Fabricated technology of biomedical micro-nano hydrogel

Yang, Shu; Wang, Fan; Han, Huijie; Santos, Hélder A.; Zhang, Yu; Zhang, Hongbo; Wei, Jie; cai, zhengwei

Published in:
Biomedical Technology

DOI:
10.1016/j.bmt.2022.11.012

Published: 01/06/2023

Document Version
Final published version

Biomedical Technology 2 (2023) 31–48

Contents lists available at ScienceDirect

Biomedical Technology

journal homepage: www.keaipublishing.com/en/journals/biomedical-technology

Fabricated technology of biomedical micro-nano hydrogel

Shu Yang [a,b], Fan Wang [a], Huijie Han [c], Hélder A. Santos [c], Yu Zhang [d], Hongbo Zhang [d], Jie Wei [b,*], Zhengwei Cai [a,**]

[a] Department of Orthopaedics, Shanghai Key Laboratory for Prevention and Treatment of Bone and Joint Diseases, Shanghai Institute of Traumatology and Orthopaedics, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, 197 Ruijin 2nd Road, Shanghai, 200025, PR China
[b] Shanghai Key Laboratory of Advanced Polymeric Materials, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, 200237, China
[c] Department of Biomedical Engineering, W.J. Korf Institute for Biomedical Engineering and Materials Science, University Medical Center Groningen/University of Groningen, Groningen, 9713 AV, the Netherlands
[d] Pharmaceutical Sciences Laboratory, Åbo Akademi University, Turku, 20520, Finland

ARTICLE INFO

Keywords:
Hydrogel
Biopolymers
Microgels
Nanogels
Tissue engineering

ABSTRACT

Micro-nano hydrogel is a novel functional material that has attracted extensive attention in various fields. Due to the size of micron and nano level, high water content and high specific surface area, the micro-nano hydrogels can achieve minimally invasive repair and are considered as promising agents in tissue repair engineering. In this review, we summarize the design and development of micro-nano hydrogels for biomedical applications, first introduce biopolymers for the synthesis of hydrogels, then introduce the preparation technologies of microgels and nanogels respectively, and systematically summarize the application characteristics and forms of different preparation technologies. Finally, the latest application progresses of microgels in local drug delivery, bone tissue repair, soft tissue repair and immunomodulation are introduced in detail, as well as the latest application progress of nanohydrogels in cartilage repair, antibacterial, antitumor/cancer nerve repair and prevention and diagnosis of diseases, and the key research directions of micro-nano hydrogel preparation technologies in the future are clarified.

1. Introduction

Hydrogel is a highly hydrophilic soft material, due to its internal 3D cross-linked network structure similar to extracellular matrix, and at the same time has the characteristics of high swelling, excellent biocompatibility and unique shape plasticity. Hydrogel has been widely used as drug delivery carriers, tissue engineering scaffolds, tissue patches, wound dressings, etc. [1–4]. However, conventional bulk hydrogels often face the risk of infection due to implantation trauma, thus their clinical use is increasingly limited, especially when specific sizes are required. Injectable hydrogels have excellent self-healing properties and can even gel in situ, which allows them to achieve point-based implantation of irregular three-dimensional structures in a minimally invasive manner [5]. However, there are still some unavoidable disadvantages here, first of all, due to the uneven shape and large size of the hydrogel, a relatively high and uneven injection force is inevitably generated during injection [1]. In addition, the release rate of encapsulated drugs in bulk injectable gels is difficult to be controlled, often leading to rapid failure of drug activity. Due to faster gelation, the process may lead to package failure and leakage prior to delivery [6].

Microgel refers to a polymer particle dispersion system with a diameter in the range of 1–1000 μm, which has great application potential in biomedical applications as an advanced microcarrier. Among them, hydrogel microspheres or microcapsules are currently the most common manifestations of microgels, and non-specific interactions *in vivo* can be reduced by modifying biomolecules such as antibodies on their surface. They are small enough to be injected through small needles and catheters, and most popularly used hydrogels are 100–300 μm in diameter, which facilitates minimally invasive delivery of stable cells and biologics [7,8]. And they often have a variety of controllable properties to allow the controlled release of drugs or active molecules, greatly improving the treatment efficiency while alleviating patient suffering. On the other hand, the microspheres are also large enough to provide sites for the adhesion growth of cells. At present, a variety of means have been

* Corresponding author.
** Co-corresponding author.
E-mail addresses: jiewei7860@sina.com (J. Wei), zhengweicai921012@163.com (Z. Cai).

https://doi.org/10.1016/j.bmt.2022.11.012
Received 12 November 2022; Received in revised form 21 November 2022; Accepted 22 November 2022

developed for the preparation of microgels. Among them, the hydrogel particles prepared by spray drying have uniform particle size, low yield, and low flexibility in designability, usually the internal and external structure is relatively simple, which limits its further application in clinical practice [9]. In order to better match the needs of tissue engineering, microfluidics have been developed, which are highly tunable and can enable the preparation of hydrogel particles with multiple functional responses [9]. Moreover, the emergence of 3D bioprinting technology has made it possible to prepare highly homogeneous multi-layer, multi-functional microgels.

Nanogels are dispersions of polymer particles formed by chemical or physical crosslinking of polymer chains, with an internal three-dimensional network structure, and particle sizes typically ranging from 1 to-200 nm, making them an advanced nanodelivery carrier [10]. Compared to microgels, nanogels have a smaller particle size and a larger specific surface area, which is beneficial for further biocoupling. In addition, nanogels can overcome *in vivo* physiological barriers more effectively, and remain stable in the internal circulation, have high bioavailability, and can effectively concentrate bioactive substances *in vivo* [11]. Especially in the administration of special sites such as cartilage, nanogels can overcome the cartilage barrier and effectively achieve drug delivery [12]. Notably, the most important property of nanogels, that is the response to external stimuli (such as temperature or pH changes), makes it promising as an intelligent delivery system. Moreover, because the nanoscale size, it is easier for nanogels to interact with cells and even be internalized, showing good application prospects in biomedicine.

In this review, we systematically summarize the previous research results, discuss the preparation methods of biomedical micro-nano hydrogels, and emphasize the great advantages of micro-nano structures in tissue engineering from the perspective of minimally invasive functional repair (Fig. 1). Firstly, we introduce common materials used to prepare biohydrogels, which are mainly divided into two categories, natural polymers and synthetic polymers. We then discussed the means of preparing technologies for micro hydrogels, including spray drying, electrohydrodynamic spraying, microfluidics and 3D bioprinting. Secondly, methods for preparing nanohydrogels are described, including microemulsion, dispersive polymerization, precipitation polymerization, physical self-assembly, and supramolecular host-guest interaction. Finally, we investigate the main biomedical applications of microgels, including topical drug delivery, bone tissue repair, soft tissue repair, and immunomodulation, as well as nanogels for tissue repair, including cartilage repair, anti-infection, anti-tumor/cancer, nerve repair and prevention and diagnosis of diseases. In summary, this review summarizes the principles and methods of micro-nano hydrogel preparation, with the aim of introducing the application potential of these materials in biomedicine and providing new opportunities for the design and development of advanced biomaterials in the future.

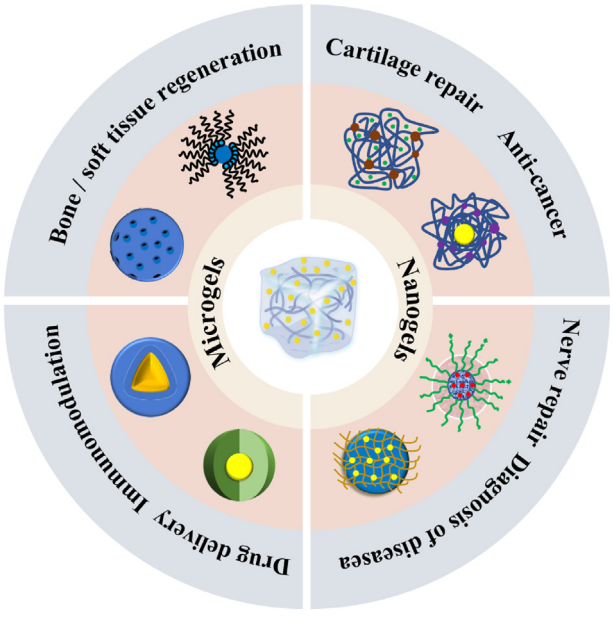

Fig. 1. Schematic diagram of the preparation method of microgels and nanogels.

2. Materials used for the fabrication of biomedical hydrogels

A rich library of polymers have been used for the design and fabrication of biomedical hydrogels. These polymers can be broadly categorized into natural polymers and synthetic polymers. According to the preparation method and mechanism, the method of forming hydrogel is mainly divided into chemical method and physical method, chemical method includes covalent crosslinking and dynamic covalent crosslinking, such as free radical polymerization, Schiff base reaction, etc., physical methods include peptide self-assembly, host and guest left and right, polymer chain entanglement, hydrogen bonding, etc. In this section, we will briefly discuss the commonly used natural polymers and synthetic polymers (Table 1).

2.1. Natural polymers

2.1.1. Cellulose

Cellulose is a linear polysaccharide composed of repeating disaccharide D-glucose units linked by β-1,4 linkage [13]. Cellulose is the main component of plant cell wall and is considered to be the most abundant organic polymer on earth [14]. In addition, seaweed, fungi and some species of bacteria can also produce cellulose, that is, bacterial cellulose [15]. Microbial cellulose film can accelerate the healing process of acute, so it is often used clinically to treat chronic wounds (e.g. burns) [16]. Cellulose-based hydrogel preparation methods are divided into chemical crosslinking (formation of covalent bonds, e.g. free radical polymerization, grafting copolymerization) and physical methods (e.g. ionic interaction, hydrogen bonding) and the products are often high strength and high toughness and are promising candidates for repairing bone defects [17]. Cellulose has also been extensively researched in nanotechnology (e.g. nanofibrillated cellulose) [18,19].

2.1.2. Chitin and chitosan

Chitin is a linear polysaccharide composed of (1–4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose units [20]. Chitin is the second most abundant polysaccharide after cellulose and has similar structure to cellulose except that the hydroxyl group is replaced with an acetamide group (-$NHCOCH_3$) [21]. Chitin is a primary constituent not only in exoskeletons of crustaceans, but also in the arthropod cuticle in general. It is also found in fish scales and fungal cell walls [21,22]. In terms of bio-hydrogel applications, since chitin is almost insoluble in common solvents, it is often converted to water-soluble deacetylated derivatives [23].

Chitosan is prepared by either chemical or enzymatic deacetylation of chitin [24]. The deacetyl degree of chitosan is generally in the range from 30% to 95% [25]. Chitosan is often combined with electrospun fibers and has been broadly applied in tissue repair because of its excellent biocompatibility, antibacterial and antifungal properties, and anti-adhesion properties, as well as abundant sources and low price [26,27]. Clinically, chitosan has been used in skin wound healing and hemostatic dressings [28], as well as approved as a dietary supplement for obesity and hyperlipidemia [29].

2.1.3. Hyaluronic acid

Hyaluronic acid (HA) is a glycosaminoglycan (GAG) that is consisted of alternating disaccharide units of d-glucuronic acid and N-acetyl-*d*-glucosamine, connected specifically by β-linkages [30]. HA is found in

Table 1
Biopolymers for the preparation of hydrogels.

Materials	Source	Feature	Geling methods	Refs
Cellulose	Natural polysaccharides from plants or bacteria	Biocompatibility Biodegradability High strength High toughness	Form covalent bond by chemical method; Reversible physical interactions	[17]
Chitin	Natural polysaccharides from fish scale or Crustacean exoskeketons	Insoluble	converted to water-soluble deacetylated derivatives	[22, 23]
Chitosan	Chitin	Biocompatibility Antibacterial Anti-adhesive	Heat or pH responsive crosslinking; Enzymatic crosslinking	[30]
Hyaluronic acid	Natural component of the extracellular matrix	Biocompatibility Biodegradability Anti-inflammatory Lubrication	Exist multiple chemically active sites and is often Modified by Methacrylic anhydride to HAMA	[33]
Alginate	Natural polysaccharides from seaweed or bacteria	Biocompatibility Biodegradability Temperature dependency	Chelated with divalent cations	[41]
Gelatin	Main components of connective tissue	Biocompatibility Biodegradability Temperature dependency Support for cell adhesion	Cooling physical crosslinking; Modified by Methacrylic anhydride to GelMA	[43, 47]
Polyvinyl alcohol	–	Biocompatibility Non-toxic Bioadhesive	Physical or chemical cross-linking	[57]
Polyethylene glycol	–	Amphiphilic polymers	Light or radiationinduced free radical polymerization	[59]
Poly(N-isopropyl acrylamide)	–	Thermal responsiveness	Physical crosslinking or free radical covalent crosslinking	[64]

extracellular tissue in many parts of the body including cartilage, muscle, skin and it is also a natural component of the extracellular matrix (ECM) [14]. HA is degraded by enzymes such as hyaluronidase produced by cells and it is also subject to degradation under oxidizing conditions [31]. For the preparation of hydrogel, HA is structurally rich in carboxyl and hydroxyl groups, which provide many active sites for multiple chemical modifications. For example, hyaluronic acid can be modified by methacrylic anhydride or glycidyl methacrylate to have a reactive methacrylic acid moiety [32]. In addition, HA can also be modified with thiol, haloacetate, diacylhydrazide, aldehyde and tyramine groups through addition or condensation reactions [33].

Hyaluronic acid was first proposed by Karl Meyer in 1934 [34]. Due to its unique properties, it has been widely studied in bioengineering. For example, in organisms, HA can regulate cellular behavior by interacting with cell surface receptors (e.g CD44) [35]. HA is a signaling molecule and has a wide range of applications in biomedicine, such as tissue engineering scaffolds, drug delivery carriers [36,37]. Currently in clinics, HA is often injected as an intra-articular mucus supplement for the treatment of bone and joints Inflammation [38].

2.1.4. Alginate

Alginate is composed of blocks of (1,4)-linked β-*d*-mannuronate (M) and α-*l*-guluronate (G) residues. According to its source, typically, a block consists of three different forms of polymer segments: continuous G residues, continuous M residues, and alternating MG residues [39]. Alginate is a naturally occurring anionic polymer, usually extracted from brown seaweed and bacteria [39]. The most common method for preparing alginate hydrogels is to combine alginate with divalent cations and ionic crosslinkers (such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Ba^{2+} or Sr^{2+}) [40]. For biomedical applications, alginate has a wide range of application prospects in minimally invasive and topical treatment for it is often used to encapsulate drugs, cells, and growth factors, among others [41]. In addition, as a polymer approved by the U.S. Food and Drug Administration (FDA), alginate has been extensively used in the food industry as a stabilizer [40,42].

2.1.5. Gelatin

Gelatin is a fibrous protein with a unique sequence of amino acids which is obtained from hydrolyzed collagen [43]. Collagen is the major component of connective tissue such as skin, tendons and bones [44]. According to the treatment method, it is usually divided into acid-treated gelatin and alkali-treated gelatin [45]. Currently, the gelatin on the market is mainly derived from porcine, bovine, fish tissues [45]. *In vivo,* gelatin can be degraded by proteases, such as collagenase and metalloproteinases [46]. Gelatin chains contain arginine-glycine-aspartic acid (RGD) sequences which is a cell adhesion site [46]. Thus, adding gelatin has been an effective method to improve the biological behavior over polymers that lack these cell-recognition sites. In addition, Gelatin is also considered as a generally-regarded-as-safe (GRAS) material by FDA [47].

