] developed a novel PDMS microfluidic device with a hierarchical, multilayer, microchannel structure to fabricate microfibers with both multi-hollow and multi-compartmental properties, as shown in Fig. 7(b). The chip is comprised of three layers with the functions of a flowing inner core, a middle hydrogel scaffold, and an outer sheath, respectively. Compared with the glass capillary microfluidic device, his proposed PDMS approach has the advantages of better reproducibility and stability.

3.3. Microfiber-assembled 3D cell structures

With their features of easy-operation, stability, mild generation, and good biocompatibility, cell-laden microfibers have been assembled to fabricate 3D cellular constructs using various strategies, as shown in Fig. 8. Fukuda et al. [83] used magnetic force to aggregate the microfibers in the support models to achieve 3D structures, using a method that involved encapsulating magnetic nanoparticles in the microfibers (Fig. 8(a)). This assembly approach resulted in negligible harm to the loaded cells. Lee et al. [84] fabricated pure chitosan-based microfibers with the microfluidic spinning technology and then used an *X*–*Y* stage-based winder to twine the microfibers into a 3D structure (Fig. 8(b)). The hepatoma cells cultured on the microfiber shaped constructs self-assembled into spheroids and showed a better liver-specific function expression. Apart from the above two strategies, Juncker et al. [85] presented a microfluidic direct writer (MFDW) approach for building cellular constructs. The solidified alginate microfibers were extruded out of the monolithic microfluidic head of the MFDW, which was mounted on an automatically controlled motorized stage (Fig. 8(c)) [85]. 3D structures with encapsulated cells were constructed layer by layer with the MFDW. Takeuchi et al. [86] built a laboratory-made microfluidic weaving machine to apply functional cell fibers as building blocks for the higher-order cellular assembly of various 3D macroscopic cellular constructs, as shown in Fig. 8(d). The basic principle of this method was to eject the microfibers through capillaries. With the help of the weaving machine, a cellular architecture with a size of around 2 cm by 1 cm could be mechanically woven. Besides weaving, the layer-by-layer stacking strategy can be used to achieve microfiber-based structures, as shown in Fig. 8(e) [81]. Bioprinting is a promising technology for building 3D cellular architecture. By combining multiple microfluidic channels with bioprinting, multi-compartmental fiber-assembled structures can be generated, as shown in Fig. 8(f) [87]. A microfluidic bioprinting head featuring multiple inlets can typically be employed (Fig. 8(f-iii)). More complex engineered constructs similar to the tissues observed *in vivo* could be fabricated using this method.

4. Applications

Microfluidic generation of droplets or fibers presents distinctive benefits in terms of a simple and cost-effective fabrication process, easy size modulation, and flexible component and geometry manipulation. In addition, the generation process can be performed under mild conditions without requiring high voltages or temperatures. Thus, sensitive biological components including cells can be loaded into droplets or fibers without loss of function. These unique characteristics enable the microfluidic fabrication of cell-laden microcarriers or microfibers for various applications in medical additive manufacturing [88–112].

4.1. Cell guidance

The morphogenesis and topographic alignment of various types of cells are generally influenced by the extracellular matrix (ECM) scaffold [88–90]. Extensive research on different forms of cell-type guidance using microfluidic spun microfibers has been reported, covering myoblasts, fibroblasts, cardiomyocytes, and neuron cells. Scheibel et al. [91] analyzed the morphologies of neuronal cells (NG108-15) cultured on collagen microfibers after incubating for 72 h. Neuronal NG108-15 cells were observed to undergo axon growth (up to 100 μm long) in the direction of the microfiber axis.

