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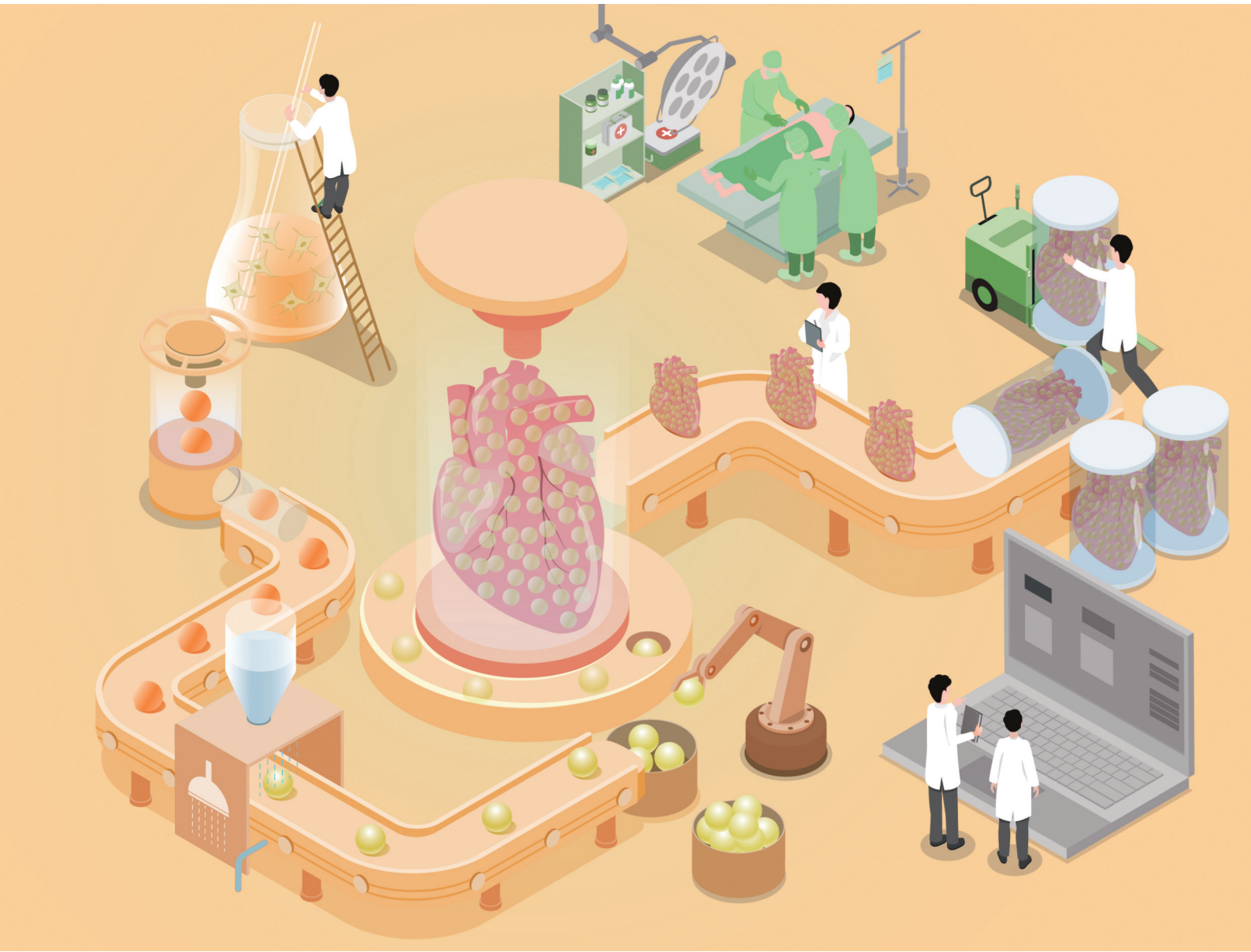

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Microgels for bioprinting: recent advancements and challenges

Mingjun Xie,[a,b,c,d,e] Ji Wang,[a] Sufan Wu,[a] Sheng Yan*[a] and Yong He*[b,c,d,e]

Microgels have become a popular and powerful structural unit in the bioprinting field due to their advanced properties, ranging from the tiny size and well-connected hydrogel (nutrient) network to special rheological properties. Different microgels can be fabricated by a variety of fabrication methods including bulk crushing, auxiliary dripping, multiphase emulsion, and lithography technology. Traditionally, microgels can encapsulate specific cells and are used for *in vitro* disease models and *in vivo* organ regeneration. Furthermore, microgels can serve as a drug carrier to realize controlled release of drug molecules. Apart from being used as an independent application unit, recently, these microgels are widely applied as a specific bioink component in 3D bioprinting for *in situ* tissue repair or building special 3D structures. In this review, we introduce different methods used to generate microgels and the microgel-based bioink for bioprinting. Besides, the further tendency of microgel development in future is introduced and predicted to provide guidance for related researchers in exploring more effective ways to fabricate microgels and more potential bioprinting application cases as multifunctional bioink components.

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1. Introduction

In recent years, 3D bioprinting technology[1–6] has been developed within the field of biomedicine. In order to realize specific biomedical applications, a series of bioprinting methods have been proposed[7–10] and various biomaterials[11–14] have been selected as a bioink component. 3D bioprinting can be categorized into three distinct groups according to the American Society for Testing and Materials (ASTM) standards, namely, jetting-based,[15] extrusion-based,[16] and vat polymerization-based[17] bioprinting. The promising printability and biocompatibility of hydrogels make them one of the most significant biomaterials in bioprinting.[18–21] Among all of the bioprinted hydrogel structures, microgels have emerged as one of the most important structures utilized in the biofabrication field. Microgels[22] refer to hydrogel structures (hydrogel microparticles (HMPs)) at the micron scale, which can form multifunctional structural units suited to different application scenarios based on various hydrogel prepolymers and related biological components. As mentioned above, currently, jetting-based and vat polymerization-based bioprinting has been applied for the fabrication of microgels. Compared with large-size bulk hydrogels, microgels have their own special excellent properties to meet different requirements in the biomedical field. Some of the excellent key properties of microgels are as follows:

Tiny size: Microgels have a small overall size, and their size can be adjusted by the preparation process parameters. Besides, compared with the same volume of bulk hydrogels, microgels have a larger specific surface area, which greatly promotes the exchange of substances between the internal and external environment of the hydrogel and provides better living conditions for the cells loaded inside. The tiny size enables the microgel to be injected or implanted into a narrow part of the patient's body to repair damaged tissue.[23]

Well-connected hydrogel network: The hydrogel network can be used as a semi-closed system to achieve the controlled release of functional molecules (such as drugs or growth factors). After crosslinking the hydrogel prepolymer, hydrogels with different material types and concentrations will have different internal pore distributions. Therefore, specific molecular controlled release systems can be established according to the different requirements of drug delivery. In addition, hydrogel networks also play an important role in providing a reliable 3D culture microenvironment for encapsulated cells.[24]

Recently, apart from the application as an independent unit, such as cell encapsulation and a drug carrier, microgels

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have gradually become a kind of bioink component for bioprinting. Aggregated microgel systems can show special rheological properties. On the one hand, the system is composed of solid particles, which allows it to exhibit elastomer characteristics that can maintain its original shape or undergo reversible elastic deformation when less stress is applied to the system. On the other hand, when a large stress is applied to the system, the aggregated microgel system tends to exhibit certain fluid characteristics and has good fluidity. This non-Newtonian fluid is known as Bingham plastic fluid,[25–27] and its special rheological properties allow it to be used as a bioink component in specific bioprinting and biomedical applications. Besides the basic printability, compared to traditional bioink which is prepared with hydrogel prepolymer, microgel-based bioink has irreplaceable properties in lots of bioprinting cases, such as extrusion in a changeable environment, deposition on rigorous receivers (injury with blood), simplifying the printing process and biofunction enhancement (Fig. 1).

In this review, we discuss the developing status of microgels in the bioprinting field, including the fabrication methods and application approaches. Firstly, we introduce and compare different methods applied to generate microgels, including bulk crushing, auxiliary dripping, multiphase emulsion and lithography technology. Then, we discuss current bioprinting application approaches of microgels, including sacrificed space occupying and secondary assembly. Finally, the further development tendency of microgels in future is introduced and predicted. This review aims at summarizing the current development of microgels and providing guidance for related researchers in exploring more effective ways to fabricate microgels and more potential bioprinting application cases.