Gelatin can be gelled by various physical or chemical crosslinking. For example, the aqueous gelatin solution can be physically crosslinked into a gel under cooling condition [48]. For chemical cross-linking, some covalent cross-linkers have also been used to react with gelatin such as glutaraldehyde [48]. In addition, gelatin modified with methacrylamide is normally cross-linked by radical polymerization *via* photoinitiationto [49]. For practical applications, gelatin has been widely used in many industries including food, pharmaceuticals, and tissue engineering [50, 51].

2.2. Synthetic polymers

2.2.1. Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a linear synthetic polymer obtained by partial or complete hydrolysis of ethyl polyacetate [52]. PVA is recognized as one of the very few vinyl polymers that are soluble in water, but almost insoluble in organic solvents [53]. PVA can form stable and elastic hydrogels through either physical or chemical cross-linking, such as radiation crosslinking, freezing-thawing, chemical reaction with glyoxal and glutaraldehyde [54–56]. PVA hydrogels have been extensively studied and used in biomedical applications such as articular cartilage replacement and regeneration [57].

2.2.2. Polyethylene glycol/polyethylene oxide

Polyethylene glycol (PEG) is a water-soluble synthetic viscous amphiphilic polymer, and is usually obtained from the anionic or cationic polymerization of ethylene oxide [58]. There are various methods to cross-link PEG polymers into hydrogels. The most common approach to make PEG hydrogels is photopolymerization, which utilizes light to convert liquid PEG macromer solutions into solid hydrogels at physiological temperature and pH [59]. PEG can also form hydrogels by electron beam irradiation *via* radiation-induced free radical processes. PEG hydrogels typically exhibit minimal or no intrinsic biological activity due to the nonadhesive nature of PEG chains [59]. Thus, for biomedical applications, PEG hydrogels can also be modified with various bioactive conjugations such as growth factors and cell-adhesive peptides [60,61].

2.2.3. poly(n-isopropyl acrylamide)

Poly(N-isopropyl acrylamide) (PNIPAm) is a well-known stimulus-responsive copolymers, and is composed of amide (-CONH-) and propyl (-$CH(CH_3)_2$) moieties in the monomer structure [62]. The PNIPAm can be covalently cross-linked by cross-linkers such as bis-acrylamide derivatives through the radical polymerization process [63]. The PNIPAm is a thermoresponsive hydrogel, when temperature is low, the hydrophilic amide group is solvated by the water molecules thus the polymer is soluble [62]. While if the temperature is elevated, it becomes insoluble because of the strengthening of hydrophobic groups. The reversible thermoresponsive behavior happens at a critical temperature of at around 34 °C [63]. The thermoresponsive PNIPAm hydrogels can be used as actuators for soft robotics, injectable scaffolds for tissue engineering, and thermoresponsive substrates for on-demand detachment of cell sheets and have a wide range of applications in smart responsive drug release [64].

3. Preparation techniques for micro hydrogels

Microgel diameters are typically in the range of 1–1000 μm and they can encapsulate various active factors for tissue regeneration or self-assemble in situ to form irregular structures. In this section, we describe the methods usually used to prepare micro hydrogels, including spray drying, electrohydrodynamic spraying, microfluidics and 3D-bioprinting.

3.1. Spray drying

Spray drying method involves the use of a spray dryer, mainly consisting of an atomizer and drying chamber. Solutions and suspensions of drugs, polymers, and particles are atomized to fine droplets. A stream of hot air induces quick evaporation of solvent from the droplets in the drying chamber, resulting in the formation of microspheres or microgels with a diameter of 1–10μm [65]. Chemical crosslinking during spray drying offers great potential for the preparation of hydrogel microspheres. Compared with other methods for preparing microspheres, spray drying technology has the advantages of low cost and green environmental protection. Through further modifications, spray-dried hydrogel microspheres can be used to encapsulate a variety of drugs and cells. For example, E-Sherbiny *et al.* prepared special PLGA nanoparticles loaded with curcumin and then encapsulated these unique nanoparticles into amphiphilic hydrogel microspheres through spray drying based on a PEG-chitosan graft copolymer [66]. The PLGA nanoparticles and the hydrogel microspheres encapsulating the curcumin-loaded PLGA nanoparticles showed average size of 221–243nm and 3.1-3.9μm, respectively. Another example, a composite hydrogel was prepared by combining a chitosan-alginate hydrogel with microspheres composed of a hydrophobic polymer PLGA and a hydrophilic drug DFO [67]. The results showed that the particles contained in the gel were monolithic and spherical in shape with no collapse. Upon dissolution, crystals were found in the hydrogel, indicating that the composite hydrogel was able to trap the drug released from the microspheres and further delay its release. Although spray drying technology has shown excellent promise for the preparation of microsphere-loaded drugs, the low yield of products and the low efficiency of preparation have always been challenging problems.

3.2. Electrohydrodynamic spraying

Electrohydrodynamic spraying is an advanced method of liquid atomization in which a high electric field is used to overcome the surface tension of the liquid. In brief, the polymer solution is extruded through a metal needle tip/nozzle with a high voltage, under the influence of a high electrostatic field, the droplets in the needle connected to the high-voltage generator are transformed into Taylor cones, and subsequent formed micron-sized capsules through cross-linking [68,69]. Here, common droplet formation include dripping, pulsating, cone-jet and multi-jet modes. Since the droplets formed are highly charged, they are generally collected by ring electrodes or grounded solutions [69]. Therefore, it is commonly used for rapid gelation procedure based on ionic cross-linking mechanisms, such as alginate particles [70]. For example, Xu *et al.* fabricated sodium alginate microspheres embedded with magnetic carbon-copper nanoparticles by combining electrostatic droplet (ESD) with copper ions as crosslinking agents [71]. Notably, this method can be implemented under ambient conditions without the need to add additional reagents, which can protect cell viability, especially, the structural integrity of the protein [72]. It is therefore a promising technology for the preparation of microcapsules for minimally invasive tissue repair. In addition, in this method, the size of the final droplet can be changed by adjusting the voltage, the distance between the collection device and the syringe pump, the inner diameter of the injection needle and the pushing speed, etc.

3.3. Microfluidics

Droplet microfluidics is an ideal platform for the synthesis of hydrogel microspheres and are developed on the basis of emulsion methods. In the microfluidic emulsion system, two or more immiscible fluids are connected through geometric channels (such as T-shaped and Y-shaped connections) (Fig. 2 a-d) and then droplets are formed [73]. After formation, the droplets are crosslinked and cured by physical or chemical induction methods to construct micron-sized hydrogel microspheres [9]. This method is relatively simple and can prepare relatively homogeneous microspheres, which are mainly divided into glass capillary microfluidics and soft lithography [9]. Capillary microfluidic device regulate droplets *via* the capillary effect, is less expensive, easy to manufacture, and operate. Large-scale homogenous device preparation, on the other hand, remains a challenge. Soft lithography is used to create microfluidic chips (Fig. 2 e-f), which allows for greater flexibility and resolution (to the micron level). Microfluidic chips frequently include complex internal channels that can be constructed, allowing three-dimensional systems to be transformed rather than being constrained to two dimensions [74]. And in the lithography method, photopolymerization is used to template the production of hydrogel microspheres on a microscale. However, the lithography method limits the complexity of the external or internal structure of hydrogel microspheres, and the yield is low [9,75]. In practice, the suitable preparation process can thus be selected based on the unique needs. Common microfluidic techniques are shown in Fig. 2.

Compared to other microsphere preparation methods, droplet microfluidic methods have the following advantages: 1) By adjusting the device channel size and fluid flow rate, products often have high monodispersity and a wide range of size adjustment which is benefit for the release and degradation behavior of the microgel. 2) By regulating the flow rate of the microfluidic oil aqueous phase and channel geometry, high-throughput, multi-variety, uniform size hydrogel particles can be prepared. 3) The mild manufacturing condition will not destroy the drug activity, which is suitable for in situ encapsulation of bioactive molecules. 4) It is promising to prepare novel multi-functional smart hydrogel

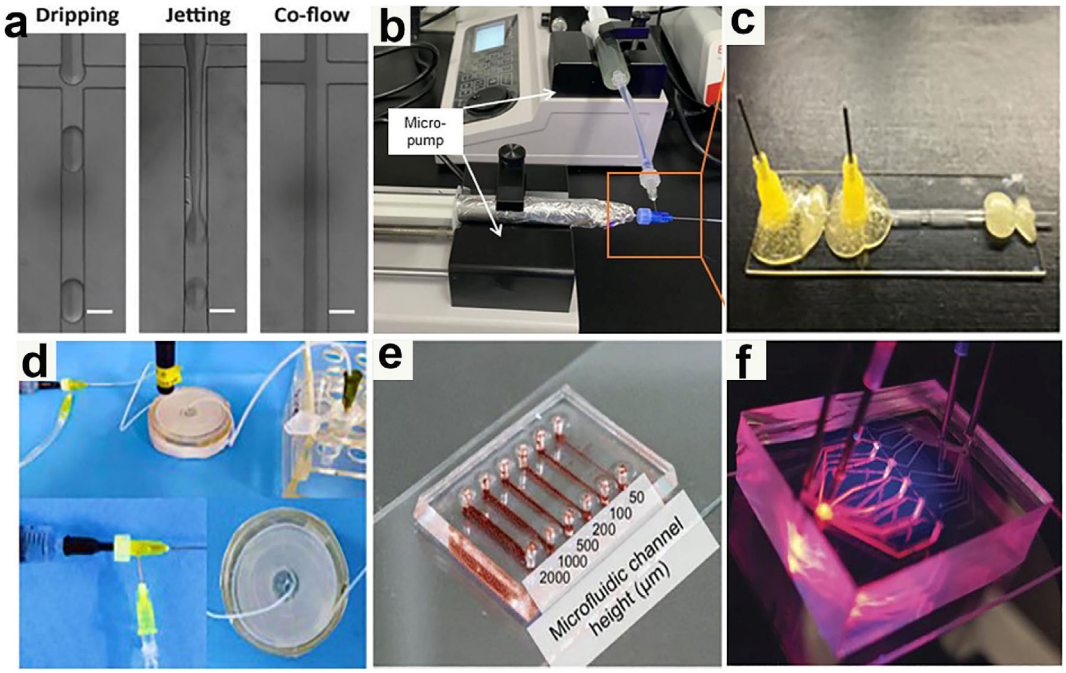

Fig. 2. Common droplet microfluidic devices. a) Flow patterns of liquids in microfluidic devices. Reprinted with permission from Ref. [73], Copyright 2018, Wiley-VCH. b-d) Original figures showing capillary microfluidic devices. Reprinted with permission from Ref. [80], Copyright 2020, Wiley-VCH. Reprinted with permission from Ref. [77], Copyright 2016, Wiley-VCH. Reprinted with permission from Ref. [78], Copyright 2020, Elsevier B.V. e-f) Original figures showing microfluidic chips. Reprinted with permission from Ref. [74], Copyright 2015, Springer. Reprinted with permission from Ref. [82], Copyright 2018, Springer.

with multi-structured and multi-component through the precise improvement of microfluidic devices [9,76]. For example, Zhao *et al.* prepared an injectable hydrogel microsphere loaded with mesenchymal stem cells and growth factors, this gentle gelling condition minimizes damage to the incorporated cells and proteins [77]. Similarly, Wu *et al.* [78] created injectable homogeneous porous microspheres with a particle size of 300 μm and a pore size of 50 μm using a microfluidics simultaneous cross-linked technique. Crosslinking strength was controlled using synchronous crosslinking, which avoided fusion and inhomogeneous crosslinking. One recent study showed that the researchers prepared spherical and rod-shaped particles respectively by appropriately adjusting the microfluidic channel [79]. Here, they studied the effect of particle shape on hydrogel performance and cell invasion conditions, and the results showed that anisotropic rod-shaped particle hydrogels exhibited better cell growth behavior. In another example, inspired by the super-lubricity of ball bearings and the adhesion of mussels, Yang *et al.* [80] prepared ultra-lubricating microspheres in microfluidics with an average diameter of 100 μm (70–140 μm), which are well in line with the knee joint space of the rat and can be used in alleviating osteoarthritis. Han *et al.* [81] fused 3D printed stents and microspheres for drug delivery. The micropore diameter of the 3D printed stent was 452 ± 51 μm, and the diameter of the microspheres was 200 ± 30 μm. Therefore, the microspheres of suitable diameter can evenly penetrate the pores of the scaffold through injection, effectively constructing a composite scaffold with a biomimetic lotus structure and promoting bone regeneration. Moreover, microfluidics techniques have promising potential for bionic applications. A parallel droplet microfluidic device for high throughput cell encapsulation has been reported [82]. Blood vessels play an important role in tissue repair because they can transfer nutrients to cells. Inspired by the rapid angiogenesis of natural microvessels *in vivo*, researchers design engineered customizable microtubes (ECMTs) through coaxial microfluidics (Fig. 3 a) [83]. ECMT can be introduced into the implants by combining with techniques such as injection to form daughter vascular networks for desirable tissue repair (Fig. 3 b). All in all, microfluidics technology has provided great convenience in tissue engineering.

3.4. 3D-bioprinting

As we all know, the efficient and convenient preparation and collection of homogenized microspheres has always been a great challenge. 3D-bioprinting technology is based on pre-designed structures that is, computer-aided patterning and assembly of living and non-biological materials with defined 2D or 3D architectures to prepare tissue-like structures for bioengineering [84]. Generally speaking, materials are crosslinked into a pre-gel by various physicochemical methods, sheared and deposited on the substrate layer by layer, and then solidified under the stimulus of light or heat. The advantage of this technology is the high precision, therefore, 3D bioprinting technology is often widely used in tissue engineering as a good solution to the problem of different tissue defect models, such as multilayered skin, bone, vascular grafts, tracheal splints, heart tissue and cartilaginous structures [85].

Due to its high stability, excellent flexibility and the high degree of manipulability 3D bioprinting is also a very promising technology for the preparation of homogeneous microspheres (Fig. 4 a), especially as a loading medium for active substances such as stem cells.

For example, Xie *et al.* [86] innovatively developed an electro-assisted bioprinting method to rapidly prepare uniform GelMA droplets of about 100 μm in size. By regulating the printing parameters (e.g., voltage, gas pressure, nozzle size, etc.), they designed several different printing states, including microdripping, taylor jet, oscillating jet, and shaking spindle (Fig. 4 b). Notably, in bioprinting, bioink are mixtures of synthetic or natural materials with cells or biomolecules for tissue reconstruction and they can seal cells well and provide support to avoid damage from physical stimuli during printing, so bioinks often require a degree of bioreactivity and rheological properties. And depending on the deposition method, bioprinting processes can be typically divided into three categories: extrusion-based bioprinting, inkjet/droplet-based bioprinting and laser-based bioprinting [87].

