Microfluidic-generated biopolymer microparticles as cargo delivery systems

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Abstract: Droplet microfluidics offers precise and simultaneous control of multiple fluids at microscale, which enables synthesis of large quantities of novel microparticles with compositional and structural diversity in a controllable way. The morphology and functionality of generated microparticles can be well designed by modulating the hydrodynamic profile as well as geometric structures within microfluidic system. The synergistic combination of droplet microfluidics with biodegradable materials makes it possible to encapsulate actives/drugs inside microparticles at high efficiency for drug delivery. The utilization of these microfluidic-generated microparticles with the characteristics of easy biodegradability and good biocompatibility in the field of drug delivery has made considerable progress in recent years. In this review, we introduce the commonly used structures of microchannel and methods to generate biodegradable microparticles with droplet microfluidics. In addition, we discuss and summarize recent advances of biodegradable microparticles in the application of drug delivery with the focus on two kinds of biopolymers for preparing biodegradable microspheres, natural biopolymers and synthetic biopolymers. Next, environment-sensing microencapsulation systems have been discussed because of their ability to release drug upon external stimulation, thereby allowing on-demand drug delivery. Finally, we point out current challenges of utilizing microparticles in drug delivery and provide some perspectives for the future direction in research and applications.

Keywords: droplet microfluidics; biodegradable; microparticles; drug delivery; environment-sensing

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1 Introduction

With the rapid development of biotechnology, various small molecule compounds, peptides, and protein drugs have been produced on a large scale and used to treat human diseases in the form of diverse pharmaceutical administration. [1-6] However, there are several problems that need to be solved in order to achieve better treatments, such as low drug utilization in vivo, toxic and side effects, as well as frequent medications to maintain drug efficacy *in vivo*. [7] Nowadays, the pharmaceutical research has gradually shifted to the development and exploration of new drug release systems as traditional pharmaceutical administration such as injections, tablets, capsules, patches, and aerosols can no longer meet the needs of clinical use. Drug release system refers to the combination of actives and biological carrier materials, which allows drugs to be released to target tissues after oral/injection by functionalizing the surface of the microparticles with molecules or antibodies, and it has facilitated the diagnosis and therapy of cancer. [8, 9] For example, developments in liposome-based drug delivery systems can provide reduced side effects, improved pharmacokinetics and increased tumor uptake for pancreatic cancer therapy; [10] Progress in 5-Fluorouracil-loaded magnetic core-shell nanoparticles allows active therapy with digestive carcinoid; [11] Developments in folate-decorated maleilated pullulan–doxorubicin conjugate provides enhanced cellular uptake and higher cytotoxicity for stomach cancer therapy; [12] Advancement of lipid–polymer hybrid microsystem allows site-specific release of encapsulated drugs to treat breast cancer. [9]

In general, the carrier materials of drug release system often employ synthetic or natural polymers which normally offer great biodegradability, stimuli-responsiveness for controlled release, and good biocompatibility. Typical examples include chitosan, collagen, polylactic acid (PLA), polyglycolic acid (PGA), lactic acid-glycolic acid copolymer (PLGA), polycaprolactone (PCL), etc. [13-16] These carrier materials are continuously degrading after administration, and the controlled release of the drug can be achieved through diffusion, swelling, erosion or biodegradation. Drugs or biological actives can be either physically or chemically encapsulated in such polymer materials to prepare microparticles with size at the micron scale (1 to 500 μm). [17-19] Currently, common methods used to fabricate drug-loaded microparticles mainly consist of solvent evaporation and spray drying. [20] These traditional methods rely on mechanical agitation or ultrasonic vibration to synthesize microparticles, which leads to drawbacks

of high polydispersibility, low drug encapsulation and uncontrollable drug release. [21]

Microfluidic-based droplet generation is a well-known technique for preparing monodisperse microparticles under mild processing conditions. Comparing with the traditionally used high-pressure or high-shear homogenization that requires high consumption of energy, microfluidic technique is especially suitable for encapsulating cells or biomolecules inside the biodegradable microparticles in a safe manner, while not losing any biological activity of biomolecules. [22, 23] The increasing popularity of microfluidics as microdroplet-generating platform has provided a new route to fabricate biopolymer microparticles with desired structure and release characteristics. [24] For example, the microfluidic technique can easily tailor the dimensions of biomolecule-laden microparticles and/or functionalize them with stimuli-sensitive groups or targeting ligands, thereby allowing desired drug release upon external stimulation. [9, 25]

This review summarizes and discusses the recent development of biodegradable microparticles prepared via microfluidic techniques for drug delivery. First, we introduce the commonly used structures of microchannel and methods to generate biodegradable microparticles with droplet microfluidics. Subsequently, recent advances of biodegradable microparticles in the application of drug delivery are presented with the focus on two essential types of biopolymers, namely natural biopolymers and synthetic biopolymers. Moreover, environment-responsive microencapsulation systems have been discussed because of their ability to release drug upon external stimulation for on-demand drug delivery. Finally, we point out current challenges of microparticles as drug delivery systems and provide some perspectives.