2. Fabrication methods of microgels

To realize the accurate and controllable generation of microgels for the biomedical field, researchers have invented a series of fabrication methods. In terms of bioprinting with microgels, there is no special requirement that needs to be considered to fabricate microgels as long as the microgel size can match the printing nozzle (especially extrusion printing nozzle) and the fabrication method can maintain high cell viability when cell-laden microgels are applied. Thus, traditional methods for generating microgels can be applied in bioprint-

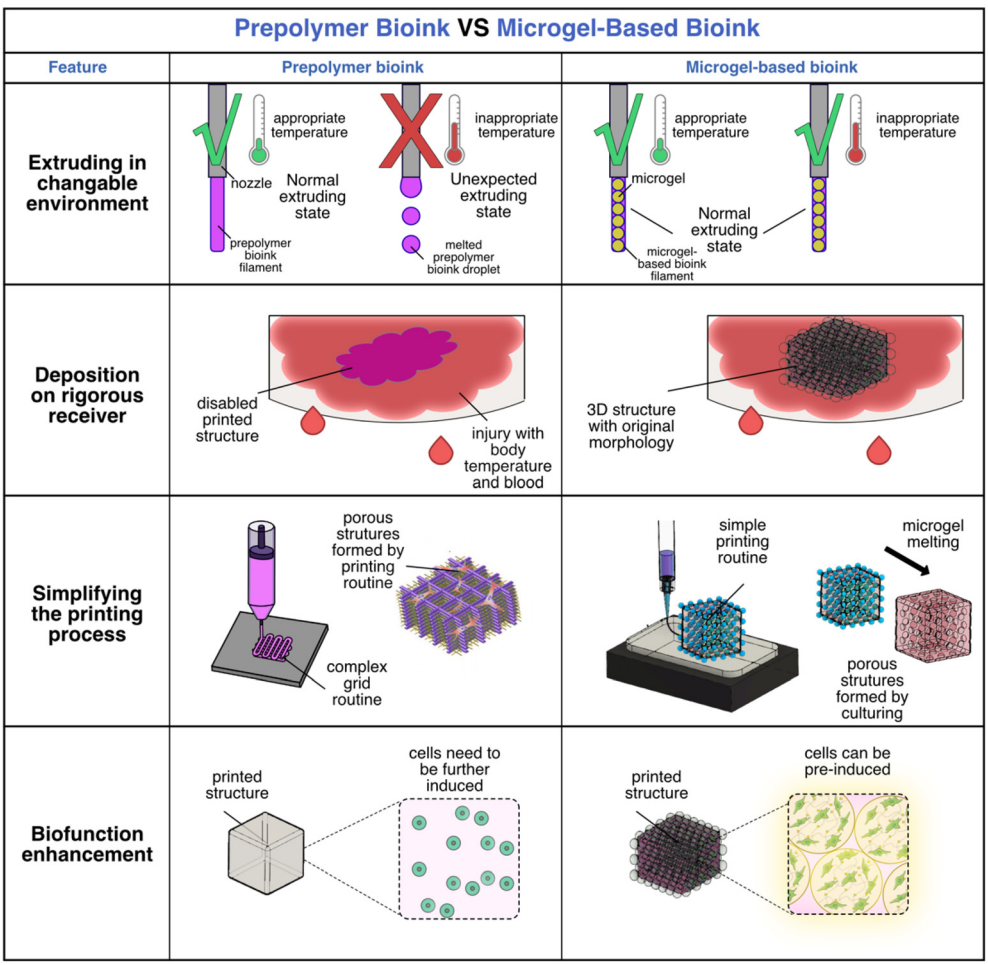


Fig. 1 Advantages of microgel-based bioink compared to traditional prepolymer bioink in 3D bioprinting.

ing with microgels. This review highly generalizes various fabrication methods and re-classify them into four main classes, namely, bulk crushing, auxiliary dripping, multiphase emulsion and adapted lithography (Fig. 2).

2.1 Bulk crushing

Roughly, microgels can be fabricated by the method of bulk crushing, that is, making hydrogel bulk into irregular fragments with micro-scale sizes. A bulk hydrogel is formed in a certain container. By adding compressive or shear force, the bulk hydrogel would be broken up and turned into microgels (Fig. 3a). Bulk crushing is one of the easiest microgel fabrication methods in that it could be manually operated. However, the size uniformity is always not promising and the surface morphology is always uneven, so that this method is usually applied in the situation where the microgel morphology can be ignored.

Stirring method: A. Ellis *et al.*[28] reported the method of bulk crushing to generate agarose microgels (Fig. 3b). The bulk agarose was placed in distilled water and blended initially using a standard food blender. Then, a silver high speed rotor-stator mixer with a fine emulsor screen was used to further reduce the microgel size by shearing. The overall diameter of the microgels was about 100 μm for the higher bulk agarose concentrations, with smaller sizes found for the bulk gels with lower concentrations. Yang *et al.*[29] applied a similar method to fabricate agarose microgels. The cooled bulk agarose was directly placed into the food mixer, followed by being stirred to obtain broken agarose microgels.

Extrusion method: In the work of Sinclair *et al.*,[30] an easy-operated strategy was proposed to generate injectable zwitterionic polycarboxybetaine microgels. Bulk polycarboxybetaine zwitterionic hydrogels were extruded through progressively finer micronic steel meshes with a stainless-steel piston and cylinder apparatus (Fig. 3d). To ensure the size uniformity, the initially generated microgels were screened by the final mesh size several times. Shao *et al.*[31] proposed a similar method to form gelatin microgels (Fig. 3c). The gelatin precursor was gelated at low temperature in the syringe, followed by being crushed into microgels continuously and pushed out through the syringe nozzle. Their sizes were relatively uniform but their shapes were irregular.

2.2 Auxiliary dripping

In nature, continuous water dropping phenomenon can be often found, which is always caused by gravity. Briefly, water tends to accumulate on some surfaces (eaves, leaves, *etc.*) or outlets (tubes, faucets, *etc.*). Due to the effect of gravity, the surface tension cannot maintain the initial state when the volume reaches some value, resulting in dropping and forming a droplet. This phenomenon can be applied to form microgels if the hydrogel precursor is applied. However, the size cannot reach the micrometer level with the gravity alone. Therefore,

Microgel Fabrication Method

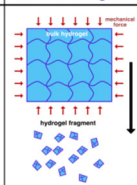
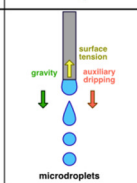
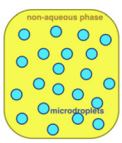
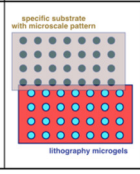
Class	Schematic diagram	Class Description	Subclass	Subclass Description	Advantage	Disadvantage
Bulk Crushing		Bulk hydrogel is formed in certain container. By adding compressive or shear force, the bulk hydrogel would be broken up and turned into microgels.	Stirring method	With blender.	Easy operation	Irregular microgel shape
			Extruding method	With syringe.		
Auxiliary Dripping		Dripping droplets are added various auxiliary force to realize the generation of microdroplets.	Piezoelectric-assisted method	With piezoelectric nozzle.	Uniform microgel size and high production speed	Complex fabrication system
			Laser-assisted method	With laser printing system.		
			Electric-field-assisted method	With high-voltage electric field.		
Multiphase Emulsion		A liquid is added into another immiscible medium. Once some disturb is added into this two-phase system, the liquid would be dispersed into microdroplets.	Liquid-liquid emulsion	Hydrogel precursor is added into non-aqueous liquid.	Uniform microgel size and high production speed	High-cost fabrication device
			Gas-liquid emulsion	Hydrogel precursor is attatched to flowing gas.		
Adapted Lithography		With the lithography method, microdroplets can generated on the specific substrate.	In-mold casting	With mold with microscale holes.	Uniform microgel size and high production speed	Material limitation
			Discrepant wettability coating	With substrate owning different wettability patterns.		
			Selected irradiation space	With projection-based printing (PBP) system.		

Fig. 2 Schematic diagrams and descriptions of different microgel fabrication methods.