Although the microspheres prepared by 3D bioprinting technology have the advantages of high efficiency and controllable deposition position, there are still some inevitable defects, such as high cost and non-negligible damage to cells caused by transient high temperature or high

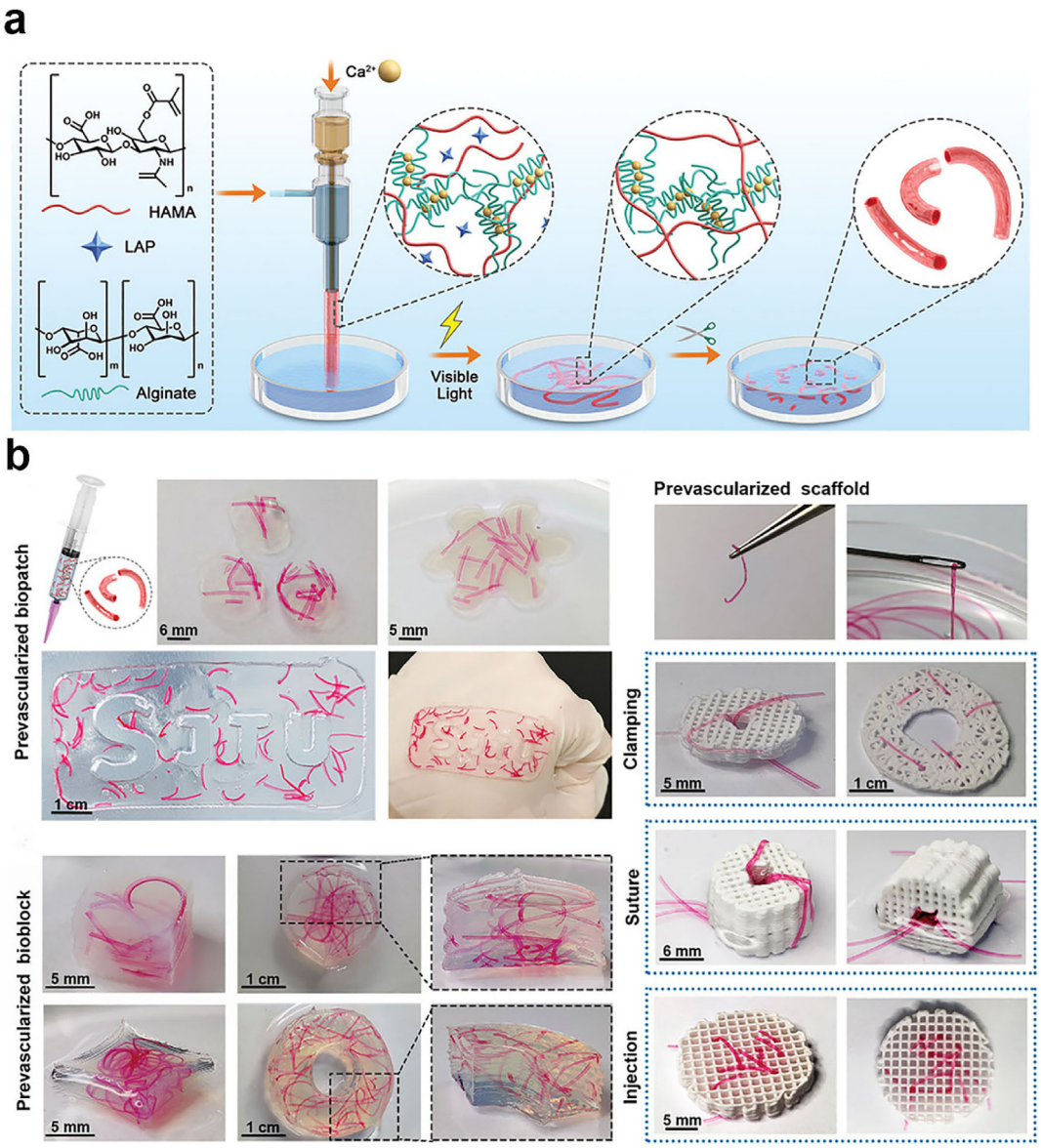

Fig. 3. Schematic diagram of ECMT preparation. a) Schematic depiction of HAMA/Alginate based microtubes prepared using coaxial microfluidic extrusion technique. b) Preparation of patient-specific soft/hard tissue repair models containing ECMT. Reprinted with permission from Ref. [83], Copyright 2021, Wiley-VCH.

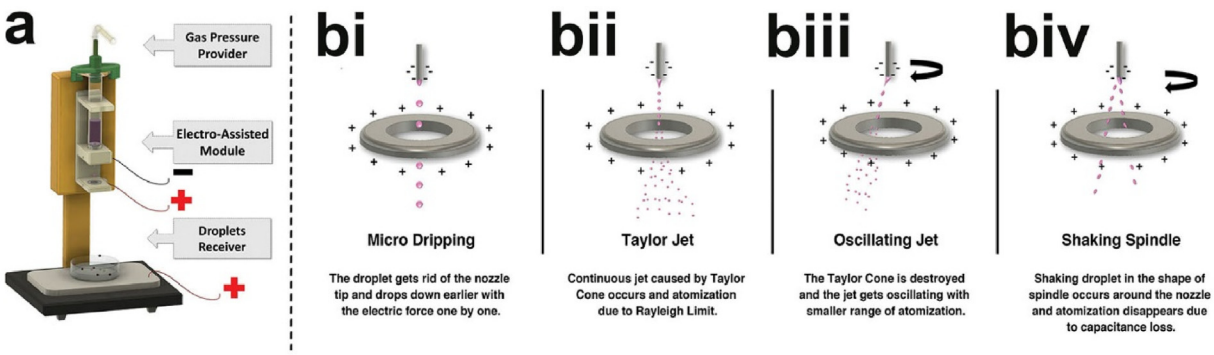

Fig. 4. Schematic diagram of the electro-assisted bioprinting devices. a) Device construction. b) Four printing forms. Reprinted with permission from Ref. [86], Copyright 2019, Wiley-VCH.

pressure in the process. Recently, laser-based bioprinting technology has a wide range of applications due to its ability to maintain high cell viability(above 95%) [88]. In this project, a crucial part of the system is the donor layer that responds to laser stimulation, and during the printing process, under the stimulation of the laser pulse, a part of the donor layer evaporates, creating high-pressure bubbles at the interface of the bioink

layer and thrusting suspended bioink droplets which are collected on the receiving substrate and subsequently crosslinked [88]. However, laser bioprinting often requires high equipment costs and is complex to operate, and many parameters (such as influencing droplet size and quality) have not yet been researched definitely. Moreover, the selection of bioinks for the preparation of stem cell spheroids is still very limited, and there is also a lack of a more complete preparation system.

4. Preparation techniques for nano hydrogels

Nanogels are physical or chemically crosslinked polymer particles (usually recognized as spherical) that have attracted widespread interest in multidisciplinary fields such as nanotechnology and biotechnology due to their excellent stability, viscoelasticity, and high loading and adjustability. In this section, we describe the methods usually used to prepare nano hydrogels, including microemulsion, dispersion/precipitation polymerization, physical self-assembly and supramolecular host-guest reaction.

4.1. Microemulsion

The microemulsion method is one of the most commonly used methods for preparing nanogels. The system generally consists of four components: oil phase, water phase, surfactant and co-surfactant, sometimes also containing an initiator or crosslinker. Most of the commonly used surfactants are either lipophilic Span or hydrophilic Tween or a mixture of both. The hydrogel particle prepared by this method is generally between tens and hundreds of nanometers in size, the operation is simple, and the size and morphology of the nanogel can be regulated by changing the microemulsion structure parameters (such as the type of initiator, the molar ratio of the aqueous phase to the surfactant) and the emulsification method (such as mechanical stirring, ultrasound), and the prepared hydrogel particle has good monodisperse and dispersion [89]. In addition, the nanogel surface is similar to an active membrane and can be modified with various functional groups to achieve the desired properties [90]. According to the distribution structure of the system, there are two types, oil-in-water and water-in-oil. If the oil phase is the continuous phase and the water phase is the dispersed phase, then it is the oil-in-water type; conversely, it is the water-in-oil type. If in the system, in the presence of a large amount of oil-soluble surfactant, the aqueous phase is dispersed in a hydrocarbon-based continuous phase, it forms an inverse microemulsion (Fig. 5 a). Nanogels prepared by the inverse microemulsion method are typically less than 100 nm in diameter [91].

Depending on the mechanism of gel preparation, there are generally two methods: microemulsion polymerizsation and microemulsion cross-linking. For microemulsion polymerizsation, an initiator is added to a microemulsion system and the polymerizsation reaction takes place under chemical or light initiation [89,92]. For microemulsion cross-linking, the cross-linking agent is introduced on the basis of a microemulsion system, cross-link water-soluble polymers, which are modified with reactive groups to obtain a stable gel-like nano-network [93]. Hydrogel particles produced by the microemulsion method have a uniform particle size and are often used as nano-drug carriers in biomedical applications. However, because the emulsification condition often requires vigorous agitation, which affects the loaded drug activity, the final treatment effect is reduced in many cases.

4.2. Dispersion polymerization and precipitation polymerization

Reversible addition-fragmentation chain transfer (RAFT) dispersive polymerization is considered as a universal strategy for synthesizing thermally responsive nanogels with controlled composition, function, and structure. This system usually includes monomers, initiators, stabilizers (dispersants) and sometimes cross-linking agents and surfactants according to the needs. Simply, when polymerization begins, the polymer chains begin to grow until they reach an insoluble critical chain length and eventually result in the formation of a stable distribution of polymer nanogels (Fig. 5 b) [94]. It is worth noting that the RAFT dispersion polymerization method is commonly used to prepare nucleating polymers with specific responsiveness, such as PNIPAm, and corresponding preparation strategies can be designed for the response performance of different polymers [95]. On the other hand, this method also has limitations, that is, water-soluble polymers other than reactive polymers cannot be used for nanogel synthesis, which limits its further applications.

Similarly, precipitation polymerization is the same homogeneous nucleation mechanism and is a common method for preparing monodisperse nanogels. Under the action of the initiator, the monomer begins to polymerize to form low radicals, and the subsequently grown polymer

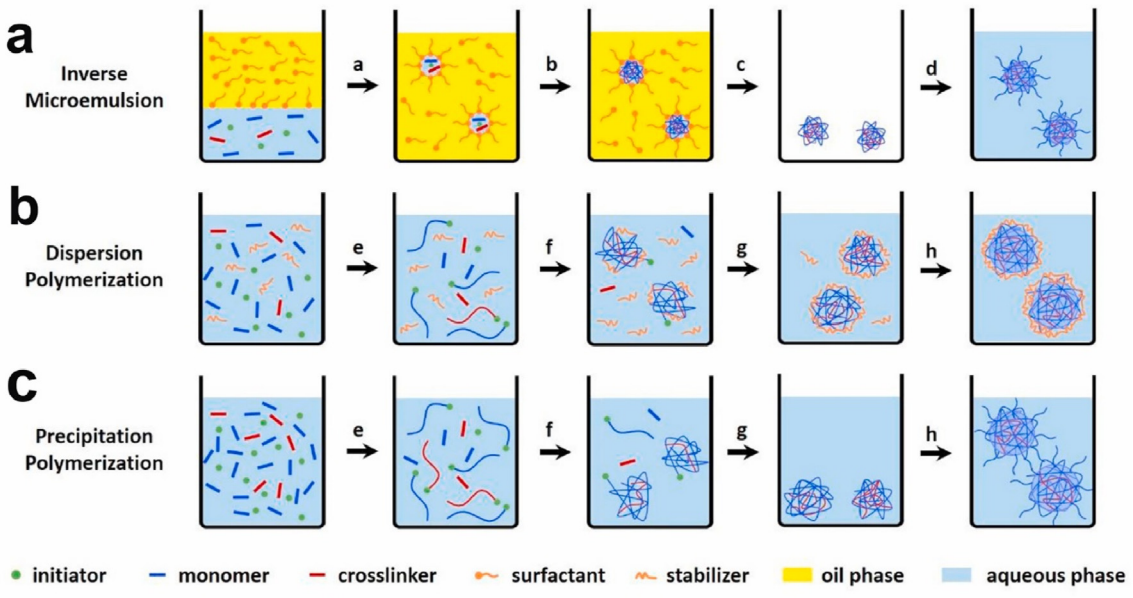

Fig. 5. Schematic diagram of the formation of nanogels by polymerization reactions. a) Inverse Microemulsion. b) Dispersion Polymerization. c) Precipitation Polymerization. Reprinted with permission from Ref. [94], Copyright 2021, Elsevier B.V.

chains subsequently collapse and form precursor particles when they reach critical lengths (Fig. 5 c). And the formed precursor particles can be formed by aggregating with other precursor particles to form larger colloidal particles, which can be grown by depositing onto the surface of existing particles or by further addition of monomers [94]. When the nanogel particles reach a critical size, the polymer chains precipitate out of the continuum to form homogeneous particles. Recently, modified precipitation polymerization methods including distillation precipitation polymerization and reflux precipitation polymerization have also been studied [96]. There are some advantages to the preparation of aqueous nanogels by precipitation polymerization: 1) There have less impurities and no additional surfactants or stabilizers are needed to obtain pure polymer nanogels; 2) The size of the nanogel is controllable; 3) High adjustability, the required performance and complex structure can be obtained by adjusting the formula; 4) The particle size of nanogels is relatively uniform. However, there is still a huge challenge in terms of how to increase productivity.

4.3. Physical self-assembly

Physical self-assembly is undoubtedly one of the simplest and most commonly used methods on synthesizing nanogels. In brief, hydrophilic polymers self-assemble in aqueous systems through electrostatic interactions or hydrophobic or molecular chain hydrogen bonds. Compared with chemical crosslinking, nanogels prepared by this physical crosslinking exhibit new functions due to their dynamic and reversible non-covalent interaction properties, with potential for processing, recovery and self-healing [97]. In general, physical self-assembly is carried out under mild conditions of aqueous media. The size of the nanogel is controlled by polymer concentration, amphiphilia, functional groups,