2 Droplet microfluidics for preparation of microparticles

Developed in 1990s, microfluidics is a technique that processes or manipulates fluids (in nanoliters to liters) through microchannels with a diameter from ten to hundreds of micrometers, which has been widely employed in various fields including food science, cosmetic manufacture, and biomedical testing. Microfluidic-based droplet generation is a well-known technique for preparation of microparticles with advantages of mild processing and high monodispersity. Because of its ability to accurately manipulate minimal amount of liquid, microfluidic system can generate highly uniform single droplets or multi-emulsions with designed structures once the viscous force and interfacial

tension are balanced. [26, 27] These microdroplets can subsequently serve as templates for formation of latter drug-loaded microparticles preparation after selective methods of solidification.

Microfluidic device with precisely designed structure and good biocompatibility is the prerequisite to prepare droplets/emulsions with expected functionality. PDMS-based microfluidic device fabricated by soft lithography and capillary-based microfluidic device represent two mainstreams of devices for droplet generation (**Fig. 1**). [28-30] Soft lithography allows the preparation of PDMS-based microfluidic device with high accuracy and reproducibility in a scalable manner. In addition, such device has low toxicity, good biocompatibility and high permeability, which can be even used for long-term *in situ* cell culture. Capillary-based microfluidic devices have also been extensively used in preparing emulsions because of its excellent chemical resistance and easy surface treatment, thus being able to manufacture emulsions with diverse morphologies and compositions. However, the capillary device requires manual alignment of capillary tubes, which leads to poor reproducibility and less robustness, thus restricting its scale-up and parallelization in industry. In addition to capillary-based and PDMS-based microfluidic device, many other polymers have been applied to fabricate microfluidic devices, such as PMMA, cyclic olefin copolymer, or solvent resistant elastomers perfluoropolyether (PFPE). [31, 32] These polymer devices are also very important for manufacturing microdroplets or microspheres since such devices are transparent and maintain excellent compatibility with various organic solvents.

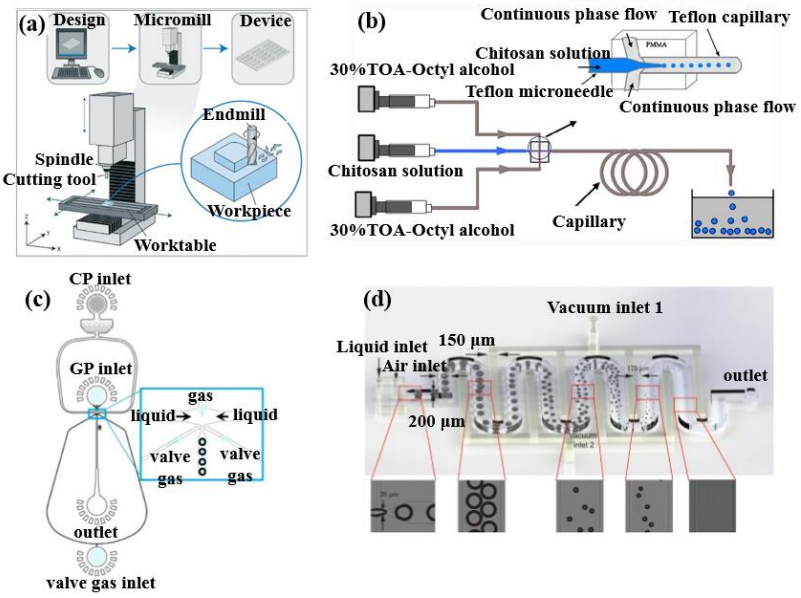

Fig. 1. (a) Schematic diagram illustrating the micromilling process. (b) Capillary-based microfluidic device. (c) & (d) PDMS-based microfluidic device. [28-32] Reprinted with permission from ref. 30. Copyright 2020, Wiley.

Various geometries, such as co-flow, cross-flow, and flow-focusing, have been used to generate droplets with single or multiple cores (**Fig. 2**). [33-35] In cross-flow microfluidic device, two phases are separately injected from the inlets and the dispersed phase is sheared by the continuous phase at the junction with various angles of intersection. [36-38] Both of the physical properties and flow rates of two phases determine the morphology and size of generated microparticles. The cross-flow structure can be subdivided into T-junction and Y-junction depending on the angle of contact between two phases. In addition, more complex structures with more than two inlets, for example, double T-junction, double V-junction, and double K-junction, are derived from these simple structures. Among all these microchannel geometries, T-junction is most commonly employed as it can generate more monodispersed and uniform emulsions. [39-41] In flow-focusing microfluidic device where continuous and dispersed phases flow in a coaxial fashion, the dispersed phase with lower flow rate is pinched-off into microdroplets and emulsions under the action of interfacial tension and shear force when passing through the characteristic contraction region [42-43] The droplet size can be precisely controlled by regulating flow rates of two phases or physical parameters. For example, the droplet size decreases as the flow rate of continuous phase increases, and the flow rate of dispersed phase exhibits opposite effect to that of the continuous phase. Adjusting the interfacial tension or viscosity of two phases is also an effective way to change the droplet size. Moreover, multiple flow-focusing in series, T-junctions in cascade, and T-junction and flow-focusing in series can be manufactured by combining single structures, which enables the accurate and precise preparation of double emulsions. To sum up, microfluidic technique is promising in preparing single droplets or multi-emulsions with controllable size and variable cores, and these droplets/emulsions are important templates for the subsequent synthesis of cargo-loaded microparticles. [44] Specifically, preparation of core-shell microparticles, porous microparticles and anisotropic microparticles via microfluidics are highlighted in following subsections.