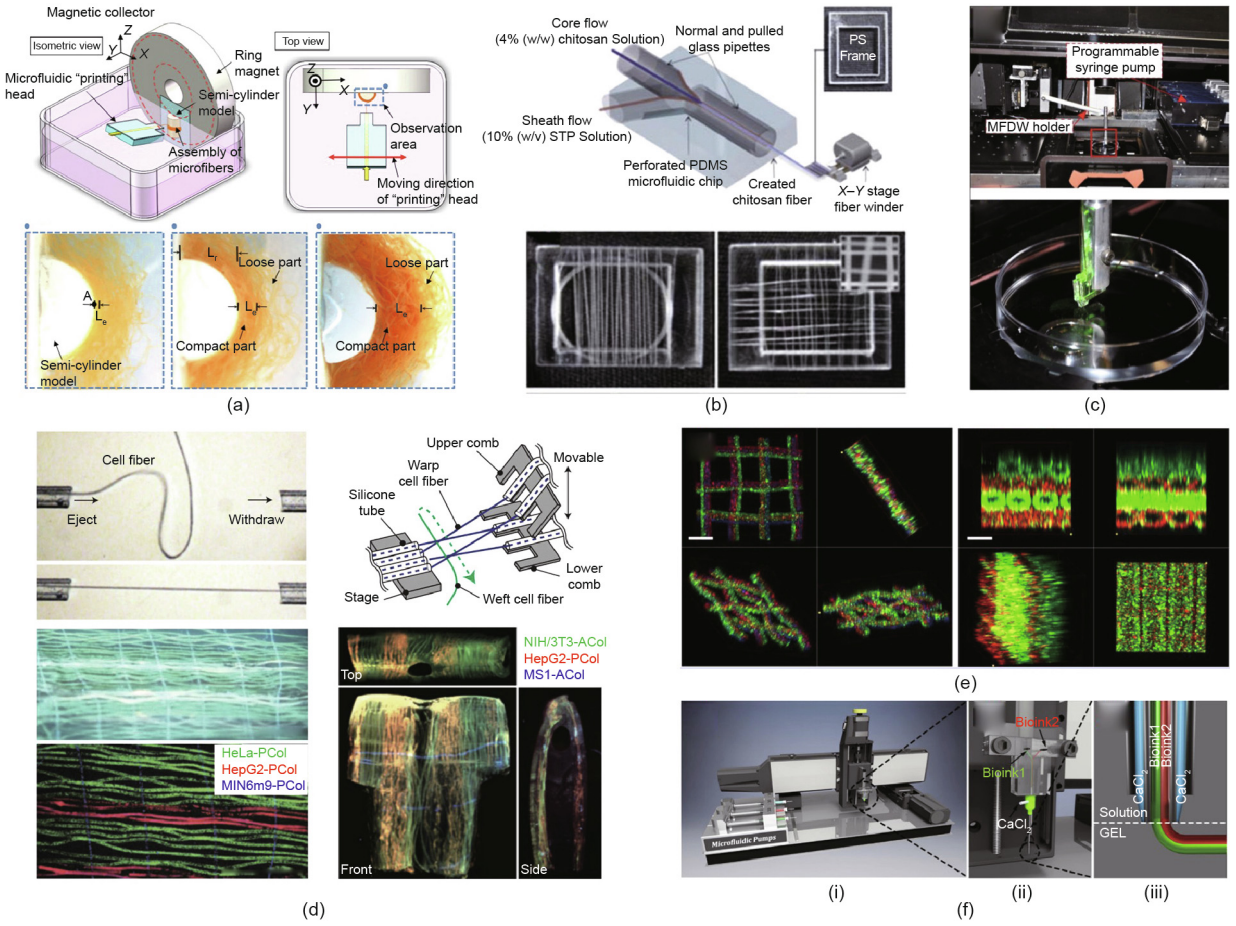

Fig. 8. Various strategies for assembling microfibers into 3D cellular constructs. (a) Magnetic force; (b) an *X*–*Y* stage-based winder; (c) a microfluidic direct writer; (d) a microfluidic weaving machine; (e) layer-by-layer stacking; (f) bioprinting. STP: sodium triphosphate pentabasic. (a) Reproduced from Ref. [83] with permission of Springer Nature, ©2015; (b) reproduced from Ref. [84] with permission of Royal Society of Chemistry, ©2010; (c) reproduced from Ref. [85] with permission of Springer Nature, ©2014; (d) reproduced from Ref. [86] with permission of Springer Nature Limited, ©2013; (e) reproduced from Ref. [81] with permission of ACS Publications, ©2016; (f) reproduced from Ref. [81] with permission of Elsevier B.V, ©2017.