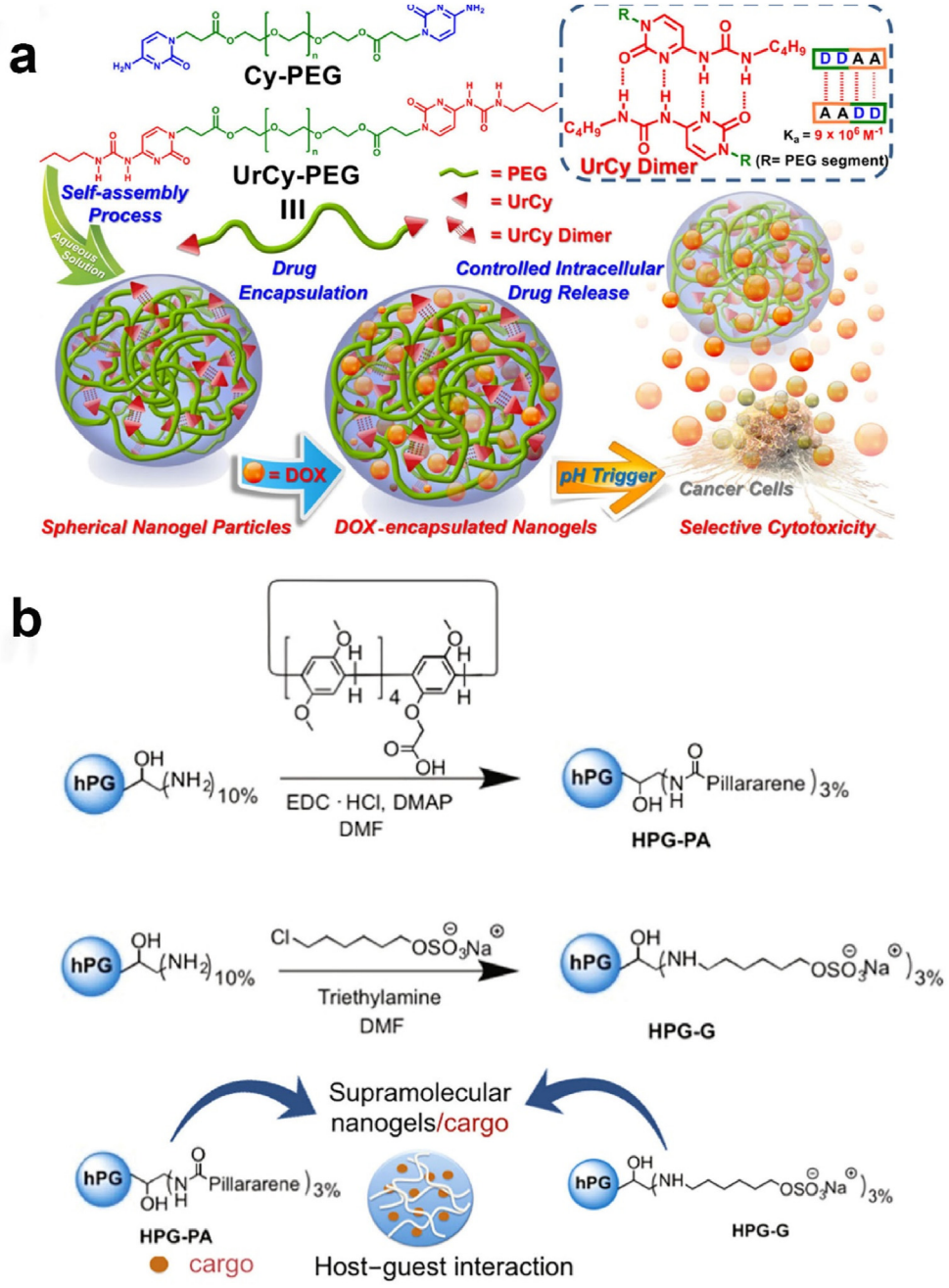

Fig. 6. Schematic of nanogels prepared by physical reaction. a) physical self-assembly. Reprinted with permission from Ref. [99], Copyright 2021, American Chemical Society. b) supramolecular host-guest reaction. Reprinted with permission from Ref. [101], Copyright 2019, Elsevier B.V.

pH, ionic strength, and temperature [98]. For example, the researchers successfully developed two kinds of poly(ethylene giycol)-based telechelic polymers Cy-PEG and UrCy-PEG (Fig. 6 a), with double and quadruple hydrogen bonds, respectively, that can self-assemble directly into spherical nanogels in water [99].

4.4. Supramolecular host-guest reaction

Recently, the use of supramolecular host-guest reaction is a relatively novel method for preparing nanogels. Because of its simple synthesis and preparation, and the multifunctional modification of side groups, it is often used to prepare intelligent functionally responsive nanogels. Common supramolecular host molecules include cyclodextrins, crown ethers, and hyperbranched polymers [100]. For example, the researchers successfully prepared supramolecular polymer nanogels using the host-guest interaction between the conjugated groups pillar arene and alkyl chains on the hyperbranched polyglycerol (hPG) backbone (Fig. 6 b) [101]. Here, the host polymer is used as the main chain (arene) and the guest part (alkyl chain) based on the hPG backbone is the guest. Compared to traditional radical polymerization nanogels, it is easier to improve the chemical or physical properties of supramolecular nanogels by adjusting the proportion of raw material formulations or introducing different stimulatory response groups. Moreover, supramolecular nanogels are often characterized by dynamic and reversible non-covalent action [101]. We have also summarized techniques about the preparation of micro-nano hydrogels in Table 2.

5. Biomedical applications of micro-nano hydrogels

Traditional bulk hydrogels often lack precise control of the microenvironment around the incorporation of cells. And for tissue engineering, very few biological clues, such as growth factors, are required. To overcome these challenges, microgels and nanogels can provide more elegant solutions. They can intelligently release the payload in response to light or thermal or electrical or magnetic stimulation through simple modifications. In this section, we describe the biomedical applications of microgels and nanogels, respectively.

5.1. Biomedical applications of microgels

Generally, hydrogel particles with a diameter of microns are defined as microgels. Due to its small size, it can be injected, and it is soft enough to adhere to the surface of the tissue by modifying its surface. With large specific surface area and high loading rate, it has great application potential in local delivery of therapeutic agents or partially assembled into a granular structure scaffold. Here, we provide an overview of microgels for biomedical applications, including topical drug delivery, bone regeneration, soft tissue repair, and immunomodulation.

5.1.1. Microgels for topical drug delivery

Hydrogels are widely used as delivery vehicles for drugs and various bioactive agents due to their excellent porosity, biocompatibility and high-water content. Traditionally, however, block hydrogels usually have a large external size and may not be ideal for applications where injection or smaller sizes are required. Injectable bulk hydrogels, on the one hand, are often unevenly injected due to their uneven shape and large size. On the other hand, they often require special methods, including in situ and shear thinning crosslinking, because gel formation is a rapid process, which can cause drug molecules to leak during gelation and results in fail delivery [6]. Microgels are an advanced delivery vehicle for drugs and proteins because of their high specific surface area and, unlike conventional hydrogels, they offer significantly enhanced injectability due to their relatively uniform size, avoiding uneven injection. And the drug molecules can also be encapsulated in microgel and prepared in the form of microcapsules. This microcapsule can also encapsulate cells, which protects them from environmental stress and thus better supports cell viability and function. Depending on the mechanism of action, therapeutic molecules are generally incorporated into microgel in three ways: The drug molecules are physically or chemically (covalently coupled) bound to the surface of the microgel (Fig. 7 a); or encapsulated directly in microgel (Fig. 7 b); or the drug molecules are first bound to nanoparticles and then encapsulated in microgel, forming a “nano-micron” structure (Fig. 7 c). Similarly, the means by which spheroids load cells are mainly divided into four types [9]: a) suspension deposition, where a cell suspension is added to a solution containing hydrogel microspheres; b) active fundraising, that is the recruitment of surrounding cells to migrate into the microspheres by encapsulating and continuously releasing certain biologically active substances; c) aqueous phase mixing, that is cells are mixed directly in the microfluidic aqueous phase, producing cell-loaded microspheres through the cleavage of the oil phase and the aqueous phase. d) swelling absorption, i.e., dropping the cell suspension onto lyophilized hydrogel microspheres, which efficiently absorb cells in solution by absorbing water and swelling.

Multiple loading means have been investigated for drug delivery *in vivo*. For example, enlightened by the adhesion of mussels and the limitations of inner ear anatomy, Chen *et al.* [102] constructed viscous injectable hydrogel microspheres that can be locally adhered to the injection site to achieve topical administration by grafting polydopamine (PDA) on the surface of methacrylonitrile-encapsylated gelatin microspheres (GM) and then loading eboselenoline liposomes into GelMA microspheres by physical adsorption. Prinda *et al.* [93] prepared an enzymatically degradable PEG-peptide microgel for the delivery of intracellular drugs to the deep lung by Michael addition reaction using four-arm-poly(ethylene glycol)-acrylate as a cross-linking agent, and this drug carrier exhibited rapid enzyme-triggered release, to avoid long-term clearance by macrophages. However, due to problems such as instability and rapid degradation of the drug molecules, repeated dosing is required to achieve a therapeutic dose, but this can sometimes have toxic side effects which can affect the effectiveness of the treatment [9]. Therefore, how to ensure the precise release of drug molecules at the target location and thus improve drug utilization is a key issue in current research. To overcome this problem, Zhao *et al.* [103] prepared a pH-sensitive composite hydrogel-loaded apigenin by water-in-oil microemulsion method to achieve drug release by regulating pH conditions. Similarly, Su *et al.* [104] developed a dextran-based microgel *via* the Schiff base reaction between aldehyded dextran and ethylenediamine, the results show that microgels containing Schiff bases exhibit significant pH dependence and can be used as promising drug delivery systems for biomedical applications. Considering the degradation and cytotoxicity of polymers, some natural polymers (such as dextran) have also been used to prepare hydrogel particles in the microemulsion method. Vashist *et al.* [90] synthesized auto-fluorescent micro/nano hydrogel particles of chitosan and hydroxyethyl cellulose by water-in-oil emulsion polymerization technique and modified hydrophobically with linseed oil-based polyol. These features can make it possible to be a potential theranostic tool for image-guided therapy. Additionally, to prolong drug activity and achieve sustained release, the incorporation of drug-laden nanoparticles into hydrogel particles is a novel promising strategy to avoid rapid drug

Table 2
Common means of preparation of micro-nano gels.

Methods	Structural precision	Form	Refs
Spray drying	1–10 μm	Microgel	[65]
Electrohydrodynamic spraying	249–3000 μm	Microgel	[68,69]
Microfluidics	50–500 μm	Microgel	[78–81]
3D-bioprinting	90–2200 μm	Microgel	[86]
Microemulsion	50–500 nm	Nanogel	[126]
Dispersion/precipitation polymerization	100–1000 nm	Nanogel	[126]
Physical self-assembly	40–200 nm	Nanogel	[99]
Supramolecular host-guest reaction	4–45 nm	Nanogel	[101]

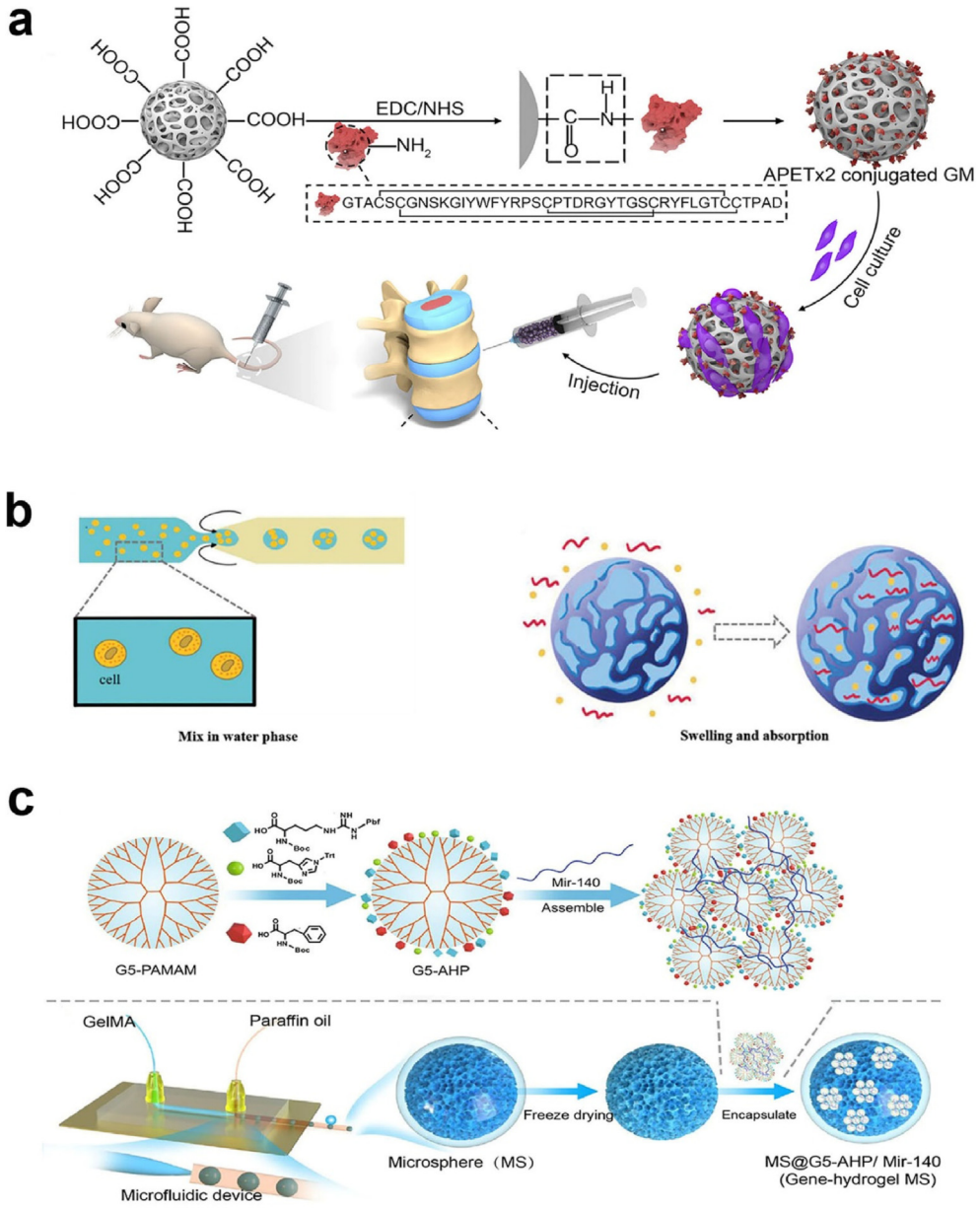

Fig. 7. Common drug packaging models. a) Drug is chemically coupled to the microspheres. Reprinted with permission from Ref. [112], Copyright 2021, American Chemical Society. b) Drug is physically encapsulated directly into the microspheres. Reprinted with permission from Ref. [9], Copyright 2021, Wiley-VCH. c) Drug encapsulation in the form of "nano-micron" structures. Reprinted with permission from Ref. [6], Copyright 2022, Springer.

clearance *in vivo*, thus ensuring drug activity. For example, a copolymer (PEG-g-PHCs) was prepared by grafting polyethylene glycol onto phthaloyl chitosan and then self-assembled with ciprofloxacin to form drug-loaded nanoparticles, which were encapsulated in alginate microgel particles along with free drug molecules [105]. The results show that drug-loaded nanoparticles with a particle size of 218 nm are encapsulated in 3.9 μm micro-hydrogel particles. The dried nano-micron hydrogel particles exhibited rapid initial swelling and showed sustained drug release within 2 min minutes. In summary, the multiple drug loading methods of microgels have greatly enriched the development of biological microcarrier technology, and further pushed minimally invasive treatment to the climax of research.

5.1.2. *Microgels for bone regeneration*

Microcarriers with osteogenic properties have shown great potential in facilitating the repair of bone defects, which can be implanted into the body in a minimally invasive manner, minimizing the patient's pain. Due to its micro-nano size, it is also very effective in filling irregularly shaped bone defects. For example, Zhao et el [106]. constructed bisphosphonate-functionalized injectable hydrogel microspheres (GelMA-BP-Mg) that can effectively capture Mg^{2+} to promote cancellous bone reconstruction in osteoporotic bone defects. And the microspheres have the characteristics of minimally invasive injection, sustained release, and bone targeting, realizing "integrated multi-purpose". Moreover, combining microgels with other materials (such as porous scaffolds) to form composite systems is also a promising repair strategy. For example, a research group has reported a porous bioceramic scaffold with a "lotus seed pod" structure, which achieves both internal vascularization and osteogenesis by using DFO-loaded hydrogel microspheres as "lotus seeds" (Fig. 8 a) [81]. In this study, the 3D printed scaffold provides mechanical support and has a large porosity to provide a three-dimensional plastic structural space for bone reconstruction, while the hydrogel microspheres inside the pore can control the release of DFO for vascularization and stable osteogenesis (Fig. 8 b-c).