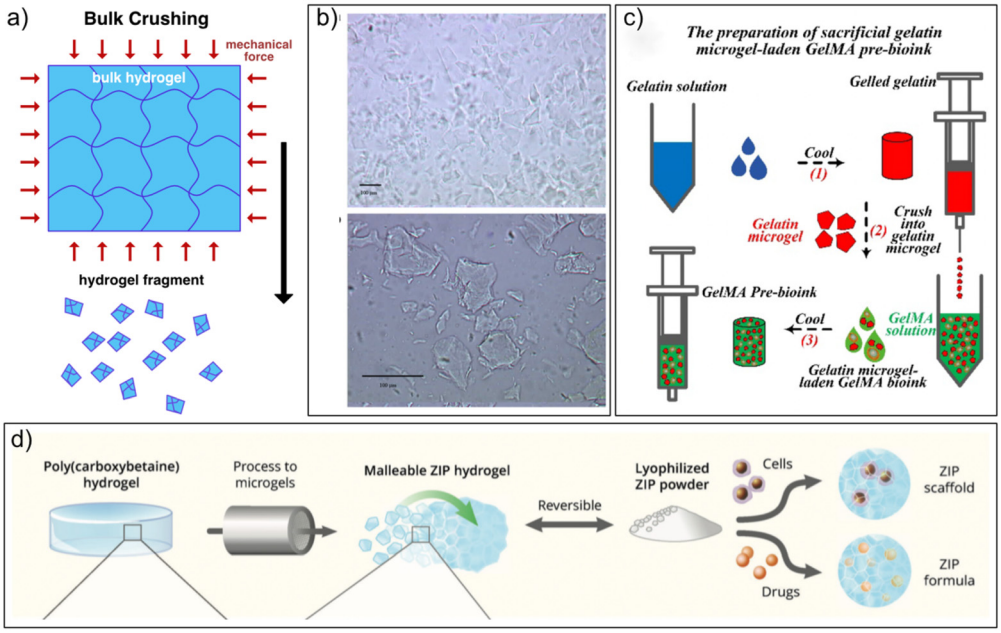


Fig. 3 Bulk crushing method for microgel fabrication. (a) Schematic of the bulk crushing method. (b) Agarose microgels generated by using a food blender and silver high speed rotor-stator mixer with a fine emulsor screen. Reproduced from ref. 28 with permission from Elsevier, copyright 2009. (c) The schematic of the fabrication of injectable zwitterionic polycarboxybetaine microgels. Reproduced from ref. 31 with permission from Springer, copyright 2020. (d) The schematic of the fabrication of gelatin microgels. Reproduced from ref. 30 with permission from Wiley-Blackwell, copyright 2018.

researchers have been trying to add various auxiliary forces to the hydrogel to realize the fabrication of microgels (Fig. 4a).

Piezoelectric-assisted method: Traditional 2D printing technology provides technical support for the development of 3D printing. In the early stages of 3D printing, researchers directly modified traditional piezoelectric-assisted 2D printers to print 3D structures, and microdroplets were the printing units in these processes. According to the principle of piezoelectric effect, the electrical pulse signal is transmitted to the piezoelectric nozzle, and the electrical energy is converted into mechanical energy and pulses the bioink inside the nozzle, resulting in microdroplets. Teo *et al.*[32] created fine microgels in a single step by in-air collision of the precursor and cross-linked droplets using the piezoelectric-assisted method (Fig. 4b). The sodium alginate precursor and calcium cross-linked droplets were generated from two piezoelectric printheads. Zhang *et al.*[33] applied a piezoelectric inkjet printer to fabricate arrayed PEGDA microgels onto glass slides at highly defined positions (Fig. 4c). By dripping the hydrogel precursor and initiator, respectively, in two steps, the microgels could form on the substrate.

Laser-assisted method: Laser-assisted printing is another common 3D printing method. Through laser assistance, the laser focused on the absorber sheet pushes the hydrogel precursor droplet to the collector to achieve printing, which has high resolution and high cost. Due to the high pulse repetition rate of the applied laser, the generating speed of microdroplets can be very fast. Thus, researchers have published related work on microgel fabrication by this method. Gruene *et al.*[34] introduced the laser-assisted method for the cell-laden microgel fabrication (Fig. 4d). Microdroplets with 10 to 7000 picolitres volume could be fabricated by adjusting the viscosity and thickness of the applied hydrogel layer in combination with the laser pulse energy. In another work of Gruene *et al.*,[35] microgels were fabricated and located as arrays to visualize cell–cell and cell–environment interactions using a fibrin-based layer-by-layer approach with the laser-assisted method, which provided a proof of concept for imaging interactions between ASCs and ECFCs (Fig. 4e).

Electric-field-assisted method: The combination of special hydrodynamic principles with traditional 3D printing technology has also become a trend in 3D printing processes in recent years. Among them, the principle of electrohydrodynamics (EHD) occupies an important position in the manufacture of 3D structures at the micro/nano scale. Electrohydrodynamics is the study of the rapid flow or atomization behavior of fluids in an electric field.[36,37] This discipline is not only an important sub-discipline of electromagnetism and fluid dynamics, but also an important cost-effective technology in engineering. The target liquid flows out through a small nozzle connected with the electrode and is exposed to a high-voltage electric field, which will be induction charged or contact charged at the nozzle end, and gradually elongated into a thin jet or atomized into tiny droplets under the combined action of electric field force, gravity and surface tension. By adjusting process parameters such as applied voltage, fluid flow, ambient temperature, nozzle model, *etc.*, the size and shape of the jet or droplet can be adjusted. In the work of Xie

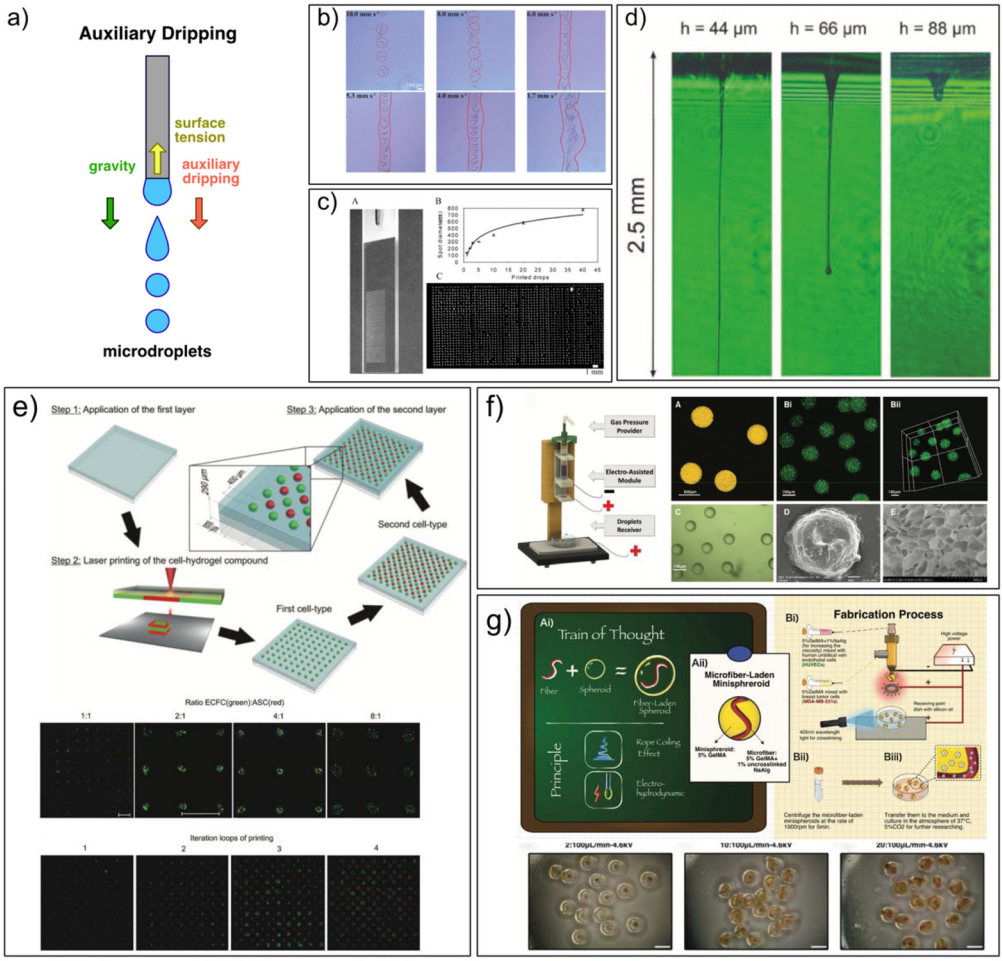


Fig. 4 Auxiliary dripping method for microgel fabrication. (a) Schematic of the auxiliary dripping method. (b) Microscopy images of fabricated alginate microgels on glass substrates. Reproduced from ref. 32 with permission from American Chemical Society, copyright 2020. (c) The PEGDA microgel array on the glass slide and the variation of spot diameter with the microgel quantity. Reproduced from ref. 33 with permission from the Royal Society of Chemistry, copyright 2008. (d) The laser-assisted method setup for cell-laden microgels and the dripping hydrogel precursor at different layer heights. Reproduced from ref. 34 with permission from BioMed Central, copyright 2011. (e) The schematic illustration of the generation of cell arrays based on fibrinogen-hyaluronic acid by the laser-assisted method and the fluorescence images illustrating the variation of cell–cell ratios (scale bars = 800 mm). Reproduced from ref. 35 with permission from Mary Ann Liebert, copyright 2011. (f) The electric-field-assisted method setup for cell-laden GelMA microgels and the morphology of the GelMA microgels. Reproduced from ref. 38 with permission from Wiley, copyright 2019. (g) The electric-field-assisted method setup for GelMA heterogeneous microgels and the morphology of the GelMA heterogeneous microgels. Reproduced from ref. 39 with permission from the Royal Society of Chemistry, copyright 2020.