Lee et al. [92] presented the continuous microfluidic fabrication of grooved microfibers and compared the neuron cell growth on grooved fibers to that on smooth fibers, as shown in Fig. 9(a). Most neuron cells were found to align along the ridges of the grooved fibers, while many neuron cells formed randomly directed neuritis on the smooth fibers. The results indicated that directed alignment of the neuron cells was stimulated by the grooved microfiber surface. The researchers also explored the influence of microfiber size on the orientation of adhered cells [93]. It was found that the orientation of the fibroblast cells increased as the size of the PLGA microfiber decreased.

Besides grooved microfibers, patterned microfibers fabricated by other methods have demonstrated cell guidance capability as well [94,95]. Zhang et al. [94] constructed alginate microfibers with aligned submicron topography based on Kelvin–Helmholtz instability waves during generation. The oriented submicron topography of the spun hydrogel was tunable by adjusting the perfuse-in and spin-out rates of the fibers. PC12 cells seeded on the surface of the shear-patterned fibers presented an ordered spreading tendency along the fiber axis, while cells on the Petri dish or on smooth fibers were randomly oriented (Fig. 9(b)) [94]. A 3D microfiber network built by microfluidic printing was also shown to have the function of guiding and orienting cell growth, as shown in Fig. 9(c) [95]. The generated chitosan scaffold had programmable periodical patterns with a wrinkled surface, and was printed directly in air. L929 fibroblasts cultured on the surface of the microfiber scaffold were found to orient along the printed direction of the chitosan filaments.

4.2. 3D cell culture

Cells are known to respond to microenvironments, which affect the morphology, adhesion, migration, proliferation, and differentiation of cells [96,97]. 3D cell culture approaches show great ability to mimic cellular interactions and *in vivo* microenvironments, compared with conventional 2D culture [98,99]. Thus, 3D approaches make the recovery of 3D structures and partial functions of the original tissue physiology possible *in vitro*. 3D cell culture is therefore of vital importance in cellular biology, drug discovery, tissue engineering, and other fields. Various approaches have been proposed for 3D cell culture [100–102]. The microcarriers or microfibers that generate from microfluidics have emerged as novel 3D cell culture platforms with the features of biomimetic 3D cell support, high-throughput fabrication, good size and geometry modulation, and controllable cell microenvironments. A great deal of research effort has been dedicated to this area.

Wang Y and Wang J [103] developed a droplet microfluidic approach for the fabrication of multicellular tumor spheroids with alginate and matrigel mixed hydrogel microcarriers. Human cervical carcinoma (HeLa) cells were encapsulated in microcarriers and closely linked to each other. The HeLa cell spheroids that formed in the microcarriers were tested with anticancer drugs, indicating the