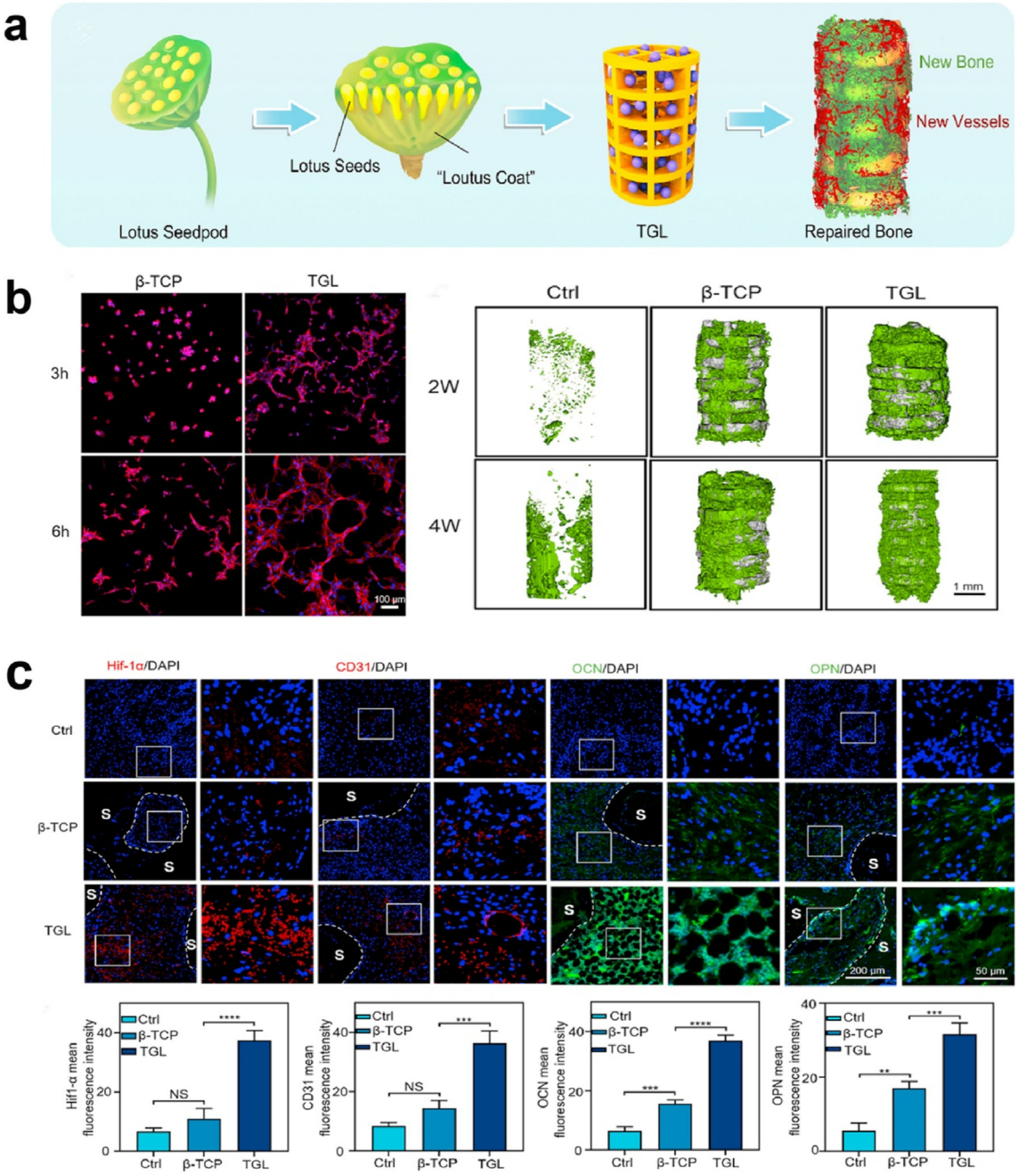

Fig. 8. Schematic diagram of porous bioceramic scaffold structure of "lotus seed pod". a) The unique biological structure of lotus seedpod and inspirational. b) *In vitro* vascularization and *in vivo* bone regeneration performance assessment. c) Histological analysis of newly regenerated tissue in the defect area after stent implantation: Expression of angiogenesis and osteogenesis-related genes and proteins. Reprinted with permission from Ref. [81], Copyright 2021, Elsevier B.V.

5.1.3. Microgels for soft tissue repair

Soft tissue remodeling is a complex process that requires the provision of a variety of dynamic physical and chemical signals to guide cell behavior [107,108]. Especially in the reconstruction of loose soft tissues, balancing the functional and mechanical properties of the material has been a challenging clinical problem. It has been shown that for implant materials, providing the necessary nutrient and oxygen supply to the cells is prerequisite for tissue repair [109]. Blood vessels transport oxygen and nutrients for cell growth and without them, cells may lose their intrinsic function and consequently fail in tissue repair engineering. Zhang *et al.* [110] used hydrogels of corresponding shapes to create micro hollow tubules (MHTs) with various ductal structures, and then co-cultured MHTs with HUVECs to prepare bionic microvessels of 50–500 μm in diameter. Studies showed that they performed well in promoting microcirculation and improving the survival rate of random flaps. ECM provides the mediator for cell behavior and is essential for tissue repair engineering. However, disruption of ECM homeostasis can lead to a variety of degenerative changes in the soft tissues, including cardiosclerosis, skin disease and tumour metastases [111]. Jin *et al.* [111] developed a gene therapy for ECM regeneration. Here, they prepared pLOXL1-Lipo@PLCL-HA by loading pLOXL1 into nanoliposomes *via* microfluidic chips and then encapsulated it into the core layer of core-shell nanofibers *via* microsolvated electrostatic spinning (Fig. 9 a). And the results reveal that pLOXL1-Lipo@PLCL-HA plays a balancing function in controlling ECM degradation/remodeling, which makes it possible to repair ECM defects as cleverly as a "patch" (Fig. 9 b-c).

5.1.4. Microgels for immunomodulation

There are complex biological signaling pathways between cells and cells and between cells and biomaterials, numerous specific mechanisms and the change of cell function activated by biomaterials have been investigated. By modulating the signal network, changes in the microenvironment can be regulated *in vivo*, which helps to improve the efficiency of damage repair and functional recovery. Because the preparation method of microgel is relatively mild, researchers can efficiently load active molecules (such as probiotics, etc.), and prepare a variety of modes of action (oral or injection), e.g. to the immune microenvironment *in vivo*. For example, Bian *et al.* [112] prepared GelMA microspheres by microfluidics and then covalently coupled APETx2 on the surface of the microspheres and further loaded myeloid cells to construct an injectable "peptide-cell-hydrogel" structure for the regulation of local inflammation. The intestine is the largest immune organ of the human body and contains a rich and diverse intestinal flora, which has a non-negligible role in the entire immune system. Liu *et al.* [113] innovatively designed a colon-targeted adhesive core-shell hydrogel microsphere (HAMs) for the regulation of the intestinal immunobiome to achieve drug delivery by oral administration (Fig. 10 a). In this study, the core-shell hydrogel microspheres protect the drug from premature exposure, can collapse in a specific location, and accurately release HMs (Fig. 10 b). And the results showed that released HMs accumulate in the inflamed colonic mucosa, modulating intestinal inflammation by inhibiting the secretion of pro-inflammatory cytokines and inducing M2 differentiation to dominate the immune response (Fig. 10 c). In conclusion, microgels have shown great advantages in immunomodulation and the combination of microgels with immunomodulatory properties has become an important novel platform for tissue repair engineering.

5.2. Biomedical applications of nano hydrogel

Due to the advantages of stable size, superior hydrophilicity and biocompatibility and responsiveness to specific environments, in particular, the low non-specific interaction of nanogels with blood proteins, which avoids the occurrence of immune responses, nanogels are considered to be potential candidates for biomedicine. In this section, we briefly introduce the latest applications of nanogels in cartilage repair, antibacterial anti-tumor/cancer, nerve repair and prevention and diagnosis of diseases.

5.2.1. Nanogels for cartilage repair

Osteoarthritis due to cartilage damage remains a major clinical repair challenge because it has no structures such as blood vessels and cells, and is often difficult to cure. Due to its high water content and strong lubricity, hydrogels show good application potential in cartilage repair. Among them, nano-gels, as the most advanced nanocarriers, show excellent application prospects. The nanoencapsulation system has strong tissue permeability and can achieve good targeted release in some structurally complex tissues or organs, which is conducive to improving drug utilization. For example, Qi *et al.* [114] reported a novel dynamic nanocomposite microgel system for the repair of articular cartilage. In this system, they first prepared cyclodextrin nanogel particles loaded with kartogenin (KGN) and further wrapped a "protective shell" on their surface by droplet microfluidics. Finally, the formed microgel component is injected into the articular cartilage defect by minimally invasive treatment. The results showed that this microgel nanocomposite system showed good tissue adhesion to natural cartilage/bone tissue, and significantly promoted articular cartilage repair *in vivo*. However, the high density of extracellular matrix around the cartilage and the negative electrostatic barrier effect are the main reasons for the difficulty in drug penetration. To solve this issue, Lin *et al.* [12] prepared injectable

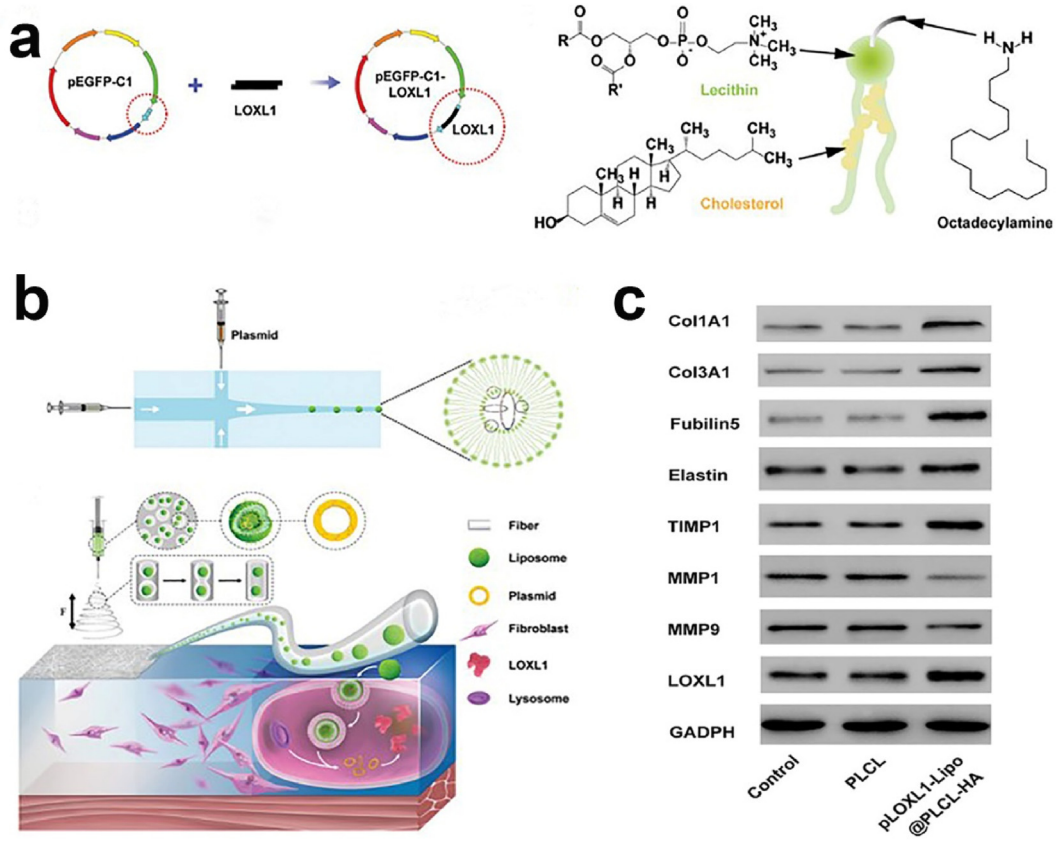

Fig. 9. Microgel composite system repairs ECM defects. a) The construction of recombinant pEGFP-C1-LOXL1 and the composition of pLOXL1-Lipo. b) Diagram of the action of the gene electrospun fibers. c) WB of related genes. Reprinted with permission from Ref. [111], Copyright 2021, Wiley-VCH.

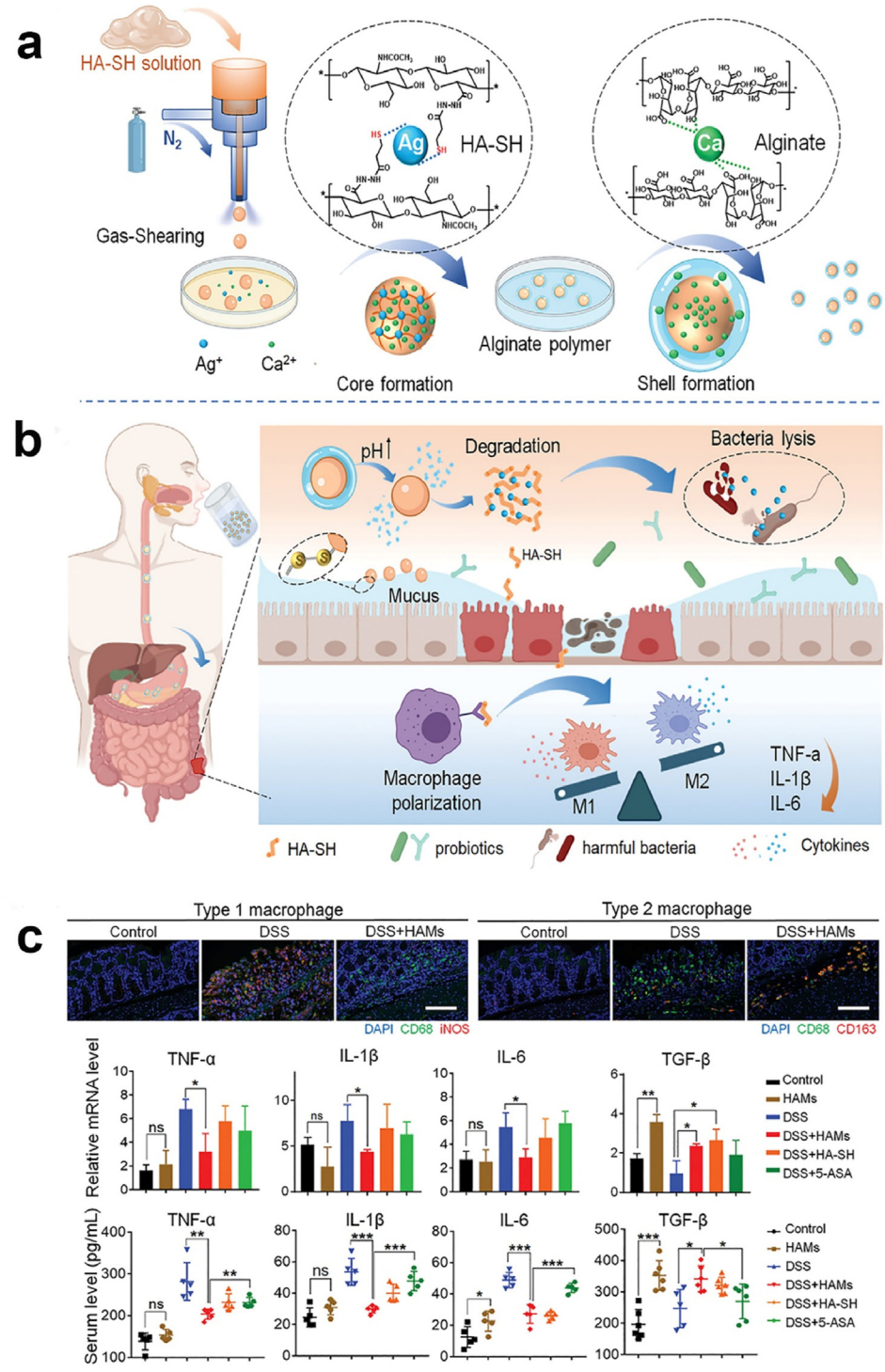

Fig. 10. The microgel complex regulates intestinal inflammation *in vivo*. a) The HA-SH-Ag hydrogel microsphere (HMs) with uniform size is fabricated through The gas-shearing technology. b) Schematic of the principle of action of oral HA-SH-Ag/alginate-Ca microspheres (HAMs). HMs released by HAMs accumulate in inflamed colon mucosa, regulate gut inflammation by suppressing the secretion of pro-inflammatory cytokines and inducing type 2 (M2) macrophage differentiation dominated immune response. c) Immunofluorescence analysis of macrophages in colonic tissue (Type 1 macrophages: iNOS red, CD68 green, and DAPI blue and type 2 macrophages:CD163 red, CD68 green, and DAPI blue); Colonic mRNA expression and serum concentrations of the tissue repair-associated cytokines transforming growth factor-β (TGF-β) and pro-inflammatory cytokines (including IL-6, IL-1β and TNF-α). Reprinted with permission from Ref. [113], Copyright 2021, Wiley-VCH.

mucoadhesive microspheres using positively charged nanoliposomes containing drugs as the secondary structure of hydrogel microspheres using microfluidic technology (Fig. 11 a-b). In this study, dopamine-modified structures on the surface of microspheres can firmly attach beads to the surface of cartilage, while charge-guided nanoliposomes can carry drugs to effectively penetrate the cartilage matrix and release drugs stimulated by reactive oxygen species, acting on chondrocytes (Fig. 11 c).