et al.,[38] an electric-field-assisted method was proposed to fabricate gelatin methacryloyl (GelMA) microgels (Fig. 4f). GelMA microdroplets were generated with an electric-field-assisted module and received with silicon oil, followed by photocrosslinking by 405 nm blue light into microgels. The microgel size could be controlled by voltage, nozzle size, and gas pressure. In another work of Xie *et al.*,[39] based on a similar electric-field-assisted module, a "microfiber-laden minisphere-roid" was successfully fabricated, which were two widely used microstructures in the biomedical field (Fig. 4g). Using the coaxial nozzle, the famous fluidic phenomenon, namely, the "rope coiling effect" would occur when two bioink phases were extruded from the inner nozzle and outer nozzle, respectively. As a result, the inner bioink would form a fiber-shape fluid inside the outer droplet and form heterogeneous microgels.

2.3 Multiphase emulsion

Emulsion is the action of a liquid to uniformly disperse in a very small droplet state inside another medium that is immiscible to each other. It is a kind of interface phenomenon. When a liquid is added to another immiscible medium, it tends to maintain the integrated state by the surface tension. Once some disturbance is added into this two-phase system, the liquid would be dispersed into smaller droplets. Based on this phenomenon, researchers have been trying to establish a different multiphase system to generate hydrogel precursor microdroplets, which could be further crosslinked into microgels (Fig. 5a).

Liquid–liquid emulsion: The liquid-liquid system is a common multiphase system applied in microgel fabrication.

Similar to the emulsion of water inside the oil, the water phase and oil phase would form one stable interface between each other. Smaller water droplets can be generated by simply stirring. However, due to the increase of surface attaching area, this multiphase system is always not stable, which tends to recover to the initial layered state. Thus, in the actual emulsion process, a surfactant is always applied to make it easier to maintain the emulsion state. C. L. Franco *et al.*[40] applied the liquid–liquid emulsion phenomenon to fabricate PEGDA microgels (Fig. 5b). The hydrogel precursor was added to mineral oil and stirred in a vortex generator, forming lots of microdroplets in this system. Then, the system was exposed to light to crosslink the microdroplets. The cells were further seeded on the PEGDA microgels. However, simply stirring emulsion can always lead to uneven microgel sizes. To fabricate more uniform microgels, researchers also designed various microfluidic chips to restrict the flow state of the multiphase fluids, which can generate microgels with nearly the same size. For example, in the work of Schindler *et al.*,[41] a high-throughput microfluidic device was built to generate cell-laden agarose microgels. Human pluripotent stem cells (hPSCs) were added to the agarose precursor (Fig. 5c). Monodisperse water-in-oil emulsion microdroplets were formed in the established microfluidic devices from two streams of aqueous and oil phases by break-off flow and crosslinked into cell-laden microgels.

Gas-liquid emulsion: Besides liquid-liquid emulsion, liquid can also be dispersed into small droplets in high-speed gas flow, which is commonly called atomization. Thus, microgels can also be fabricated by the gas–liquid emulsion method. For example, in the work of Pal *et al.*,[42] a versatile air-assisted co-axial device (ACAD) was established to fabricate microgels

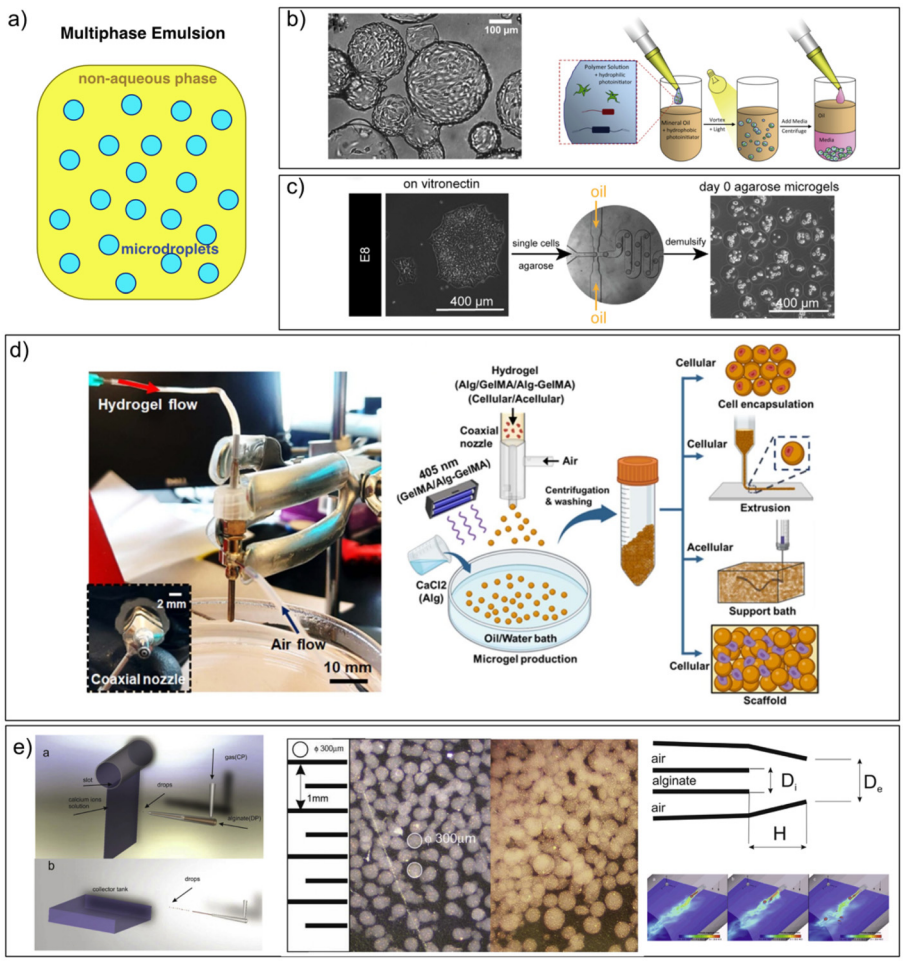


Fig. 5 Multiphase emulsion method for microgel fabrication. (a) Schematic of the multiphase emulsion method. (b) The diagram of the vortex-induced emulsion encapsulation method and the cell attaching on the PEGDA microgels. Reproduced from ref. 40 with permission from Elsevier BV, copyright 2011. (c) The established microfluidic chips and the generated cell-laden agarose microgels. Reproduced from ref. 41 with permission from Cell Press, copyright 2021. (d) The air-assisted co-axial device setup and the schematic of microgel fabrication from alginate, GelMA and alginate-GelMA using the proposed device. Reproduced from ref. 42 with permission from IOP Publishing, copyright 2023. (e) The air–liquid emulsion devices applied in the experimental setup, the schematic representation of the two experimental setups, namely, with and without vertical liquid screen ($CaCl_2$) and the effect of misalignment of inner capillary axes on alginate microdroplet formation and interaction mechanism. Reproduced from ref. 43 with permission from Elsevier, copyright 2017.

(Fig. 5d). The system setup had a co-axial nozzle for the hydrogel precursor and air flow. To demonstrate the feasibility of the method, microgels composed of various biomaterials and their combination were generated, including alginate, GelMA, and alginate-GelMA microgels. Besides, Marra *et al.*[43] proposed the gas–liquid emulsion method to generate alginate microgels and analyzed the flow behavior during the emulsion process by computational fluid dynamics (Fig. 5e). The results demonstrated that the proposed gas–liquid emulsion method represented a feasible solution for high-throughput fabrication of alginate microgels.

2.4 Adapted lithography

The lithography method was developed from the early lithography transfer method, and the depiction on the transcribed paper and then fell on the plate to become a reverse grain, and then printed on the paper surface as a normal pattern. This method has been widely applied in the field of publication printing such as newspapers, posters, *etc*. In this method, the printing plate is simple to manufacture and low cost. The color binding is accurate and the printing content can be obtained with a large yield. Essentially, lithography technology is a kind of method to operate small microdroplets, so that this method can be potentially applied in the fabrication of microgels and researchers have proposed different adapted lithography technologies for it (Fig. 6a).