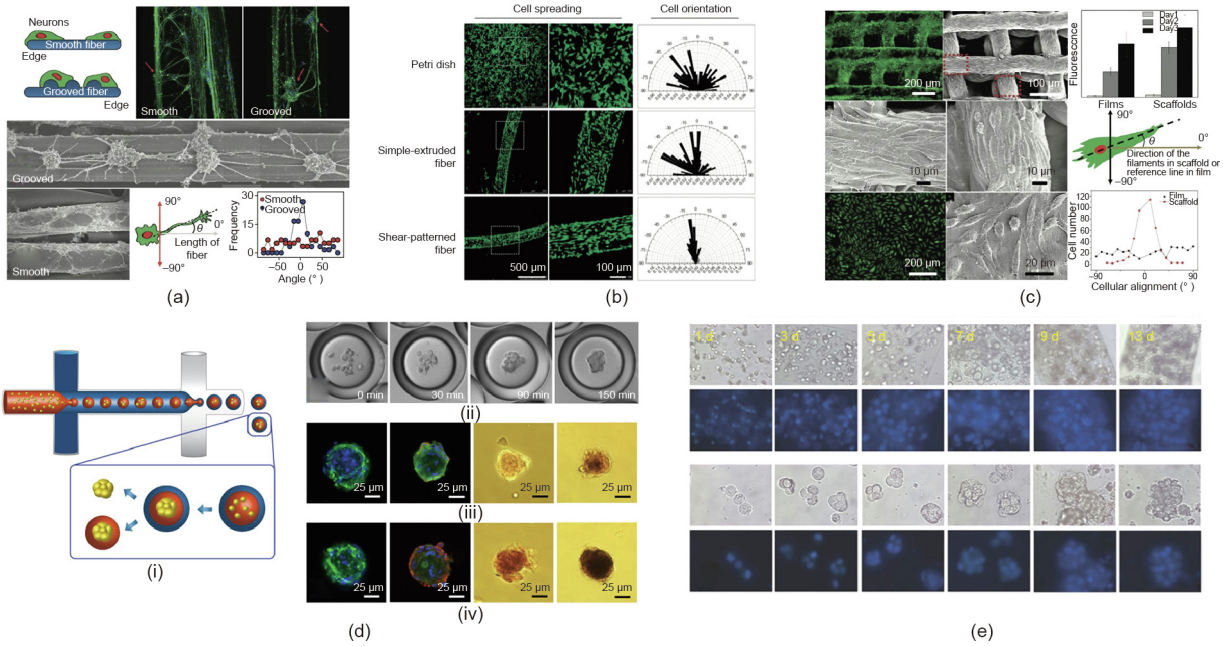

Fig. 9. Applications of cell guidance and 3D cell culture in medical additive manufacturing. (a–c) Cell guidance; (d,e) cell spheroid fabrication. (a) Reproduced from Ref. [92] with permission of John Wiley & Sons, Inc., ©2012; (b) reproduced from Ref. [94] with permission of John Oxford University Press, ©2017; (c) reproduced from Ref. [95] with permission of John Wiley & Sons, Inc., ©2017; (d) reproduced from Ref. [106] with permission of Springer Nature Limited, ©2013; (e) reproduced from Ref. [75] with permission of John Wiley & Sons, Inc., ©2014.

ability of the tumor cell spheroids to mimic the properties of tumors *in vitro*. This microfluidic approach to produce multicellular tumor spheroids could be used for cellular interaction research, oncotherapy, and high-throughput screening of anticancer drugs. Core–shell structured microcarriers with embryonic stem (ES) cells encapsulated inside were also fabricated from microfluidics by He et al. [104]. Their microcarriers showed excellent ability for the 3D culture of ES cells to promote their aggregation and proliferation. Higher pluripotency of ES cells was also maintained in comparison with 2D culture. In addition, the researchers encapsulated cells in core–shell microcarriers in order to roughly investigate the influence of the cellular microenvironment on 3D cell culture by replacing the previous core material with different kinds of biocompatible polymers [105]. This 3D biomimetic platform is anticipated to find broad application in developmental biology and high-throughput drug screening. Leong et al. [106] carried out similar but more detailed work on microcarriers with cell spheroids encapsulated for microenvironment evaluation (Fig. 9(d-i)). The researchers replaced the hydrogel shell with an oil layer, which simplified the encapsulation process and decreased the viscosity requirements of various phases. In combination with an amphiphilic surfactant in the oil, this feature induced rapid spheroid formation after 150 min in the droplet system, versus 1–4 d with other existing technologies, as shown in Fig. 9(d-ii) [106]. The hMSCs were encapsulated to form spheroids in the cores with alginate and alginate-arginine-glycine-aspartic acid (-RGD), respectively, which exhibited enhanced osteogenic differentiation in the latter case (Figs. 9(d-iii) and (d-iv)) [106]. This result indicated that the inner core materials of the droplet system could be changed to fine-tune the microenvironment for desired differentiation.