5.2.2. *Nanogels for antibacterial*

The formation of bacterial biofilms is currently one of the biggest causes of implant failure in clinical practice, leading to multiple complications and even death. Due to the advantages of stability and drug targeting, the nanogel carrier can achieve local high concentration release, and has great application potential in antibacterial and anti-infection. For example, Liu *et al.* [115] designed and synthesized silver thiolide nanoclusters (AgNCs) and further impregnated them in chitosan, successfully preparing multifunctional nanogels. Compared with unencapsulated AgNCs, it exhibits enhanced antimicrobial activity (approximately > 10-fold). Similarly, the researchers encapsulated thymol in a nanogel through UV-induced crosslinking for antifungal applications in the oral cavity [116]. And the permeability and bioavailability of thymol

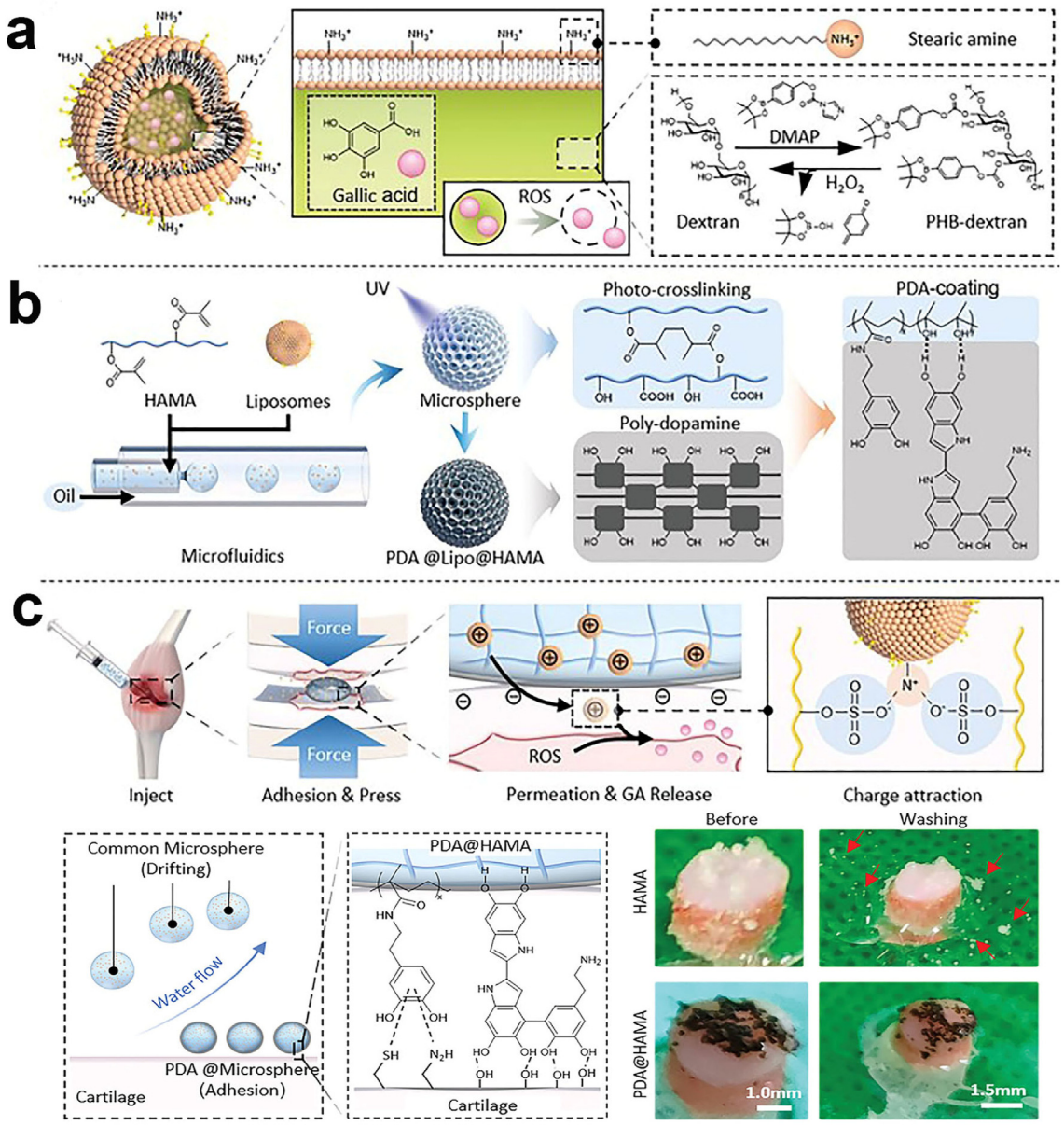

Fig. 11. Nano liposomes penetrate the barrier by charge force to repair cartilage. a) Synthesis of positively charged liposomes loaded with GA. b) Diagram of microsphere preparation using microfluidics. c) Schematic of the mechanism of action of charge-guided micro-nano-microspheres adhering to cartilage. Reprinted with permission from Ref. [12], Copyright 2021, Wiley-VCH.

are significantly enhanced due to the nano-size of the nanogel. In addition to acting as an antibacterial microcarrier, the nanogel has also shown satisfactory results on antibacterial coatings.

5.2.3. *Nanogels for anti-tumor/cancer*

As a nanoscale delivery carrier, nanogel effectively prolongs the blood circulation time of the drug and avoids the immune response due to its excellent biocompatibility and hemocompatibility, which is currently an effective way to deliver chemotherapy drugs to cancer sites to improve the effect of chemotherapy [117]. Moreover, its high load and stability characteristics also effectively enhance the anti-tumor activity of the drug, in addition to improving the tumor microenvironment, it also shows great application potential in imaging diagnosis. For example, Lin *et al.* [118] developed a novel multifunctional hyaluronic acid nanogel (mHA-GC) by encapsulating gold clusters and DOX for early diagnosis and treatment of tumors. Here, mHA-GC nanogels can exhibit specific release to the glutathione (GSH) tumor microenvironment with a high reducing agent concentration. And thanks to the introduction of gold nanoclusters, mHA-GC nanogels can be used for *in vivo* and *in vitro* imaging under the influence of near-infrared light. More importantly, the fluorescence signal of the hybrid nanogel increases with the increase of GSH concentration, which further improves the accuracy and targeting of tumor diagnosis and treatment. On the other hand, photodynamic and photothermal therapy (PTT) are currently recognized as effective means of treating tumors, and satisfactory progress has also been made by further combining with nanogels to form a composite system. Li *et al.* [119] have for the first time developed a PTT-induced, oleanolic acid (OA) -based self-assembled nanoregulator ORM for remodeling the tumor microenvironment (TME) and delivering drugs to deep tumor tissue. Under the action of near-infrared light, physical "thermal" treatment of the tumor can remodel the ECM to allow further penetration of the released target drug. And the results show that this "hot" immune microenvironment has the anti-tumor efficacy of long-term immune memory. Additionally, in order to achieve a good treatment of tumors, an

effective simulation model is indispensable. Smart nanogel drug carriers have similarly demonstrated superior therapeutic efficacy in multicellular spheroid tumor models *in vitro* [120].

5.2.4. Nanogels for nerve repair

Due to the complexity of the underlying disease mechanisms and the unique biological microenvironment, the current clinical treatment of the central nervous system remains extremely limited. Nanogels have also shown promise in repairing nerve damage for their unique microstructure and mechanical properties. A recent study showed that using nanogels as a carrier can effectively deliver various active molecules (especially peptide drugs) into dorsal root ganglia intracellularly, significantly enhancing axon regeneration after CNS injury [121]. Moreover, the researchers prepared heat-sensitive nanogels that triggered drug release through external near-infrared light stimulation for prolonged nerve block [122]. And because of the reversible nature of heat-sensitive nanogels, the *in vitro* release of drugs can be repeatedly triggered to achieve controlled pulse release of drugs, so as to achieve the effect of spatiotemporal control. In summary, due to the characteristics of high loading, targeting and prolonging the duration of drugs, nanogel carriers also have outstanding application advantages in neural repair.

5.2.5. Prevention and diagnosis of diseases

Recently, more and more researchers have focused on nanodiagnostics. Due to their excellent biocompatibility, large specific surface area and intelligent response, nanogels have also shown great application potential in cancer diagnosis, such as phototriggered imaging (including fluorescence imaging (FLI), photoacoustic imaging (PAI), photothermal imaging (PTI)) and magnetic resonance imaging (MRI) contrast agents [123]. For example, recently, the researchers prepared ultra-small size nanogels (5–20 nm) for MRI contrast agents using gelatin as the matrix, and confirmed the non-cytotoxicity and effective relaxation of the nanogels *in vivo* experiments [124]. Similarly, Zhang *et al.* [125] used emulsion polymerization to prepare magnetic nanogels (MNLs) as multifunctional carriers for breast cancer MRI imaging and chemotherapy. The results show that MNLs with high magnetic content have a potential advantage in MRI for breast cancer diagnosis.

6. Conclusion and outlook

Micro-nano hydrogels overcome the limitations of traditional hydrogels to enable a wider and more far-reaching delivery of drugs or biomolecules. In the past two decades, the technical means and engineering design of micro-nano hydrogel preparation have made significant progress, and adapted or realized a variety of biomedical applications, opening up a world full of possibilities for tissue repair engineering.

Although many studies have fully demonstrated the great advantages of micro-nano hydrogels in biomedical applications, there are still some key issues to be solved, especially in the realization of scale and industrialization, the diversity and richness of structural design, and the interaction between biomaterials and cells, need to be explored more deeply through more research. Here, we briefly describe some of the challenges that need to be addressed in order to better inform future research:

(1) Production and industrialization are essential for the widespread use of micro-nano hydrogels in tissue engineering. Efficient and rapid homogenization of microparticles remain a great challenge. Therefore, the future research direction should be committed to designing more convenient preparation methods, reducing the technical cost of equipment, and achieving yield production.
(2) Research and design of micro-nano hydrogels are limited. For example, at present, the preparation of hydrogel microspheres is mainly based on droplet microfluidic preparation technology, which has low throughput and is difficult to achieve mass production; Moreover, due to the influence of mechanical effects in the device, spherical particles are usually generated, and other geometries are difficult to achieve. Therefore, future research should focus on the design of particle shapes, developing novel techniques to simulate the effects of different shapes of hydrogel particles on cell behavior. These simulations may have important implications for the rational design of biomaterials that promote endogenous remediation.
(3) Attention should be paid to the interaction between biological materials and tissues in the body. When current materials enter the body to function, they still do not match normal physiological functions well. In the future, while developing new and excellent biomaterials, we can also focus on the design of dynamic materials, and design biomaterials that can simulate natural physiological processes and thus respond to the release according to the differences in the microenvironment at different stages in the body.
(4) The overall preparation procedure of materials used in bioengineering is relatively complex. At present, the mainstream of tissue engineering generally requires materials to be carefully designed and combined with a variety of active molecules, layer by layer, and finally achieve the purpose of treatment. The preparation process is cumbersome, and the release of active molecules and the maintenance of cell activity in the process are difficult to control, and for some special drugs, there is even a threat of drug resistance. Therefore, in the future, we can focus on the development of new materials and truly realize the “integration” of material functions.
(5) Tissue repair is a complex process that involves multiple signal interactions. Nerves play an important role in signaling, but research designs for neural repair are still relatively limited. Therefore, future research can combine advanced micro-nano manufacturing technology to make “true efforts” in the preparation of intelligent functional neural repair micromaterials.
(6) Finally, tissue engineering research needs to be accompanied by technological developments. Several studies have been reported on the use of hydrogel microactuators for biomimetic applications. In the future, it is believed that micro-nano hydrogels can be more combined with electricity or magnetism to act as “intelligent” biomimetic devices, opening up new ways for biosensors, drug diagnosis, targeted drug delivery, etc.

Acknowledgements

This research is funded by National Natural Science Foundation of China General Program (32171340, 32101104), Shanghai Municipal Health Planning Commission (202140127).

References

[1] Y. Han, J. Yang, W. Zhao, H. Wang, Y. Sun, Y. Chen, J. Luo, L. Deng, X. Xu, W. Cui, H. Zhang, Biomimetic injectable hydrogel microspheres with enhanced lubrication and controllable drug release for the treatment of osteoarthritis, Bioact. Mater. 6 (10) (2021) 3596–3607.
[2] Y. Li, J. Wang, Y. Wang, W. Cui, Advanced electrospun hydrogel fibers for wound healing, Compos. B Eng. 223 (2021) 14.
[3] H. Yuk, C.E. Varela, C.S. Nabzdyk, X. Mao, R.F. Padera, E.T. Roche, X. Zhao, Dry double-sided tape for adhesion of wet tissues and devices, Nature 575 (7781) (2019) 169–174.
[4] Q. Xuan, F. Jiang, H. Dong, W. Zhang, F. Zhang, T. Ma, J. Zhuang, J. Yu, Y. Wang, H. Shen, C. Chen, P. Wang, Bioinspired intrinsic versatile hydrogel fabricated by amyloidal toxin simulant-based nanofibrous assemblies for accelerated diabetic wound healing, Adv. Funct. Mater. 31 (49) (2021) 20.
[5] J.S. Lee, J.H. Cho, S. An, J. Shin, S. Choi, E.J. Jeon, S. Cho, W in situ self-cross-linkable, long-term stable hyaluronic acid filler by gallol autoxidation for tissue augmentation and wrinkle correction, Chem. Mater. 31 (23) (2019) 9614–9624.
[6] B. Li, F. Wang, F. Hu, T. Ding, P. Huang, X. Xu, J. Liang, C. Li, Q. Zhou, M. Lu, L. Deng, L. Guo, W. Cui, Injectable "nano-micron" combined gene-hydrogel microspheres for local treatment of osteoarthritis, NPG Asia Mater. 14 (1) (2022) 15.