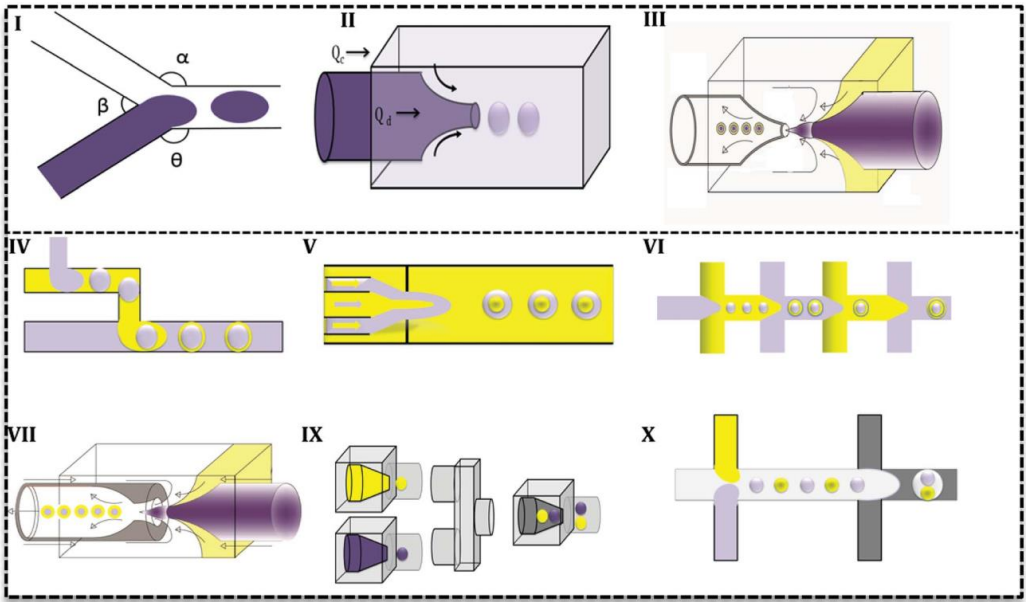

Fig. 2. Droplet-based microfluidic designs used to produce emulsions. (I) cross-flow, (II) co-flow, (III) flow-focusing, (IV) two T-junctions in a row, (V) three concentric channels with one focusing point, (VI) flow-focusing in rows, (VII) combination of co-flow and flow-focusing geometries, (IX) three co-flow, (X) combination of two T-junction and flow-focusing. [33] Reprinted with permission from ref. 33. Copyright 2019, The Royal Society of Chemistry.

2.1 Core-shell structure microparticles

In the microfluidic system, pairwise immiscible phases can form diverse droplet structures such as water-in-oil-in-water (W/O/W), oil-in-water-in-oil (O/W/O) or other more complicated structures. Microparticles with core-shell structure can be fabricated after solidification. [45, 46] The core-shell structure is usually composed of a central core and a shell coated over its surface, and the two components are connected to each other through either physical or chemical interaction. Biologically active biomolecules, drugs, and other ligands can be encapsulated within the core. Generally, the shell is made of stimuli-responsive materials (pH, temperature or magnetic responsive). Once the selective external field is applied, the controlled release of drug is achieved. [47] Gong et al. [48] and Li et al. [49] (**Fig. 3**) have successfully prepared the core/shell double emulsions with flow-focusing microfluidic device. The drug was encapsulated in the core, and the shell was made of poly N-isopropylacrylamide (PNIPAm) nanogels and magnetic iron oxide nanoparticles, which conferred the microparticles both thermo-sensitivity and magnetic-sensitivity. In addition, magnetic nanoparticles could generate heat in the presence of alternating magnetic field, which increased the shell temperature and induced the phase transition of PNIPAm, thereby realizing targeted and on-demand drug release.