In-mold casting: The printing plate used in lithography has grooves with a specific topology to print the on-demand patterns. It easily comes to mind that microgels can be fabricated by directly casting the hydrogel precursor into a printing plate or a mold consisting of duplicate microscale grooves. Zhang *et al.*[44] successfully fabricated bilayer-ribbon-shaped poly(*N*-isopropylacrylamide) (PNIPAM) microgels, which could be deformed in water using nonwetting molds as templates (Fig. 6b). A press with a quartz window was applied to the molding of microgels. The hydrogel precursor was first added on the replicated PFPE film, which was then covered by a flat PFPE film. Then, the hydrogel precursor was photocrosslinked into microgels and demolded from the mold. Andreas *et al.*[45] also explored the in-mold casting method to fabricate PEG microgels (Fig. 6c). The molds are prepared by placing a PDMS layer with a 12 mm punched hole onto a second full PDMS bottom layer. The hydrogel precursor was poured into the cavities of the mold. Excess precursor was cleaned with a polyethylene terephthalate (PET) film to prevent formation between the cavities. After crosslinking, the microgels could be collected from the mold.

Discrepant wettability coating: Besides special sunk molds, substrates with different wettability arrangement can also realize the on-demand distribution of the hydrogel precursor. When the hydrogel precursor is irregularly coated on the treated substrate with microscale hydrophilic areas, it tends to accumulate at these areas and form microdroplets, followed by being crosslinked into microgels. Li *et al.*[46] proposed the method of discrepant wettability coating for rapid heterogeneous microgel preparation (Fig. 6d). Researchers manufactured the special hydrophobic/hydrophilic contact line at the PDMS-glass interface, the micropatterns on which led to wetting of the hydrogel precursor to hydrophilic spaces and repulsion of the hydrogel precursor to hydrophobic spaces. By coating a hydrogel precursor on the discrepant wettability surface, the microdroplet array self-assembled rapidly and was transformed into a microgel array by crosslinking. Ngoc *et al.*[47] also reported a rapid method for preparing a microgel array with surfaces patterned with differential wettability (Fig. 6e). PEG hydrogel precursor was spotted onto the hydrophilic spaces of the patterned gold-coated substrates and sandwiched by the DTT-treated silanized glass coverslip. By irradiation of UV light, the microdroplets were then crosslinked into microgels.

Selected irradiation space: For photocrosslinkable hydrogels, the approach of selected light irradiation space can directly realize the microgel formation. The hydrogel precursor would be photocrosslinked into microgels at the irradiation points, while the one at the lucifugal points would remain in the liquid state. Gulfam *et al.*[48] proposed the fabrication method of PEG microgel building blocks by the selected irradiation space technique (Fig. 6f). Photomask thin films with square and hexagonal shapes were designed by AutoCAD, respectively. The hydrogel precursor was added on a glass slide and another glass slide was placed on top to form a uniformly distributed layer of hydrogel precursor. The photomask thin film was covered on the upper glass slide and irradiated with UV light. The microgels then formed in the transparent spaces of the mask. Moreover, with the development of projection-based 3D bioprinting (PBP),[49–52] the selected irradiation space method can also be realized by this technique without the complex mask manufacturing process. That is, the image signal is projected out after digital processing, and the basic principle of PBP technology is that the digital light source in the form of surface light is projected layer by layer on the surface of the liquid hydrogel precursor, which is cured layer by layer. The electric mask can be easily generated and modified by the upper computer. Liu *et al.*[53] applied a PBP printer to fabricate microgels with different 3D shapes (Fig. 6g). This method successfully made use of the advantages of PBP technology, namely, high flexibility and good controllability and provided an ideal way for the mass fabrication of customized microgels.

3. Bioprinting application approaches of microgels

Based on various fabrication methods, microgels with different functions and properties have been established. Researchers have also designed a series of application approaches for microgels. Apart from the independent application units such as cell encapsulation[54–57] and drug carriers,[58–69] in recent years, researchers have been exploring the potential applications of microgels in the bioprinting field.

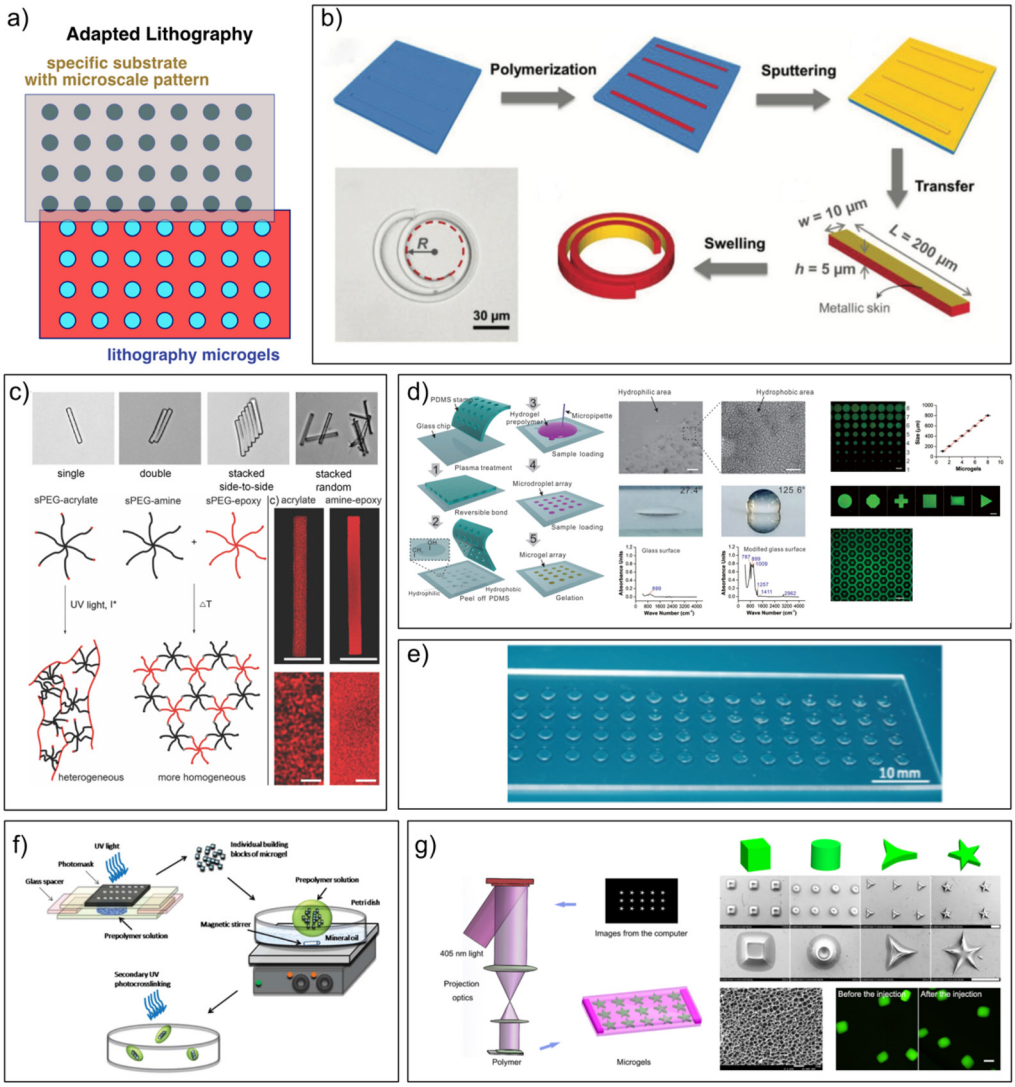


Fig. 6 Adapted lithography method for microgel fabrication. (a) Schematic of the adapted lithography method. (b) The fabrication schematic of bilayer spiral microgels. Reproduced from ref. 44 with permission from Wiley, copyright 2019. (c) The microgel gelation system and morphologies of the generated microgels (top scale bar: 20 μm; bottom scale bar: 1 μm). Reproduced from ref. 45 with permission from the Royal Society of Chemistry, copyright 2018. (d) The schematics and the morphology of the wettability-guided assembly of a microgel array. Reproduced from ref. 46 with permission from Wiley, copyright 2016. (e) The microgel array formation procedure and outputs. Reproduced from ref. 47 with permission from Elsevier BV, copyright 2016. (f) The schematic diagram of microgel preparation with a mask. Reproduced from ref. 48 with permission from Wiley, copyright 2011. (g) The schematic diagram of a PBP printer for microgel customization and the morphology of fluorescein isothiocyanate (FITC)-labeled microgels before and after the injection. Reproduced from ref. 53 with permission from American Chemical Society, copyright 2019.