[7] A.C. Daly, L. Riley, T. Segura, J.A. Burdick, Hydrogel microparticles for biomedical applications, Nat. Rev. Mater. 5 (1) (2020) 20–43.
[8] R.J. Kulchar, B.R. Denzer, B.M. Chavre, M. Takegami, J. Patterson, A review of the use of microparticles for cartilage tissue engineering, Int. J. Mol. Sci. 22 (19) (2021) 24.
[9] Z. Zhao, Z. Wang, G. Li, Z. Cai, J. Wu, L. Wang, L. Deng, M. Cai, W. Cui, Injectable microfluidic hydrogel microspheres for cell and drug delivery, Adv. Funct. Mater. 31 (31) (2021) 24.
[10] Z. Zhang, G. Hao, C. Liu, J. Fu, D. Hu, J. Rong, X. Yang, Recent progress in the preparation, chemical interactions and applications of biocompatible polysaccharide-protein nanogel carriers, Food Res. Int. 147 (2021) 14.
[11] L.C. Chen, Y.P. Kong, Y. Zheng, S.Y. Zhang, L.Y. Zhang, J.Y. Wang, Preparation of coix seed oil bioactive delivery systems based on homologous polysaccharides and proteins, Int. J. Biol. Macromol. 151 (2020) 376–383.
[12] F. Lin, Z. Wang, L. Xiang, L. Deng, W. Cui, Charge-guided micro/nano-hydrogel microsphere for penetrating cartilage matrix, Adv. Funct. Mater. 31 (49) (2021) 12.
[13] R.J. Moon, A. Martini, J. Nairn, J. Simonsen, J. Youngblood, Cellulose nanomaterials review: structure, properties and nanocomposites, Chem. Soc. Rev. 40 (7) (2011) 3941–3994.
[14] V.G. Muir, J.A. Burdick, Chemically modified biopolymers for the formation of biomedical hydrogels, Chem. Rev. 121 (18) (2021) 10908–10949.
[15] R. Portela, C.R. Leal, P.L. Almeida, R.G. Sobral, Bacterial cellulose: a versatile biopolymer for wound dressing applications, Microb. Biotechnol. 12 (4) (2019) 586–610.
[16] W.K. Czaja, D.J. Young, M. Kawecki, R.M. Brown, The future prospects of microbial cellulose in biomedical applications, Biomacromolecules 8 (1) (2007) 1–12.
[17] L.H. Fu, C. Qi, M.G. Ma, P. Wan, Multifunctional cellulose-based hydrogels for biomedical applications, J. Mater. Chem. B. 7 (10) (2019) 1541–1562.
[18] H. Du, W. Liu, M. Zhang, C. Si, X. Zhang, B. Li, Cellulose nanocrystals and cellulose nanofibrils based hydrogels for biomedical applications, Carbohydr. Polym. 209 (2019) 130–144.
[19] F. Rol, M.N. Belgacem, A. Gandini, J. Bras, Recent advances in surface-modified cellulose nanofibrils, Prog. Polym. Sci. 88 (2019) 241–264.
[20] X. Hu, Y. Du, Y. Tang, Q. Wang, T. Feng, J. Yang, J.F. Kennedy, Solubility and property of chitin in NaOH/urea aqueous solution, Carbohydr. Polym. 70 (4) (2007) 451–458.
[21] R.A.A. Muzzarelli, Chitin Nanostructures in Living Organisms, Springer, Dordrecht, 2011, pp. 1–34.
[22] W.J. Tang, J.G. Fernandez, J.J. Sohn, C.T. Amemiya, Chitin is endogenously produced in vertebrates, Curr. Biol. 25 (7) (2015) 897–900.
[23] K. Kurita, Controlled functionalization of the polysaccharide chitin, Prog. Polym. Sci. 26 (9) (2001) 1921–1971.
[24] D. Gómez-Ríos, R. Barrera-Zapata, R. Ríos-Estepa, Comparison of process technologies for chitosan production from shrimp shell waste: a techno-economic approach using Aspen Plus (R), Food Bioprod. Process. 103 (2017) 49–57.
[25] A. Di Martino, M. Sittinger, M.V. Risbud, Chitosan: a versatile biopolymer for orthopaedic tissue-engineering, Biomaterials 26 (30) (2005) 5983–5990.
[26] K. Kalantari, A.M. Afifi, H. Jahangirian, T.J. Webster, Biomedical applications of chitosan electrospun nanofibers as a green polymer – Review, Carbohydr. Polym. 207 (2019) 588–600.
[27] L. Zhu, L. Peng, Y.Q. Zhang, The processing of chitosan and its derivatives and their application for postoperative anti-adhesion, Mini Rev. Med. Chem. 15 (4) (2015) 330–337.
[28] M. Pogorielov, O. Kalinkevich, V. Deineka, V. Garbuzova, A. Solodovnik, A. Kalinkevich, T. Kalinichenko, A. Gapchenko, A. Sklyar, S. Danilchenko, Haemostatic chitosan coated gauze: in vitro interaction with human blood and in-vivo effectiveness, Biomater. Res. 19 (2015), 22-22.
[29] A. Kumar, A. Vimal, A. Kumar, Why Chitosan? From properties to perspective of mucosal drug delivery, Int. J. Biol. Macromol. 91 (2016) 615–622.
[30] Y. Li, J. Rodrigues, H. Tomas, Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications, Chem. Soc. Rev. 41 (6) (2012) 2193–2221.
[31] R. Stern, A.A. Asari, K.N. Sugahara, Hyaluronan fragments: an information-rich system, Eur. J. Cell Biol. 85 (8) (2006) 699–715.
[32] J.A. Burdick, C. Chung, X. Jia, M.A. Randolph, R. Langer, Controlled degradation and mechanical behavior of photopolymerized hyaluronic acid networks, Biomacromolecules 6 (1) (2005) 386–391.
[33] J.A. Burdick, G.D. Prestwich, Hyaluronic acid hydrogels for biomedical applications, Adv. Mater. 23 (12) (2011) H41–H56.
[34] K. Meyer, J.W. Palmer, The polysaccharide of the vitreous humor, J. Biol. Chem. 107 (3) (1934) 629–634.
[35] K.T. Dicker, L.A. Gurski, S. Pradhan-Bhatt, R.L. Witt, M.C. Farach-Carson, X. Jia, Hyaluronan: a simple polysaccharide with diverse biological functions, Acta Biomater. 10 (4) (2014) 1558–1570.
[36] S.H. Park, J.Y. Park, Y.B. Ji, H.J. Ju, B.H. Min, M.S. Kim, An injectable click-crosslinked hyaluronic acid hydrogel modified with a BMP-2 mimetic peptide as a bone tissue engineering scaffold, Acta Biomater. 117 (2020) 108–120.
[37] Q. Sun, H. Bi, Z. Wang, C. Li, X. Wang, J. Xu, H. Zhu, R. Zhao, F. He, S. Gai, P. Yang, Hyaluronic acid-targeted and pH-responsive drug delivery system based on metal-organic frameworks for efficient antitumor therapy, Biomaterials 223 (2019) 11.
[38] Y. Zheng, J. Yang, J. Liang, X. Xu, W. Cui, L. Deng, H. Zhang, Bioinspired hyaluronic acid/phosphorylcholine polymer with enhanced lubrication and anti-inflammation, Biomacromolecules 20 (11) (2019) 4135–4142.
[39] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, Prog. Polym. Sci. 37 (1) (2012) 106–126.
[40] J. Sun, H. Tan, Alginate-based biomaterials for regenerative medicine applications, Materials 6 (4) (2013) 1285–1309.
[41] H. Zhang, J. Cheng, Q. Ao, Preparation of alginate-based biomaterials and their applications in biomedicine, Mar. Drugs 19 (5) (2021) 24.
[42] I.A. Brownlee, A. Allen, J.P. Pearson, P.W. Dettmar, M.E. Havler, M.R. Atherton, E. Onsøyen, Alginate as a source of dietary fiber, Crit. Rev. Food Sci. Nutr. 45 (6) (2005) 497–510.
[43] A.A. Aldana, G.A. Abraham, Current advances in electrospun gelatin-based scaffolds for tissue engineering applications, Int. J. Pharm. 523 (2) (2017) 441–453.
[44] M.C. Gómez-Guillén, B. Giménez, M.A. López-Caballero, M.P. Montero, Functional and bioactive properties of collagen and gelatin from alternative sources: a review, Food Hydrocolloids 25 (8) (2011) 1813–1827.
[45] J.B. Rose, S. Pacelli, A.J. El Haj, H.S. Dua, A. Hopkinson, L.J. White, F.R. Rose, Gelatin-based materials in ocular tissue engineering, Materials 7 (4) (2014) 3106–3135.
[46] S.S. Ng, K. Su, C. Li, M.B. Chan-Park, D.A. Wang, V. Chan, Biomechanical study of the edge outgrowth phenomenon of encapsulated chondrocytic isogenous groups in the surface layer of hydrogel scaffolds for cartilage tissue engineering, Acta Biomater. 8 (1) (2012) 244–252.
[47] A.O. Elzoghby, W.M. Samy, N.A. Elgindy, Protein-based nanocarriers as promising drug and gene delivery systems, J. Contr. Release 161 (1) (2012) 38–49.
[48] A. Bigi, G. Cojazzi, S. Panzavolta, K. Rubini, N. Roveri, Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking, Biomaterials 22 (8) (2001) 763–768.
[49] A.I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E.H. Schacht, M. Cornelissen, H. Berghmans, Structural and rheological properties of methacrylamide modified gelatin hydrogels, Biomacromolecules 1 (1) (2000) 31–38.
[50] K.B. Djagny, Z. Wang, S. Xu, Gelatin: a valuable protein for food and pharmaceutical industries: Review, Crit. Rev. Food Sci. Nutr. 41 (6) (2001) 481–492.
[51] M. Li, Y. Guo, Y. Wei, A.G. MacDiarmid, P.I. Lelkes, Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications, Biomaterials 27 (13) (2006) 2705–2715.
[52] C.M. Hassan, N.A. Peppas, Structure and morphology of freeze/thawed PVA hydrogels, Macromolecules 33 (7) (2000) 2472–2479.
[53] E. Chiellini, A. Corti, S. D'Antone, R. Solaro, Biodegradation of poly (vinyl alcohol) based materials, Prog. Polym. Sci. 28 (6) (2003) 963–1014.
[54] H. S. Mansur, R. L. Oréfice, A. A. P. Mansur, Characterization of poly(vinyl alcohol)/poly(ethylene glycol) hydrogels and PVA-derived hybrids by small-angle X-ray scattering and FTIR spectroscopy, Polymer. 45(21) 2004 7193-7202.
[55] C.M. Hassan, N.A. Peppas, in: A. ABE (Ed.), Biopolymers/Pva Hydrogels/Anionic Polymerisation Nanocomposites, vol. 153, Springer-Verlag Berlin, Berlin, 2000, pp. 37–65.
[56] Y. Zhang, P.C. Zhu, D. Edgren, Crosslinking reaction of poly(vinyl alcohol) with glyoxal, J. Polym. Res. 17 (5) (2010) 725–730.
[57] M.I. Baker, S.P. Walsh, Z. Schwartz, B.D. Boyan, A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications, J. Biomed. Mater. Res. B Appl. Biomater. 100B (5) (2012) 1451–1457.
[58] E. Ostuni, R.G. Chapman, R.E. Holmlin, S. Takayama, G.M. Whitesides, A survey of structure-property relationships of surfaces that resist the adsorption of protein, Langmuir 17 (18) (2001) 5605–5620.
[59] J.M. Zhu, Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering, Biomaterials 31 (17) (2010) 4639–4656.
[60] T.P. Kraehenbuehl, P. Zammaretti, A.J. Van der Vlies, R.G. Schoenmakers, M.P. Lutolf, M.E. Jaconi, J.A. Hubbell, Three-dimensional extracellular matrix-directed cardioprogenitor differentiation: systematic modulation of a synthetic cell-responsive PEG-hydrogel, Biomaterials 29 (18) (2008) 2757–2766.
[61] C.N. Salinas, K.S. Anseth, Mixed mode thiol-acrylate photopolymerizations for the synthesis of PEG-peptide hydrogels, Macromolecules 41 (16) (2008) 6019–6026.
[62] M.A. Haq, Y.L. Su, D.J. Wang, Mechanical properties of PNIPAM based hydrogels: a review, Mater. Sci. Eng. C Mater. Biol. Appl. 70 (2017) 842–855.
[63] X. Zhao, X. Chen, H. Yuk, S. Lin, X. Liu, G. Parada, Soft materials by design: unconventional polymer networks give extreme properties, Chem. Rev. 121 (8) (2021) 4309–4372.
[64] S. Ashraf, H.K. Park, H. Park, S.H. Lee, Snapshot of phase transition in thermoresponsive hydrogel PNIPAM: role in drug delivery and tissue engineering, Macromol. Res. 24 (4) (2016) 297–304.
[65] J.K. Oh, D.I. Lee, J.M. Park, Biopolymer-based microgels/nanogels for drug delivery applications, Prog. Polym. Sci. 34 (12) (2009) 1261–1282.
[66] I.M. E-Sherbiny, H.D.C. Smyth, Controlled release pulmonary administration of curcumin using swellable biocompatible microparticles, Mol. Pharm. 9 (2) (2012) 269–280.
[67] G. Rassu, A. Salis, E.P. Porcu, P. Giunchedi, M. Roldo, E. Gavini, Composite chitosan/alginate hydrogel for controlled release of deferoxamine: a system to potentially treat iron dysregulation diseases, Carbohydr. Polym. 136 (2016) 1338–1347.
[68] J. Gansau, L. Kelly, C.T. Buckley, Influence of key processing parameters and seeding density effects of microencapsulated chondrocytes fabricated using electrohydrodynamic spraying, Biofabrication 10 (3) (2018) 14.
[69] A. Vossoughi, H.W.T. Matthew, Encapsulation of mesenchymal stem cells in glycosaminoglycans-chitosan polyelectrolyte microcapsules using electrospraying technique: investigating capsule morphology and cell viability, Bioeng. Transl. Med. 3 (3) (2018) 265–274.