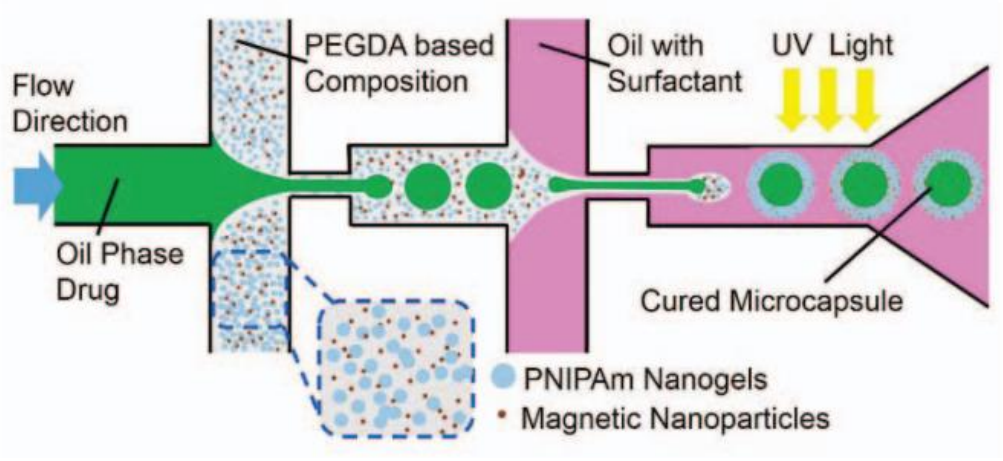

Fig. 3. Schematic diagram showing the fabrication of the microcapsules by a multi-stage microfluidic/optofluidic flow focusing process to generate core-shell droplets. [49] Reprinted with permission from ref. 49. Copyright 2015, IEEE.

2.2 Porous structure microparticles

Drug-loaded microparticles with porous structure can also be prepared with microfluidic technique in a pathway similar to that of the core-shell structure, with the addition of some pore-forming substances, such as ammonium bicarbonate, hydrogen peroxide, etc., in the internal phase (**Fig. 4**). [50-52] These porogens decompose to generate carbon dioxide, ammonia, oxygen and other gases, which are subsequently released to the surrounding environment through the shell layer, leaving drug-loaded microparticles with porous structure in place. Drugs can be loaded either on the surface or in the interior of porous microparticles. The resulted porous structure leads to large pore volume and high specific surface area. In fact, the porous structure can greatly influence the functionality of drug-loaded microparticles. For example, both the porosity, pore size and structure of pores determine the release rate of encapsulated drugs. [53] In general, the microspheres with the porous surface exhibit a burse release initially, while the microspheres with nonporous surface show a relatively weak initial burst and sustain its release during the entire time period. [53] In addition, the porous structure reduces the density of particles, which is convenient for administration by inhalation as the ideal aerodynamic properties can be obtained by adjusting the microparticle size and voids.

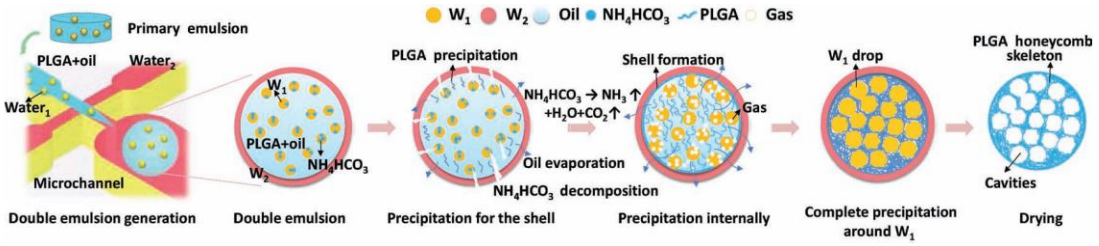

Fig. 4. Schematic diagram of the formation process of the honeycomb structure microspheres. [52] Reprinted with permission from ref. 52. Copyright 2013, Wiley.

2.3 Anisotropic structure microparticles

Anisotropic particle can possess diverse, sometimes even opposite, components, physicochemical properties, polarities, functions, and shapes within the same particle, which contributes to its important applications in various fields, such as biological materials, solid surfactants, and biosensors. Anisotropic particles are mainly divided into three categories: Janus particles, patch particles and multi-segment particles.

Janus particles are non-centrosymmetric particles with two distinct sides, which may differ in either surface wettability, or in other aspects such as optical, electrical or magnetic properties. [54-56] Depending upon the anisotropic properties, these particles can be used as emulsion stabilizers in a manner analogous to surfactant molecules, optical probes for chemical, biological, or rheological measurements, or building blocks for electronic paper. The droplet microfluidic has become an important technique for preparing Janus particles. In general, two or more dispersed phases flow inside the Y-shaped microchannel, and merge into the main channel after the first intersection by accurately regulating their relative flow rate. Then at the second junction the combined dispersed phases are sheared by the continuous phase into droplets with a double-sided or multi-sided structure. The generated droplets gradually transform into Janus particles under either photopolymerization or thermal polymerization. More features and functionalities could be further incorporated into the microparticles by selectively adding various environment responsive materials into any side of the microparticles, for example, embedding Fe_3O_4 nanoparticles into the hydrogel phase. In addition, such a microfluidic system could be utilized in combination with bulk emulsification methods, such as membrane emulsification, to realize the scale-up and mass production of Janus microparticles. [57]

In summary, various microparticles with simple or complex structure, for example, microspheres, core-shell microparticles, microcapsules, hollow microparticles, non-spherical microparticles, porous microparticles, Janus microparticles, and microparticles with multicore or higher-order compartments,

have been successfully fabricated by the methods described in this section 2.1-2.3 (**Fig. 5-6**).