Microgels are regarded as a kind of bioink component to realize specific functions that traditional bioink cannot realize.

Extrusion in a changeable environment: Traditional bioink always has thermo-sensitivity, so that the printing environment needs to be strictly set up to guarantee the bioink to be in an appropriate rheological state.[70,71] In comparison, microgels in the microgel-based bioink have been pre-crosslinked by some methods and have a relatively stable structure. Therefore, the microgel-based bioink can excellently resist the outer environment variation and meet different bioprinting conditions.

Deposition on a rigorous receiver: In some cases, bioink needs to be deposited on receivers with rigorous conditions. For example, as an emerging technology, *in situ* bioprinting has attracted attention in the bioprinting field, that is, depositing bioink directly on organ defects with blood and body temperature to improve regeneration effect. Once traditional bioink is deposited on the organ defect with a specific 3D structure, it would be destroyed by the high temperature and blood. However, microgel-based bioink could maintain the 3D structure due to the stability of pre-crosslinked microgels.

Simplifying the printing process: Microgels can simplify the printing process in some complex structure bioprinting cases. The porous structure is an important structure in the 3D bioprinting field because it can provide enough nutrient channels

for the encapsulated cells. For traditional bioink, the porous structure is usually realized by designing a specific printing routine such as grid scaffolds,[72] which increases the printing duration and complexity. By using microgels fabricated from sacrificial materials, a pore network can be easily built by post treatment. For example, by adding gelatin microgels into traditional bioink, which has thermo-sensitivity, the pore network can be simply established by heating the crosslinked structure. Besides, complex 3D structures with a suspended part always need an accompanying supporting structure in the printing process, which would extremely increase the printing duration and complexity. Microgels have been applied for solving this problem by establishing a suspension bath. Sacrificial microgels were accumulated in some container and the printing nozzle was inserted in this suspension bath. By the supporting effect, the bioink can be directly deposited in the suspension bath regardless of the structure complexity. After printing and crosslinking, the suspension bath can be easily removed by some post treatment methods.

Biofunction enhancement: Bioprinting structures are always applied in some biomedical applications such as tissue regeneration and organ models. In order to make the encapsulated cells have specific biological functions, in the bioprinting process with traditional bioink, the printed structures always require specific induction culturing,[73] which increases the application duration. As a function unit, cell-laden microgels can be mass produced and pre-inducted as a kind of production, during which the encapsulated cells are transferred to a differentiated state and have specific biological functions. Thus, structures printed by microgel-based bioink would be directly applied in a series of biomedical applications without waiting for the post induction.

3D bioprinting based on microgel-based bioink mainly uses the extrusion bioprinting method. This method involves extruding bioink from a syringe and a nozzle assembled on 3D bioprinter to form complex 3D structures. However, in extrusion bioprinting, the number of choices for bioink is limited because most soft biomaterials cannot support subsequent layers of printing without crosslinking to build complex 3D structures. However, the accumulation system established by microgels has Bingham fluid features. The system is composed of solid particles on the one hand, allowing it to exhibit elastomeric features, which can retain its original shape or undergo reversible elastic deformation when less strain is applied to the system. In contrast, when a large strain is applied to the system, aggregated microgel systems tend to exhibit some fluid characteristics and have good fluidity. In this way, the microgel-based bioink can perfectly match the demands of bioprinting, and traditional bioprinters (mainly extrusion bioprinters) can perfectly match the requirements for bioprinting methods. Due to the differences between microgel-based and traditional liquid bioinks, there are some special printing factors that need to be considered during microgel bioprinting. These factors are closely related to the flow initiation process of 3D printing and may affect printing quality and cell compatibility:

Microgel deformation: When microgel-based bioink is loaded into a printing syringe, due to the considerable size of accumulated microgels, the syringe wall and nozzle provide resistance and restrict the flow of bioink. Depending on the size of the nozzle opening, microgels would possibly be packed closer to remove aqueous solution from the gap. This results in further deformation, compression, or sometimes rupture before it can flow.

External shear stress: The physical and chemical properties of microgel-based bioink, such as microgel size and mechanical modulus, can affect the dissipation process between microgels. In order to initiate the flow of microgel-based bioink, the external shear stress must exceed the wall resistance and the yield stress of microgel-based bioink.

This review highly generalizes various application approaches focused on bioprinting applications and re-classifies them into two main classes, namely, sacrificed space occupying and secondary assembly (Fig. 7).

3.1 Sacrificed space occupying

Microgels can play a sacrificed space-occupying role in specific applications. By combining sacrificial biomaterials and the above microgel fabrication methods, microgels with special sensitivity to environmental conditions followed by solid–liquid transformation can be fabricated. This allows the microgels to maintain a solid state to occupy some space and provide mechanical support initially, and "disappear" after they have completed their mission in specific physical or chemical conditions (Fig. 8a).

Suspension bath: 3D bioprinting always needs to balance the printability and biocompatibility of the bioink. However, there is often a conflict between the two aspects. For one thing, the encapsulated cells tend to grow in a relatively soft microenvironment (low bioink concentration) to realize survival, proliferation and differentiation. For another, the higher bioink concentration can guarantee the printability due to the higher viscosity. Therefore, it has become a challenge for researchers to simultaneously settle the two questions in a bioprinting process. Suspension baths have become one of the most popular solutions to solve this problem. Sacrificial microgels are fabricated and accumulated in the big holder (printing bath). Firstly, the microgels maintained the solid state and the bioink was directly extruded from the printing nozzle. The extruded filament can be easily supported by the suspension bath and realize the 3D structure deposition. Then, the bioink is crosslinked by a specific method, which finishes the 3D structure formation. Finally, the suspension bath composed of sacrificial microgels is treated by specific physical or chemical methods, resulting in easy removal from the printed structure. Spencer *et al.*[74] used gelatin microgels, which could maintain solid state at low temperature and become liquid at high temperature, as the main component of the suspension bath (Fig. 8b). The engineered GelMA/PEDOT:PSS bioink was extruded in the gelatin microgel bath. Complex 3D structures were successfully printed by this method. Hinton *et al.*[75] reported the bioprinting method of complex 3D structures with soft protein and polysac-

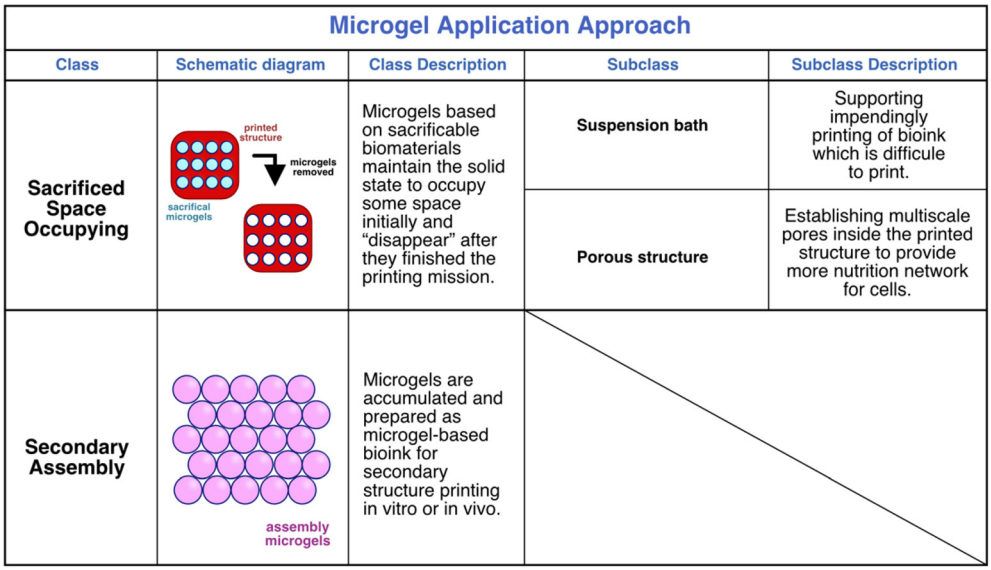


Fig. 7 Schematic diagrams and descriptions of different microgel application approaches.

charide hydrogels (Fig. 8c). The support bath also consisted of accumulated gelatin microgels. The bioink was directly printed in the suspension bath. After crosslinking, the gelatin microgels could be simply removed by heating.