[70] H. Kim, S.H. Han, Y.M. Kook, K.M. Lee, Y.Z. Jin, W.G. Koh, J.H. Lee, K. Lee, A novel 3D indirect co-culture system based on a collagen hydrogel scaffold for enhancing the osteogenesis of stem cells, J. Mater. Chem. B. 8 (41) (2020) 9481–9491.
[71] P. Xu, C. Cen, M. Zheng, Y. Wang, Z. Wu, Z. Teng, A facile electrostatic droplets assisted synthesis of copper nanoparticles embedded magnetic carbon microspheres for highly effective catalytic reduction of 4-nitrophenol and Rhodamine B, Mater. Chem. Phys. 253 (2020) 9.
[72] U. Angkawinitwong, A.J. Courtenay, A.M. Rodgers, E. Larraneta, H.O. McCarthy, S. Brocchini, R.F. Donnelly, G.R. Williams, A novel transdermal protein delivery strategy via electrohydrodynamic coating of PLGA microparticles onto microneedles, ACS Appl. Mater. Interfaces 12 (11) (2020) 12478–12488.
[73] L. Zhang, K. Chen, H. Zhang, B. Pang, C.H. Choi, A.S. Mao, H. Liao, S. Utech, D.J. Mooney, H. Wang, D.A. Weitz, Microfluidic templated multicompartment microgels for 3D encapsulation and pairing of single cells, Small 14 (9) (2018) 8.
[74] K.I. Kamei, Y. Mashimo, Y. Koyama, C. Fockenberg, M. Nakashima, M. Nakajima, J. Li, Y. Chen, 3D printing of soft lithography mold for rapid production of polydimethylsiloxane-based microfluidic devices for cell stimulation with concentration gradients, Biomed. Microdevices 17 (2) (2015) 8.
[75] B.G. De Geest, J.P. Urbanski, T. Thorsen, J. Demeester, S.C. De Smedt, Synthesis of monodisperse biodegradable microgels in microfluidic devices, Langmuir 21 (23) (2005) 10275–10279.
[76] J. Yang, Y. Zhu, F. Wang, L. Deng, X. Xu, W. Cui, Microfluidic liposomes-anchored microgels as extended delivery platform for treatment of osteoarthritis, Chem. Eng. J. 400 (2020) 12.
[77] X. Zhao, S. Liu, L. Yildirimer, H. Zhao, R. Ding, H. Wang, W. Cui, D. Weitz, Injectable stem cell-laden photocrosslinkable microspheres fabricated using microfluidics for rapid generation of osteogenic tissue constructs, Adv. Funct. Mater. 26 (17) (2016) 2809–2819.
[78] J. Wu, G. Li, T. Ye, G. Lu, R. Li, L. Deng, L. Wang, M. Cai, W. Cui, Stem cell-laden injectable hydrogel microspheres for cancellous bone regeneration, Chem. Eng. J. 393 (2020) 10.
[79] T.H. Qazi, J. Wu, V.G. Muir, S. Weintraub, S.E. Gullbrand, D. Lee, D. Issadore, J.A. Burdick, Anisotropic rod-shaped particles influence injectable granular hydrogel properties and cell invasion, Adv. Mater. 34 (12) (2022) 12.
[80] J. Yang, Y. Han, J. Lin, Y. Zhu, F. Wang, L. Deng, H. Zhang, X. Xu, W. Cui, Ball-bearing-inspired polyampholyte-modified microspheres as bio-lubricants attenuate osteoarthritis, Small 16 (44) (2020) 14.
[81] X. Han, M. Sun, B. Chen, Q. Saiding, J. Zhang, H. Song, L. Deng, P. Wang, W. Gong, W. Cui, Lotus seedpod-inspired internal vascularized 3D printed scaffold for bone tissue repair, Bioact. Mater. 6 (6) (2021) 1639–1652.
[82] D.M. Headen, J.R. García, A.J. García, Parallel droplet microfluidics for high throughput cell encapsulation and synthetic microgel generation, Microsyst. Nanoeng. 4 (2018) 9.
[83] C. Li, X. Han, Z. Ma, T. Jie, J. Wang, L. Deng, W. Cui, Engineered customizable microvessels for progressive vascularization in large regenerative implants, Adv. Healthc. Mater. 11 (4) (2022) 12.
[84] L. Moroni, J.A. Burdick, C. Highley, S.J. Lee, Y. Morimoto, S. Takeuchi, J.J. Yoo, Biofabrication strategies for 3D in vitro models and regenerative medicine, Nat. Rev. Mater. 3 (5) (2018) 21–37.
[85] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (8) (2014) 773–785.
[86] M. Xie, Q. Gao, H. Zhao, J. Nie, Z. Fu, H. Wang, L. Chen, L. Shao, J. Fu, Z. Chen, Y. He, Electro-assisted bioprinting of low-concentration GelMA microdroplets, Small 15 (4) (2019) 10.
[87] Z. Wang, W. Kapadia, C. Li, F. Lin, R.F. Pereira, P.L. Granja, B. Sarmento, W. Cui, Tissue-specific engineering: 3D bioprinting in regenerative medicine, J. Contr. Release 329 (2021) 237–256.
[88] C. Mandrycky, Z. Wang, K. Kim, D.H. Kim, 3D bioprinting for engineering complex tissues, Biotechnol. Adv. 34 (4) (2016) 422–434.
[89] R. Khorasani, S. Pourmahdian, Synthesis and characterization of hydrogel nanoparticles through inverse microemulsion polymerization of 2-Acrylamido-2-methyl-1-propanesulfonic acid, J. Macromol. Sci., Pure Appl. Chem. 51 (3) (2014) 240–248.
[90] A. Vashist, V. Atluri, A. Raymond, A. Kaushik, T. Parira, Z. Huang, A. Durygin, A. Tomitaka, R. Nikkhah-Moshaie, A. Vashist, M. Agudelo, H.,S. Chand, I. Saytashev, J.C. Ramella-Roman, M. Nair, Development of multifunctional biopolymeric auto-fluorescent micro- and nanogels as a platform for biomedical applications, Front. Bioeng. Biotechnol. 8 (2020) 16.
[91] M. Tuncel, D. Ceylan Tuncaboylu, Preparation of semi-interpenetrating silk fibroin-polyacrylamide nanogels, Polym. Bull. 78 (6) (2021) 3043–3054.
[92] Y. Wang, J. Deng, W. Zhong, L. Kong, W. Yang, Facile surface superhydrophilic modification: NVP/MBA inverse microemulsion surface-grafting polymerization initiated by UV light, Macromol. Rapid Commun. 26 (22) (2005) 1788–1793.
[93] P. Wanakule, G.W. Liu, A.T. Fleury, K. Roy, Nano-inside-micro: disease-responsive microgels with encapsulated nanoparticles for intracellular drug delivery to the deep lung, J. Contr. Release 162 (2) (2012) 429–437.
[94] D. Keskin, G. Zu, A.M. Forson, L. Tromp, J. Sjollema, P. van Rijn, Nanogels, A novel approach in antimicrobial delivery systems and antimicrobial coatings, Bioact. Mater. 6 (10) (2021) 3634–3657.
[95] K. Ma, Y. Xu, Z. An, templateless synthesis of polyacrylamide-based nanogels via RAFT dispersion polymerization, Macromol. Rapid Commun. 36 (6) (2015) 566–570.
[96] M. Fan, F. Wang, C. Wang, Reflux precipitation polymerization: a new platform for the preparation of uniform polymeric nanogels for biomedical applications, Macromol. Biosci. 18 (8) (2018) 11.
[97] X. Du, Y. Gao, Q. Kang, J. Xing, Design and applications of tumor microenvironment-responsive nanogels as drug carriers, Front. Bioeng. Biotechnol. 9 (2021) 17.
[98] Y. Yin, B. Hu, X. Yuan, L. Cai, H. Gao, Q. Yang, Nanogel: a versatile nano-delivery system for biomedical applications, Pharmaceutics 12 (3) (2020) 25.
[99] F.B. Ilhami, Y.T. Yang, A.W. Lee, Y.H. Chiao, J.K. Chen, D.J. Lee, J.Y. Lai, C.C. Cheng, Hydrogen bond strength-mediated self-assembly of supramolecular nanogels for selective and effective cancer treatment, Biomacromolecules 22 (10) (2021) 4446–4457.
[100] P. Wei, J.A. Czaplewska, L. Wang, S. Schubert, J.C. Brendel, U.S. Schubert, Straightforward access to glycosylated, acid sensitive nanogels by host-guest interactions with sugar-modified pillar 5 arenes, ACS Macro Lett. 9 (4) (2020) 540–545.
[101] L. Gao, F. Zabihi, S. Ehrmann, S. Hedtrich, R. Haag, Supramolecular nanogels fabricated via host-guest molecular recognition as penetration enhancer for dermal drug delivery, J. Contr. Release 300 (2019) 64–72.
[102] K. Chen, F. Wang, R. Ding, Z. Cai, T. Zou, A. Zhang, D. Guo, B. Ye, W. Cui, M. Xiang, Adhesive and injectable hydrogel microspheres for inner ear treatment, Small (2022) 13.
[103] X. Zhao, Z. Wang, A pH-sensitive microemulsion-filled gellan gum hydrogel encapsulated apigenin: characterization and in vitro release kinetics, Colloids Surf. B Biointerfaces 178 (2019) 245–252.
[104] H. Su, Q. Jia, S. Shan, Synthesis and characterization of Schiff base contained dextran microgels in water-in-oil inverse microemulsion, Carbohydr. Polym. 152 (2016) 156–162.
[105] J. Du, I.M. El-Sherbiny, H.D. Smyth, Swellable ciprofloxacin-loaded nano-in-micro hydrogel particles for local lung drug delivery, AAPS PharmSciTech 15 (6) (2014) 1535–1544.
[106] Z. Zhao, G. Li, H. Ruan, K. Chen, Z. Cai, G. Lu, R. Li, L. Deng, M. Cai, W. Cui, Capturing magnesium ions via microfluidic hydrogel microspheres for promoting cancellous bone regeneration, ACS Nano 15 (8) (2021) 13041–13054.
[107] Z. Cai, Q. Saiding, L. Cheng, L. Zhang, Z. Wang, F. Wang, X. Chen, G. Chen, L. Deng, W. Cui, Capturing dynamic biological signals via bio-mimicking hydrogel for precise remodeling of soft tissue, Bioact. Mater. 6 (12) (2021) 4506–4516.
[108] L. Cheng, Z. Cai, T. Ye, X. Yu, Z. Chen, Y. Yan, J. Qi, L. Wang, Z. Liu, W. Cui, L. Deng, Injectable polypeptide-protein hydrogels for promoting infected wound healing, Adv. Funct. Mater. 30 (25) (2020) 12.
[109] Q.H. Phua, H.A. Han, B.S. Soh, Translational stem cell therapy: vascularized skin grafts in skin repair and regeneration, J. Transl. Med. 19 (1) (2021) 11.
[110] L. Zhang, Y. Xiang, H. Zhang, L. Cheng, X. Mao, N. An, L. Zhang, J. Zhou, L. Deng, Y. Zhang, X. Sun, H.A. Santos, W. Cui, A biomimetic 3D-self-forming approach for microvascular scaffolds, Adv. Sci. 7 (9) (2020) 10.
[111] J. Jin, Q. Saiding, X. Wang, M. Qin, Y. Xiang, R. Cheng, W. Cui, X. Chen, Rapid extracellular matrix remodeling via gene-electrospun fibers as a "patch" for tissue regeneration, Adv. Funct. Mater. 31 (15) (2021) 14.
[112] J. Bian, F. Cai, H. Chen, Z. Tang, K. Xi, J. Tang, L. Wu, Y. Xu, L. Deng, Y. Gu, W. Cui, L. Chen, Modulation of local overactive inflammation via injectable hydrogel microspheres, Nano Lett. 21 (6) (2021) 2690–2698.
[113] H. Liu, Z. Cai, F. Wang, L. Hong, L. Deng, J. Zhong, Z. Wang, W. Cui, Colon-targeted adhesive hydrogel microsphere for regulation of gut immunity and flora, Adv. Sci. 8 (18) (2021) 12.
[114] Q. Feng, D. Li, Q. Li, S. Li, H. Huang, H. Li, H. Dong, X. Cao, Dynamic nanocomposite microgel assembly with microporosity, injectability, tissue-adhesion, and sustained drug release promotes articular cartilage repair and regeneratio, Adv. Healthc. Mater. 11 (8) (2022) 15.
[115] J. Liu, L. Liu, S. Li, Q. Kang, R. Zhang, Z. Zhu, Self-assembled nanogels of luminescent thiolated silver nanoclusters and chitosan as bactericidal agent and bacterial sensor, Mater. Sci. Eng. C Mater. Biol. Appl. 118 (2021) 10.
[116] J.B. Naik, R.L. Rajput, J.S. Narkhede, A. Mujumdar, P.B. Patil, Synthesis and evaluation of UV cross-linked Poly (acrylamide) loaded thymol nanogel for antifungal application in oral candidiasis, J. Polym. Res. 28 (1) (2021) 13.
[117] H. Wang, L. Gao, T. Fan, C. Zhang, B. Zhang, O.A. Al-Hartomy, A. Al-Ghamdi, S. Wageh, M. Qiu, H. Zhang, Strategic design of intelligent-responsive nanogel carriers for cancer therapy, ACS Appl. Mater. Interfaces 13 (46) (2021) 54621–54647.
[118] Y. Lin, C. Li, A. Liu, X. Zhen, J. Gao, W. Wu, W. Cai, X. Jiang, Responsive hyaluronic acid-gold cluster hybrid nanogel theranostic systems, Biomater. Sci. 9 (4) (2021) 1363–1373.
[119] Z. Li, H. Shi, H. Xie, Y. Yang, Y. Zheng, H. Chen, Y. Gao, Tri-component programmable nanoregulator with Three-pronged penetration boosts immunotherapy of Triple-Negative breast cancer, Chem. Eng. J. 439 (2022) 15.
[120] E.D. Abdolahinia, G. Barati, Z. Ranjbar-Navazi, J. Kadkhoda, M. Islami, N. Hashemzadeh, S.M. Dizaj, S. Sharifi, Application of nanogels as drug delivery systems in multicellular spheroid tumor model, J. Drug Deliv. Sci. Technol. 68 (2022) 11.
[121] M.J. Kwon, Y. Seo, H. Cho, H.S. Kim, Y.J. Oh, S. Genişcan, M. Kim, H.H. Park, E.H. Joe, M.H. Kwon, H.C. Kang, B.G. Kim, Nanogel-mediated delivery of oncomodulin secreted from regeneration-associated macrophages promotes sensory axon regeneration in the spinal cord, Theranostics 12 (13) (2022) 5856–5876.
[122] M. Prieto, L. Usón, S. Garcia-Salinas, C. Yus, G. Landa, T. Alejo, L. Lujan, M. Perez, S. Irusta, V. Sebastian, G. Mendoza, M. Arruebo, Light activated pulsatile drug delivery for prolonged peripheral nerve block, Biomaterials 283 (2022) 16.
[123] H. Wang, M.L. Picchio, M. Calderon, One stone, many birds: recent advances in functional nanogels for cancer nanotheranostics, Wiley. Interdiscip. Rev. Nanomed. Nanobiotechnol. 14 (4) (2022), e1791.

[124] K. Atsushi, J. Jun-ichiro, Y. Fumiya, H. Zhang, T. Yasuhiko, S. Akira, T. Mitsumasa, A. Ichio, Ultra-small size gelatin nanogel as a blood brain barrier impermeable contrast agent for magnetic resonance imaging, Acta Biomater. 125 (2021) 290–299.
[125] X. Zhang, P. Wei, Z. Wang, Y. Zhao, W. Xiao, Y. Bian, D. Liang, Q. Lin, W. Song, W. Jiang, H. Wang, Herceptin-conjugated DOX-Fe3O4/P(NIPAM-AA-MAPEG) nanogel system for HER2-targeted breast cancer treatment and magnetic resonance imaging, ACS Appl. Mater. Interfaces 14 (14) (2022) 15956–15969.
[126] K. Yonca, J.C.K. Alexander, D.L. Laura, Translating therapeutic microgels into clinical applications, Adv. Healthc. Mater. 11 (6) (2022), 2101989.