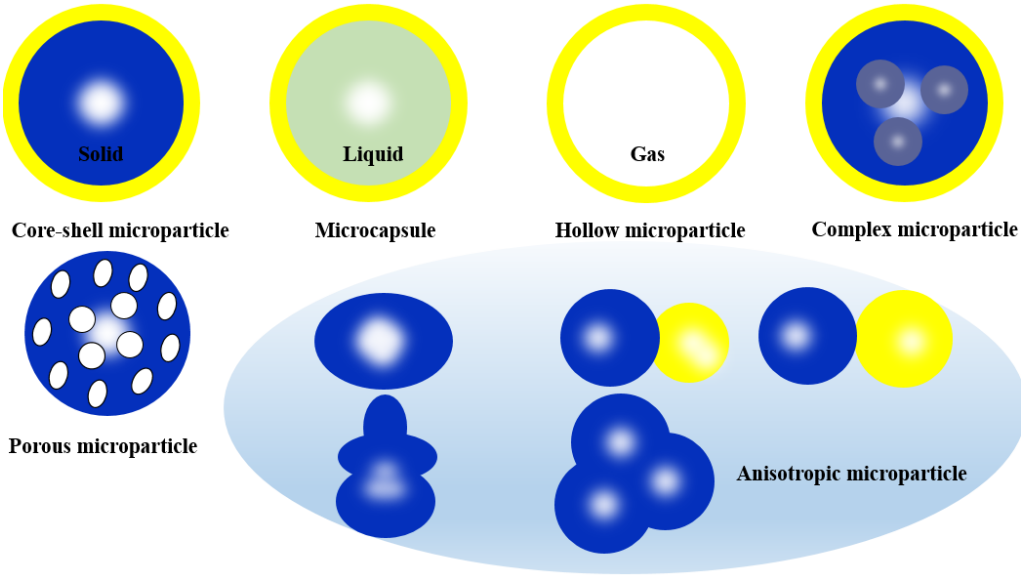

Fig. 5. Schematic illustration of the various types of biopolymeric microparticles.

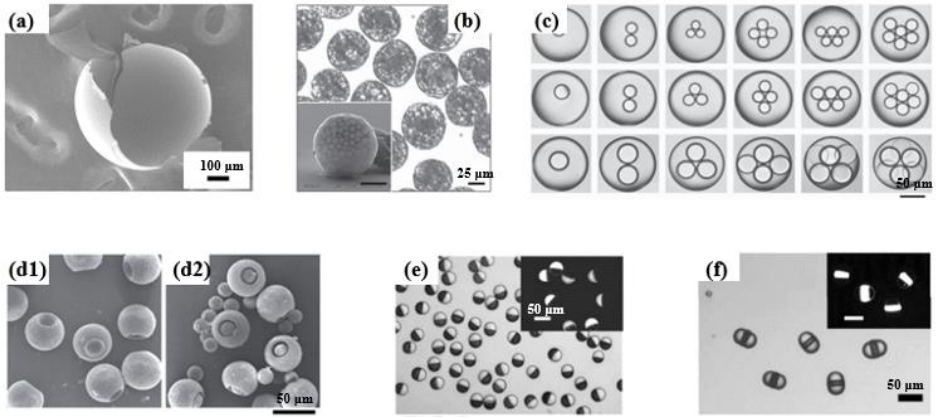

Fig. 6. Microscope images of biopolymeric microparticles. [55] Reprinted with permission from ref. 55. Copyright 2012, The Royal Society of Chemistry.

3 Natural polymer-based drug-loaded microparticles

Generally, synthetic or natural polymers with the characteristics of great biodegradability, controlled release and good biocompatibility, for example, chitosan, alginate, agarose, polylactic acid, etc., are used as materials to prepare drug-loaded microparticles (**Fig. 7**). In this section, natural polymer-based drug-loaded microparticles prepared by microfluidics for cargo are summarized.