Cell-laden microfibers also play a major role in 3D cell culture. Zhao et al. [75] encapsulated cells uniformly in alginate microfibers to provide a 3D microenvironment for the cells, as shown in Fig. 9(e). The hepatocellular carcinoma (HepG2) cells were found to proliferate and aggregate gradually to form spheroids in the microfibers. A large number of cellular spheroids was achieved, corresponding to the continuous microfluidic spinning of cell-laden microfibers. By digesting the Ca-Alg, the achieved cell spheroids were released for further applications. The authors also investigated the influence of different microfiber structures on cell performance using this microfluidic system.

4.3. Tissue assembly

Tissue-like cellular constructs can be achieved by assembling or stacking engineered microcarriers or microfibers into arbitrary 3D structures. Takeuchi et al. [107] developed a rapid construction method for achieving macroscopic 3D cellular constructs by stacking large numbers of cell-loaded collagen microcarriers into designed silicone chambers (Fig. 10(a)). 3D tissue structures could be generated rapidly, as the cells located on the surfaces of the microcarriers formed cell–cell adhesions without the addition of any adhesives. He et al. [58] fabricated 3D vascularized human mammary tumors with a similar bottom-up assembly method. The researchers first obtained avascular microtumors by encapsulating cancer cells in the core of microcarriers for miniaturized 3D culture, which were then assembled together with endothelial cells and other stromal cells to fabricate 3D vascularized tumors. The 3D vascularized tumors exhibited higher drug resistance than avascular microtumors or 2D-cultured cancer cells, which indicated the importance of the extracellular microenvironment. Zhao et al. [81] applied bioactive microfibers from microfluidics to create multiple 3D constructs. Hollow hydrogel microfibers encapsulated with human umbilical vein endothelial cells (HUVECs) were first generated. It was found that the HUVECs adhered on the inner interface of the hollow microfiber and then spread to form a monolayer cell tube, which was similar to blood vessels in terms of components and structures. A layer-by-layer microstructure or gridding architecture could subsequently be created by stacking or crisscross weaving microfibers, which showed promise in mimicking microfiber-like network tissues for further applications. Besides microcarrier/microfiber-based cellular constructs, Wallace et al. [108] applied a handheld microfluidic