Porous structure: Bioprinting of porous large-scale structures is of great significance in that the pores inside the large-scale structure can provide enough nutrition and air for the encapsulated cell growth. Because of the diffusion gradient, the concentration of nutrition or growth factor molecules from outside would gradually decrease when they diffuse into the large-scale hydrogel network. Thanks to their controllable size, sacrificial microgels have been verified to be a feasible tool to form multi-scale pores inside the hydrogel structures. By simply mixing the sacrificial microgels inside the bioink, they can firstly occupy some space in the deposited bioink. After the bioink is crosslinked, the sacrificial microgels can be treated by specific methods to transform into liquid state, so that amounts of pores can be generated inside the printed structures. In the work of Jiang *et al.*,[76] mesenchymal stem cell (MSC)-laden porous hydrogel scaffolds were successfully printed by adding sacrificial microgels (Fig. 8d). Sacrificial poly (ethylene glycol)-norbornene-dopamine (PEGNB-Dopa) microgels were generated by microfluidic technology. The crosslinked cell-laden PEGNB-Dopa microgels were subsequently added to another hydrogel precursor. After the printing and crosslinking process, the PEGNB-Dopa microgels were rapidly degraded, resulting in lots of uniform pores inside the bulk hydrogel. Furthermore, Xie *et al.*[77] applied the thermosensitive property of gelatin and added gelatin microgels with uniform sizes into GelMA precursor to establish centimeter-scale tumor tissue with angiogenesis (Fig. 8e). The thermo-crosslinked sacrificial gelatin microspheres encapsulating human umbilical vein endothelial cells (HUVECs) were mixed with GelMA precursor mixed with human breast cancer cells (MDA-MB-231s). After printing, the structure was cultured in a 37 °C environment and pores were formed inside the crosslinked GelMA hydrogel and released the encapsulated HUVECs.

3.2 Secondary assembly

In recent years, microgels have gradually evolved into a novel bioink component type due to their tiny size, on-demand functions and promising rheological properties. The accumulation system established by microgels possesses Bingham fluid features (Fig. 9a). On the one hand, the system is composed of solid particles, which allows it to exhibit elastomer characteristics, which can maintain its original shape or undergo reversible elastic deformation when less stress is applied to the system. On the other hand, when a large stress is applied to the system, the aggregated microgel system tends to exhibit certain fluid characteristics and has good fluidity. Thus, the microgel-based bioink can perfectly match the requirements of bioprinting.

So far, the number of reports on the secondary assembly of microgels has been increasing in 3D bioprinting and biomedical application fields. In the latest review of Feng *et al.*,[78] the assembly of microgels was detailedly summarized and discussed. The review summarized the fabrication methods available for microgel assembly, its advanced properties and the potential applications in the biomedical field. Moreover, Daly *et al.*[79] also reviewed the new potential application direction of microgels and predicted the huge development of microgel secondary assembly in future. Ouyang *et al.*[80] also summarized the recent progress and trends of microgel secondary assembly. The microgels were named building blocks in this report. The report provided new classifications of different living building blocks and highlighted state-of-the-art research and trends, inspiring other researchers working in the biomedical field.

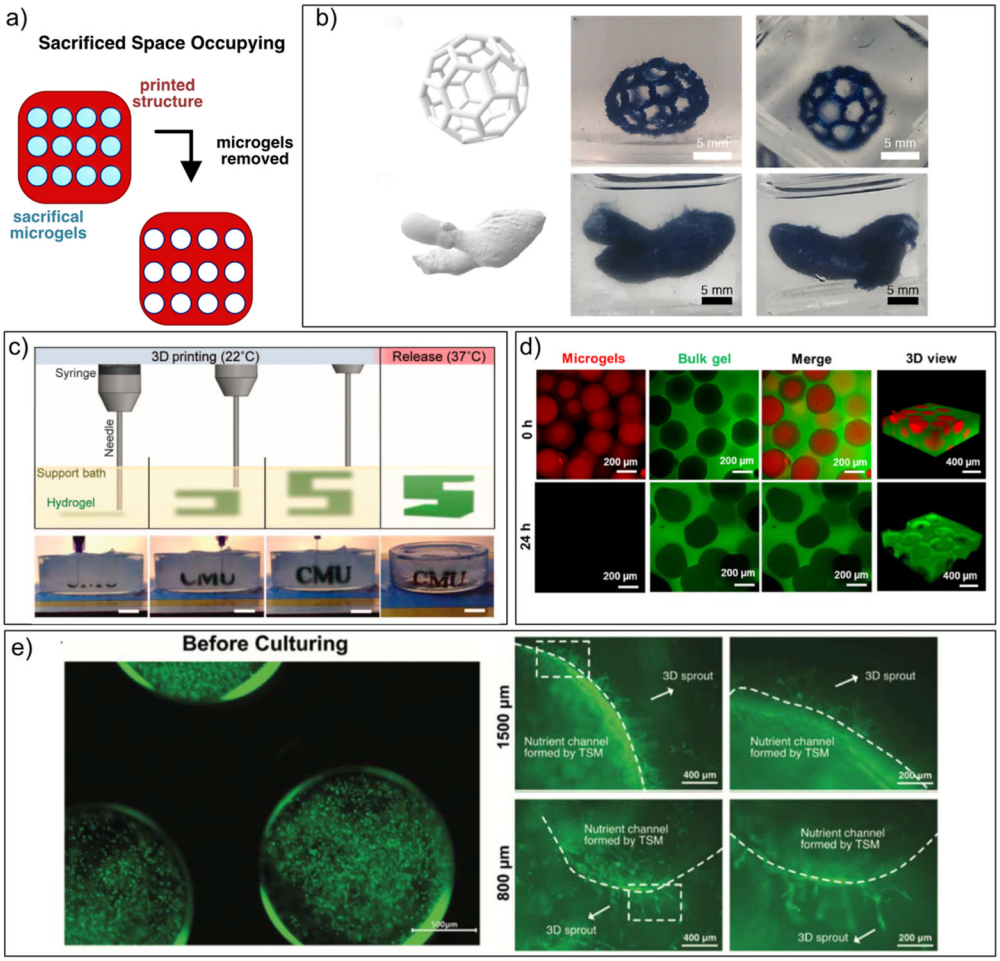


Fig. 8 Sacrificed space occupying with microgels. (a) Schematic of sacrificed space occupying application. (b) The bioprinting of complex structures using the synthesized GelMA/PEDOT:PSS bioink in the gelatin microgel suspension bath. Reproduced from ref. 74 with permission from American Chemical Society, copyright 2019. (c) The suspension printing method by depositing bioink in the thermo-reversible support bath consisting of gelatin microgels. Reproduced from ref. 75 with permission from American Association for the Advancement of Science, copyright 2015. (d) The confocal images of the microgel-templated hydrogel. The dissolution of the microgels would result in a porous structure. Reproduced from ref. 76 with permission from Elsevier BV, copyright 2022. (e) The angiogenesis process in the porous centimeter-scale structures. Reproduced from ref. 77 with permission from Whioce Publishing Pte, copyright 2022.

As a new bioink system, the printability and theoretical research of microgel-based bioink has been gradually carried out. In the work of Xin *et al.*,[81] detailed research by experimental and computational methods on the microgel assembly was reported (Fig. 9b). The external resistance from the printing apparatus and internal physicochemical properties of microgels were revealed. These results could be applied to improve the printability of microgel-based bioink and facilitate their wider applications in the biomedical field. Moreover, Song *et al.*[82] computationally analyzed the printing process of the gelatin microgel-based bioink in extrusion bioprinting in terms of the filament cross-sectional morphology and influence of the yield-stress fluid property on the structural printability, which provided more theoretical references to the related researchers (Fig. 9c).