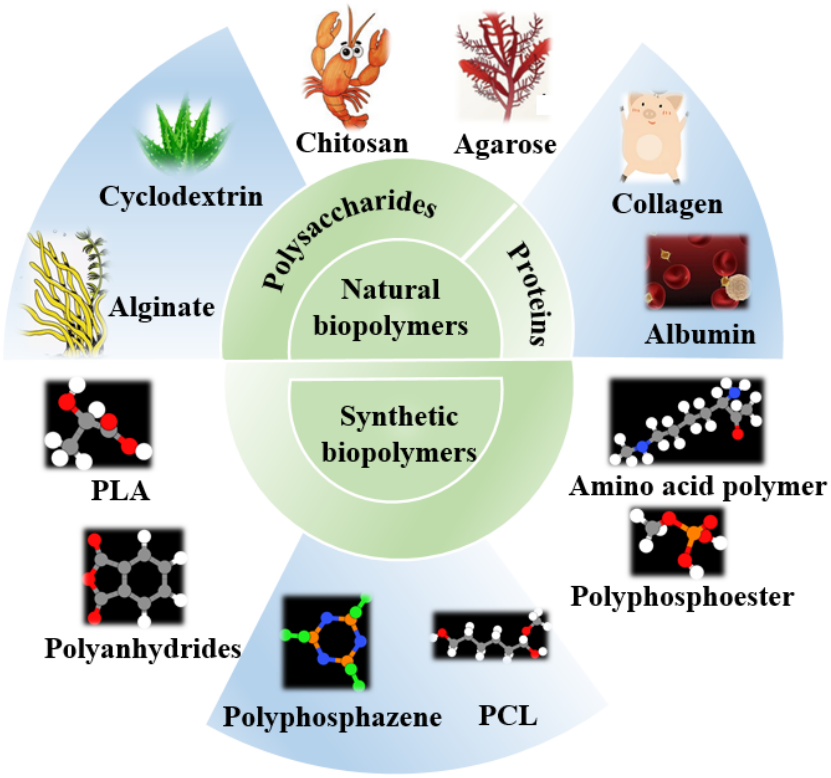

Fig. 7. Schematic illustration of the natural biopolymers including polysaccharides, proteins, and synthetic biopolymers including PLA, PCL, etc.

3.1 Chitosan-based drug-loaded microparticles

Chitosan can be obtained via enzymatic hydrolysis or N-deacetylation of chitin in the presence of alkali, which is the only natural polysaccharide containing amine. It contains N-acetyl-d-glucosamine and β - (1,4) - linked d-glucosamine at random distribution. [58] Chitosan is a weak polyelectrolyte as each monomer is composed of two free hydroxyl groups and one primary amine group. [59] Therefore, chitosan is only soluble in acid solution, but precipitates in organic solution and alkali solution. Its pKa value varies from 6.5 to 7.0, making the amino group protonated in acidic solution and enabling the fabrication of biopolymer microparticles via simple electrostatic interactions. [60] Moreover, its amino group can also chelate metal ions or react with chemical crosslinkers, thereby becoming a promising biopolymer for the preparation of microparticles. [61, 62]

The main crosslinking methods of chitosan include chemical crosslinking and ionic crosslinking, and different crosslinking methods have a great influence on morphology and sphericity of chitosan microparticles (**Fig. 8**). [63] For example, ionic-crosslinked chitosan microparticles normally exhibits poor sphericity, rough surface and loose structure in morphology, which is due to the weak electrostatic

interactions and low crosslinking ability between ionic crosslinking agent and chitosan. In contrast, the morphologies of chemical-crosslinked chitosan microspheres normally exhibit good sphericity, uniform sizes and smooth surfaces, which is attributed to the strong covalent interaction between chemical crosslinking agent and chitosan. [63] Therefore, the surface morphology and porosity of microparticles could be adjusted through crosslinking methods, and thus the regulation of release rates can be achieved.

In addition to adjusting the cross-linking method to control release rate, pH is also an important way to control release. The chitosan microspheres are pH-responsive. Before contacting with the acidic solution, these microparticles could maintain their structural integrity without any drug release. Once being exposed to the acid, the chitosan shell would decompose and both free drug molecules and drug-loaded nanoparticles are released. [47]