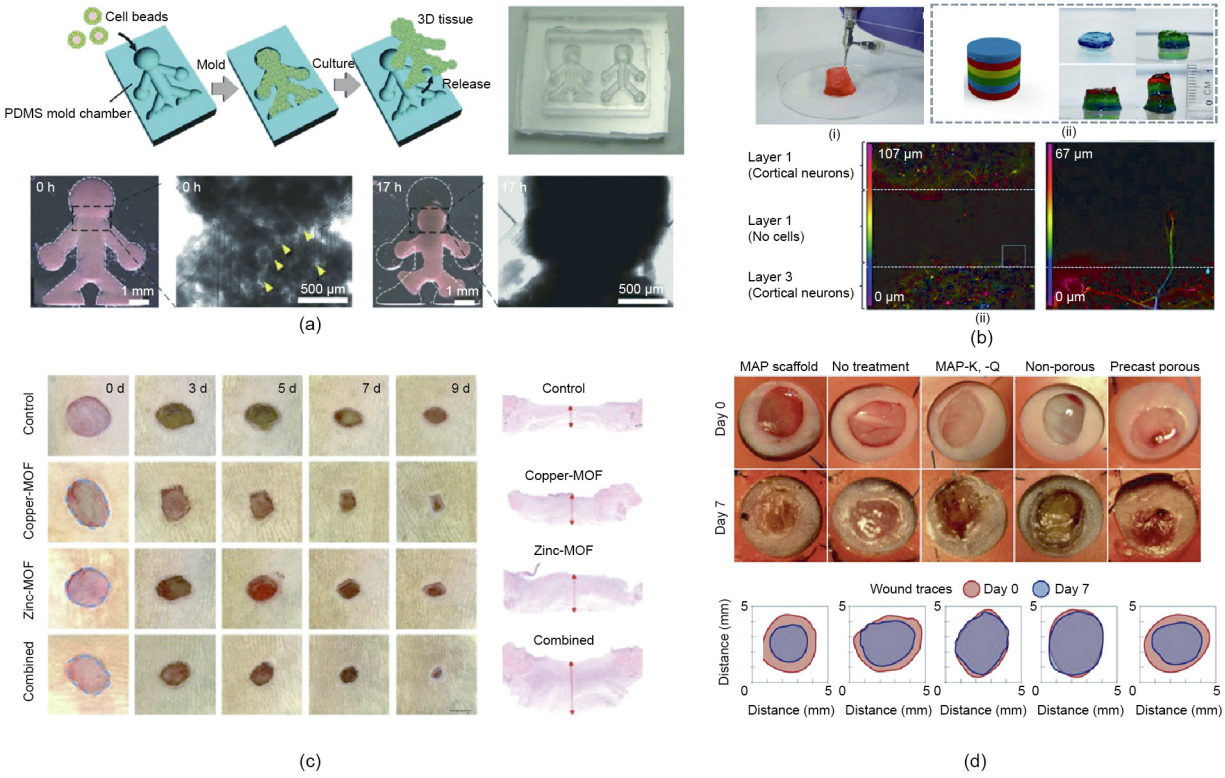

Fig. 10. Applications of tissue assembly and therapy in medical additive manufacturing. (a,b) Tissue assembly; (c,d) wound healing. MOF: metal–organic framework. (a) Reproduced from Ref. [107] with permission of John Wiley & Sons, Inc., ©2011; (b) reproduced from Ref. [94] with permission of Elsevier B.V, ©2015; (c) reproduced from Ref. [111] with permission of Royal Society of Chemistry, ©2018; (d) reproduced from Ref. [52] with permission of Springer Nature Limited, ©2015.

bioprinting approach to construct 3D brain-like structures (Fig. 10(b)). The free-formed 3D structure had a layered architecture that consisted of a peptide-modified biopolymer and encapsulated primary neural cells. A multi-layered neural circuit was formed in this 3D structure model (Fig. 10(b-iii)), paving the way for brain disease understanding and brain-related learning and memory.

4.4. Therapy

Cell-laden microcarriers or microfibers with injectable hydrogel materials, functional biological cues, and therapeutic cells can be applied in the fields of medical transplantation, wound healing, and tissue regeneration [109,110], due to their potential and promising ability for replicating natural tissue/structure functions with minimally invasive implantation procedures. Increasing attention is being paid to this area, and a variety of relevant research works have been published.

Takeuchi et al. [86] explored the applicability of cell-laden microfibers for the treatment of diabetes mellitus in medical transplantation. They first sheathed islet cell microfibers with an alginate–agarose interpenetrating network (IPN) hydrogel, which not only isolated the fibers from the fibrotic reaction, but also protected the cells from immunological attack. The generated primary islet cell microfibers were then implanted into diabetic mice, which later presented normalized blood glucose concentrations. In contrast, the bare injection of dispersed primary islet cells with the same quantity of the cells in the microfibers failed. This result demonstrated the therapeutic ability of generated primary islet cell microfibers for diabetes mellitus treatment. Zhao et al. [111] presented a kind of microfiber laden with vitamin metal–organic frameworks (MOFs) for wound-healing application (Fig. 10(c)). The MOFs were synthesized *in situ* during the spinning of core–shell structured alginate microfibers in microfluidic channels. The highly controlled core–shell structure of the microfiber allowed gentle and controllable release of the laden MOFs, which then showed antibacterial and antioxidation capabilities. The released copper ions, zinc ions, and biocompatible vitamin ligands accelerated the tissue wound-healing process when the microfibers were assembled into a film and applied to mouse full-thickness cutaneous infected wound models. Besides microfibers, Segura et al. [52] developed a novel building-block-based approach to achieve injectable microcarrier-based tissue mimetic constructs, which were delivered to the wound site for wound healing. The injectable constructs exhibited significantly faster wound closure than other control groups, as shown in Fig. 10(d) [52]. The faster wound closure was ascribed to the microscale porosity of the 3D building constructs.