Besides the basic theory research on the microgel assembly, some research has reported the initial exploration of biomedical applications for the microgel-based bioink. Song *et al.*[83] reported an injectable gelatin microgel-based bioink for scaffold printing, which was composed of solid gelation microgel and crosslinkable gelatin solution phase loaded cells (Fig. 9d). This microgel-based bioink was verified to be feasible for direct bioprinting in air and crosslinked to maintain the printed 3D morphology at room temperature. Fang *et al.*[84] proposed a cell-laden microgel-based biphasic bioink consisting of microgels in close-packed condition and a hydrogel precursor (Fig. 9e). This bioink system was tested to be useful for the printing of a series of hydrogels into complicated 3D structures with high shape fidelity. Jeon *et al.*[85] combined the microgel secondary assembly method with the suspension bath; that is, cell-laden microgels were directly assembled into well-defined 3D shapes *via* freeform reversible embedding of suspended hydrogels, which would potentially provide a significant tool for the establishment of on-demand biomimetic

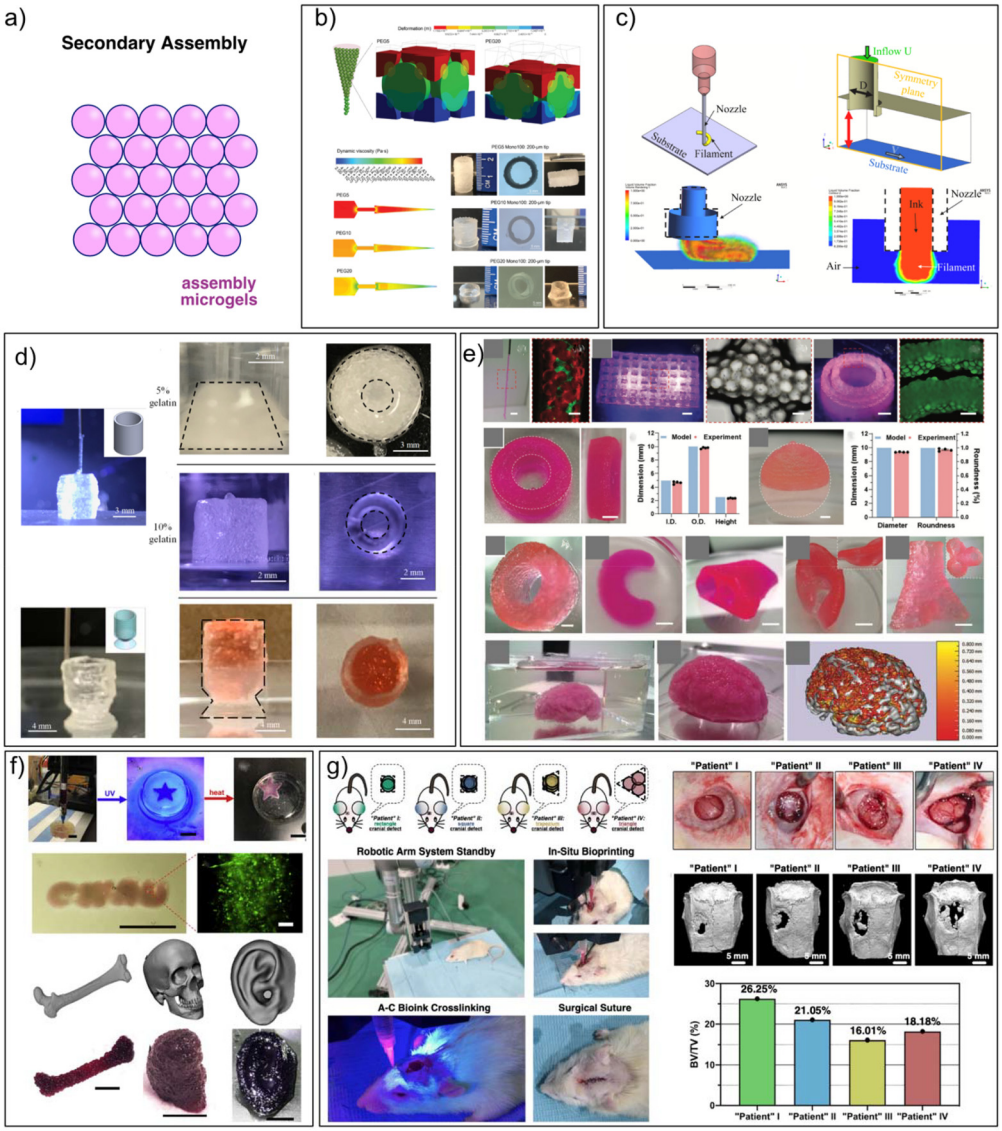


Fig. 9 Secondary assembly with microgels. (a) Schematic of secondary assembly application. (b) The computational modeling of microgel-based bioink deformation in a closed face-centered cubic with gradually increasing force. Reproduced from ref. 81 with permission from American Association for the Advancement of Science, copyright 2021. (c) The schematic of extrusion printing in air, computational model and the front/side view of a simulation of filament deposition. Reproduced from ref. 82 with permission from Elsevier BV, copyright 2021. (d) The tube-shaped and cup-shaped structures during printing with the gelatin microgel-based bioink. Reproduced from ref. 83 with permission from American Chemical Society, copyright 2020. (e) The 3D printing capability and fidelity using MB bioink. Reproduced from ref. 84 with permission from Wiley, copyright 2022. (f) The combination of microgel-based bioink secondary assembly and suspension bath. Reproduced from ref. 85 with permission from Elsevier SCI Ltd, copyright 2019. (g) The *in situ* bioprinting in rat cranial defect models with different morphologies with the proposed microgel-based bioink. Reproduced from ref. 86 with permission from Springer Nature, copyright 2022.

3D tissue *in vitro* (Fig. 9f). Besides the *in vitro* research, recently, Xie *et al.*[86] innovatively introduced microgel-based bioink into organ defect *in situ* repair, namely, *in situ* bioprinting (Fig. 9g). For *in situ* bioprinting,[87–92] the printing conditions could be extremely harsh, such as volatile environment temperature, depositing damage surface with body temperature and blood, *etc*. GelMA microgels were wet by GelMA precursor to build the novel bioink system. Taking advantage of microgel-based bioink in terms of rheological and biocompatible features, the robustness of this bioink system could perfectly match the requirements of various *in situ* bioprinting situations, indicating that this bioink would be potentially applied in clinical organ damage repair.

4. Conclusion and outlook

Through the introduction of the excellent characteristics, preparation methods and application approaches of microgels specifically for the bioprinting field, in this review, it can be

found that the application status of microgels has attracted more and more attention from researchers in related fields. However, there are still a large number of parts that need to be improved in the preparation scheme of microgels, and the structure and spatial distribution design of microgels themselves need to be further improved, and their application fields should be further explored. From our perspective, in the future, the development direction of microgels will mainly focus on the following aspects:

Stable mass production of microgels: The current production and preparation scheme of microgels is still unstable, and the yield is not high. Due to the small size of microgels, in order to meet the needs of large batches in practical applications, it is necessary to improve the production efficiency of microgels. Therefore, in the next few years, researchers should develop new and reliable fabrication devices, which can realize the mass production of microgels for clinical treatment or basic research.

Preparation of complex heterogeneous microgels: Biological tissues are often composed of a variety of microstructures, such as glomerular units, embryos, tumors, *etc*. is composed of vascular networks with microfiber structures and main tissues with spheroid structures. However, the commonly used microfluidic method and inkjet method are not easy to achieve the preparation of heterogeneous microgels with different topological morphologies. In order to better simulate the complex interactions between cells *in vivo*, more reliable solutions need to be explored in the future to prepare various complex heterogeneous microgels to study the complex interactions between tissues and cells *in vivo*.

Secondary printing of new microgel-based bioinks: As mentioned above, a very small number of studies have reported the use of cured microgels as bioink components for specific 3D bioprinting scenarios. These studies have opened up completely new application directions for microgels. However, in recent years, it has been reported that the "secondary printing" of microgels is still stuck in the basic microgel deposition, and has not further developed microgel-based bioink. At the same time, researchers have not yet obtained reliable bioinks for these two application scenarios, which are currently hot research topics in the field of bioprinting—*in vitro* printing of large vascularized tissues and *in situ* printing/repair of defective organs. Combining the unique rheological advantages of microgels, microgel-based bioinks are expected to become more reliable bioprinting bioinks breaking through the limitations and challenges in 3D bioprinting.

Printing method development: As mentioned in this review, microgel-based bioink has been applied in many research studies. However, this excellent bioink is mainly utilized in the extrusion bioprinting process. Photocuring type bioprinting has become a popular printing method due to its high efficiency and accuracy; that is, structures are printed layer by layer with light irradiation and continuously changing electrical masks. However, bioink with low mechanical module, which is regarded as an appropriate microenvironment for cell growth, is always hard to be printed in this excellent printing process in that the crosslinked part cannot maintain the original shape and would cause obvious error to the further layer printing. Microgel-based bioink has the potential to solve this contradiction in the photocuring printing process in that the gathered microgel system simultaneously has certain fluidic and solid profiles. In future, microgels can be fabricated into smaller sizes and the components of microgel-based bioink can be optimized to meet the requirements of photocuring bioprinting and open a brand-new page of bioprinting.

Author contributions

Conceptualization: Yong He, Mingjun Xie; Investigation: Mingjun Xie, Sheng Yan; writing – original draft: Mingjun Xie; writing – review & editing: Yong He, Sheng Yan, Sufan Wu, Ji Wang.

Conflicts of interest

All authors declare no financial/commercial conflicts of interest.

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