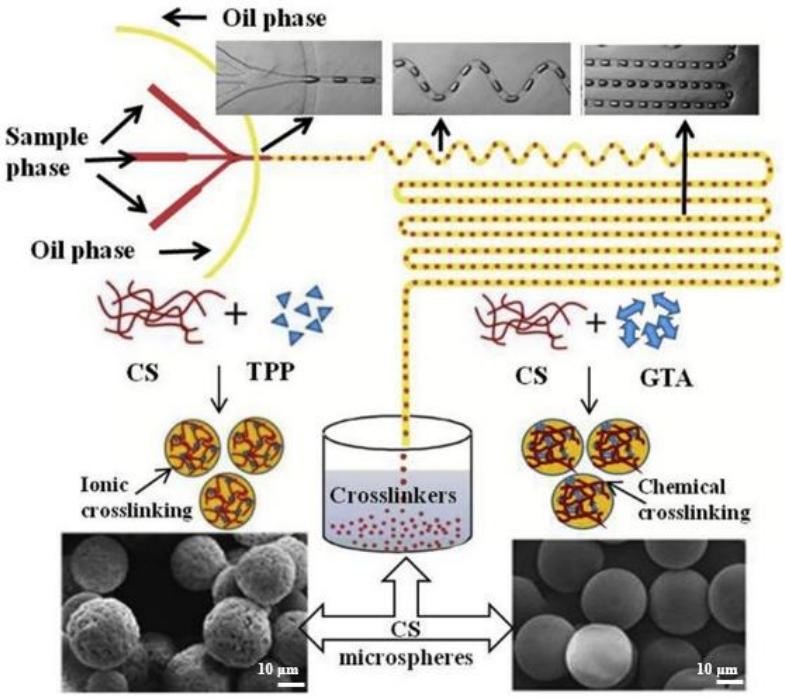

Fig. 8. Schematic illustrations of microfluidic chip emulsification coupled with the crosslinking reaction process between CS and crosslinker TPP/GTA. [63] Reprinted with permission from ref. 63. Copyright 2020, Elsevier.

3.2 **Alginate-based drug-loaded microparticles**

Alginate is extensively used in the field of biomedical engineering, which is obtained by treating brown alga Phaeophyceae in the presence of alkali. [64] Alginate, a hydrophilic and anionic polysaccharide, is composed of α-lguluronic acid (G-block) and 1,4-linked β-d-mannuronic acid (M-block) with a linear structure. The production process and source of alginate as well as G-block and M-block distribution influence the molecular weight of alginate significantly, which subsequently

influences the physical property of alginate.

When preparing alginate microparticles, the polymer could quickly gelate via ionic crosslinking once adding divalent cations, of which calcium ions are the most widely used. [65] The combination of microfluidic technique and sedimentation strategy makes it possible to fabricate various shapes and sizes of alginate-based microparticles, for example, microsphere, tail-shaped and mushroom-shaped, for the application of sustained and targeted drug delivery. [66, 67]

Alginate hydrogels have the advantages of high safety, non-toxicity, and good biocompatibility. It has been reported that implantation of highly purified alginate hydrogels into animals will not cause any obvious foreign body reaction. [67] However, pure alginate hydrogels lack cell adhesion sites, and exhibit slow biodegradability and poor mechanical properties, which greatly limits their practical application. In order to improve their mechanical property, the composite alginate-chitosan microspheres are prepared via a new type of microfluidic device (**Fig. 9**). [68, 69] Interestingly, the device contains a steplike barrier structure that is designed to limit the motion of microparticles in the detection region. These calcium alginate-chitosan microparticles can keep their structural integrity in the acidic environment of the stomach, thus preventing their premature decomposition in the stomach and improving the therapeutic effect.

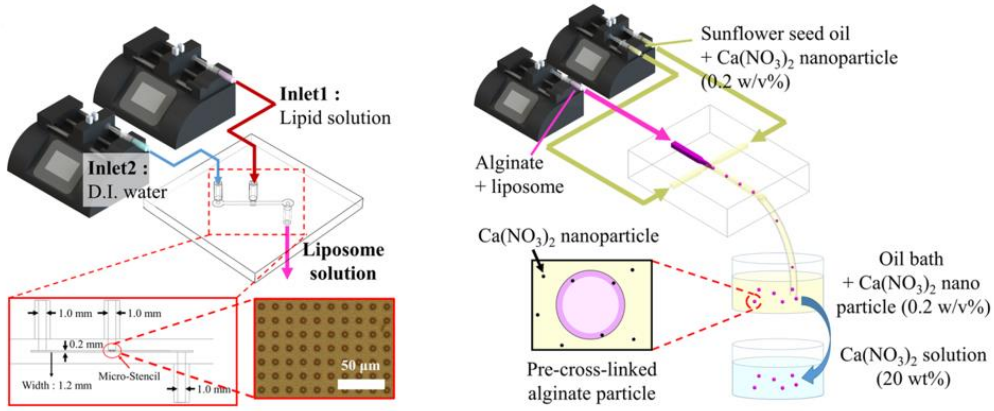

Fig. 9. Schematic diagram of amphiphilic liposome preparation. [69] Reprinted with permission from ref. 69. Copyright 2020, American Chemical Society.

3.3 Agarose-based drug-loaded microparticles

Agarose, an alternating neutral polysaccharide of α-1-4-linked 3,6-anhydro-1-galactose and β-1,3-linked-d-galactose, is sourced from red algae.[70] As agarose cools down, a thermally reversible hydrogel is formed because of the hydrogen bonding interactions in spiral area. [71] Due to its protein adsorption

resistant, highly bio-inertness and controllability over its mechanical property, it has been extensively applied in the field of cell culture. [72] A simple and novel microfluidic device which consists of droplet formation module and droplet trapping module has been manufactured. [73] This device enables the mass production and trapping of agarose microgels via the combined effects of surface tension and hydrodynamic force. In short, this simple device integrates droplet fabrication via flow-focusing T-junction, immobilization with trapping structure and gelation by lowering the temperature. The synergistic action of agarose and integrated device provides a new approach in real-time monitoring of cellular behavior, which is particularly useful in the fields of tissue engineering or material synthesis.