Stem cells have shown promising properties for cell microcarriers or microfibers and the application of cell-based therapy. The injectable stem-cell-laden microcarriers or microfibers are able to degrade quickly, causing the cells to be released *in vivo*. For example, human umbilical cord mesenchymal stem cells (hUCMSCs) were released from the microcarriers after being injected for 4 d, and showed excellent proliferation and osteogenic differentiation [112]. Stem-cell-laden microcarriers or microfibers with good biocompatibility can also be anticipated to serve as novel platforms for the long-term delivery of therapeutic factors. Taken together, microcarriers or microfibers loaded with functional cells (whether therapeutic cells or stem cells) have shown significant importance in cell-based therapy and regenerative medicine.

5. Conclusions and outlook

This review summarizes recent research progress in the microfluidic generation of cell-laden microcarriers and microfibers and their applications in the field of medical additive manufacturing. Starting with a mechanism-based introduction to droplet

microfluidics and microfluidic spinning technology, we overviewed the generation of different kinds of microcarriers and microfibers with distinctive morphologies and structures from microfluidics. Various strategies for assembling cell-laden microcarriers and microfibers into complex 3D architectural cellular structures were highlighted in this paper. With the benefits of stable mechanical property, excellent processability, and good biocompatibility, cell-laden microcarriers and microfibers were demonstrated to have a broad range of applications, such as cell guidance, 3D cell culture, tissue assembly, and cell-based therapy.

Despite numerous exciting and compelling achievements in microfluidic technology for medical additive manufacturing, challenges remain to be addressed and there is potential for further development in this area. Thus, the following three important issues are suggested for consideration. First, although cell-laden microcarriers with complicated morphologies and structures play a pivotal role in building biomimetic 3D cell constructs, their microfluidic production on a large scale remains challenging at present. This difficulty is ascribed to the intrinsic properties of the microfluidic fabrication devices. As described above, PDMS and glass capillaries are the most commonly used materials for the fabrication of microfluidic devices. PDMS chips have the advantage of scaled droplet generation, while glass capillary devices can stably achieve droplets with more complex structures. Combining the benefits of these two kinds of devices could spur on innovation in novel microfluidic devices, which might hold potential for the scaled production of microcarriers with complicated morphologies and structures. Second, a huge gap exists between assembled 3D cellular constructs and *in vivo* tissue structures. On the one hand, efforts should be addressed to mimic the heterogeneous structures of tissues, including the types, morphologies, alignments, and corresponding positions of the cells. On the other hand, extracellular microenvironments and cell–cell interactions play a significant role in maintaining the physiological functions of tissues *in vivo*, which could be better mimicked by incorporating suitable biological cues such as ECM in assembled 3D cellular constructs. Third, the current assembly strategies are limited in the spatial resolution and poor controllability of building complex and miniaturized 3D cellular architectures. Robotic techniques are suggested as one of the best choices to overcome this difficulty, considering their manipulating precision. Bioprinting is a kind of robotic approach for building macroscopic cellular structures, which can be upgraded by replacing the nozzle with a microfluidic chip head for better control over multiple fluids. Apart from these three issues, the development of cell-laden microcarriers or microfibers for the long-term delivery of therapeutic factors or differentiated cells such as a stem cell niche, as well as the applications of organs on chips, is worthy of anticipation.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2020YFA0908200), the National Natural Science Foundation of China (22002061, 52073060, and 61927805), the Natural Science Foundation of Jiangsu (BE2018707), and the Jiangsu Agricultural Science and Technology Innovation Fund (CX(20)3051).

Compliance with ethics guidelines

Jie Wang, Changmin Shao, Yuetong Wang, Lingyun Sun, and Yuanjin Zhao declare that they have no conflict of interest or financial conflicts to disclose.

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