The combination of agarose with other natural biopolymers used as drug carriers is also a growing trend. For example, the mixture of chitosan and agarose confers the composite hydrogels both pH and thermal sensitivity. [74] (**Fig. 10**). In addition, the elastic modulus of composite structure could be flexibly tuned by adjusting the concentration of agarose, and it was measured to be 1 kPa when the concentration of agarose was 2%. Compared with agarose microgel, the composite microgel showed stronger resistance to acid and higher stability in acidic solution. This composite is a promising drug carrier, and it may provide a new approach to prepare biocompatible and biodegradable materials with cooperative properties.

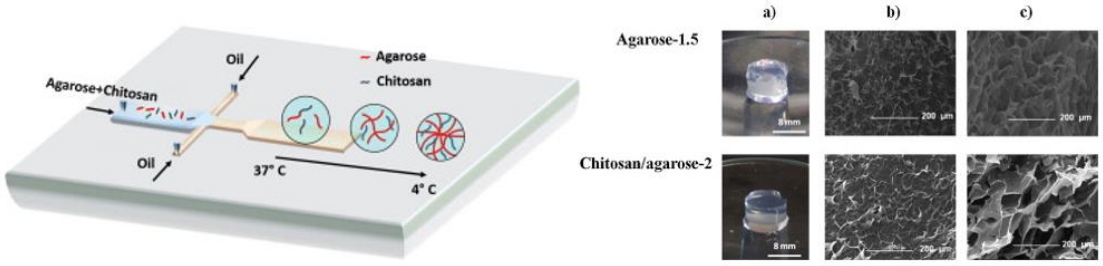

Fig. 10. Schematic of the microfluidic preparation of microgels from chitosan/agarose solutions. [74] Reprinted with permission from ref. 74. Copyright 2014, Elsevier.

3.4 Albumin-based drug-loaded microparticles

Albumin, which is denatured upon heating and slightly soluble in salt solution, is a globular protein. Albumin microspheres encapsulating protein drugs can be produced by crosslinking bovine serum albumin (BSA) at the interface. [75] The obtained BSA microcapsules allow the free passage of small molecules such as fluorescent sodium salt, but block the diffusion of large molecules like fluorescent BSA in and out microcapsules. The BSA microcapsules are designed to be enzyme-degradable, which can release macromolecules upon enzymatic digestion.

Above all, the comparison of various natural polymer-based microparticles is as follows (**Tab. 1**):

Tab. 1. Natural polymer-based microparticles

Natural polymer	Microfluidic device	Microparticle structure	Curing method	Encapsulated drug	Results	Ref.
chitosan	flow-focusing	microsphere	ionic crosslinking & chemical	5-Fluorouracil	pH-responsive	63
	dual-coaxial	core shell	ionic crosslinking	oleophilic curcumin	programmed sequential drug	47
alginate	step-like	core shell	ionic crosslinking	RIS-anti-osteoporotic	pH-responsive	68
	crossed-shaped	microsphere	ionic crosslinking	liposomes	chemical and mechanical stimuli	69
agarose	flow-focusing	microsphere	low-temperature	5-Fluorouracil	pH and temperature responsive	73
	flow-focusing	microsphere	low-temperature	adenoid cystic carcinoma	high throughput	74
albumin	flow-focusing	microsphere	low-temperature	fluorescein sodium salt	selective permeability	75

4 Synthetic polymer-based drug-loaded microparticles

4.1 Polylactic acid (PLA)-based drug-loaded microparticles

Polylactic acid has been proven to exhibit good biocompatibility and biodegradability [76, 77] The degradation products of PLA, i.e. carbon dioxide and water, can be directly consumed by the body's metabolism. The asymmetric carbon chain in the polylactic acid molecule has a regular configuration, which can form a semi-crystalline polymer and exhibits excellent mechanical property. PLA is mainly used as fractures internal fixator. Recently, it has become one of the most valued materials in the field of biomedical degradable materials, and has been approved by FDA as a biodegradable medical material.

The synergistic combination of droplet microfluidics with biodegradable PLA makes it possible to encapsulate actives/drugs inside monodispersed microparticles at high efficiency (**Fig. 11**). [78] The drug release rate from the monodispersed microparticles is much slower than that from the non-uniform

microparticles prepared by conventional method. Most of drugs are likely to be trapped or adsorbed on the surface of the microspheres generated via conventional method, while drugs tend to be encapsulated inside the core of microfluidic-based microparticles, thereby resulting in the lower release rate of microfluidic-based microparticles. In addition, the initial burst of monodispersed microparticles is much slower than those with a broader size distribution. All of these results confirm that the combination of PLA biopolymer and microfluidic technique is valid and applicable in sustained drug release.

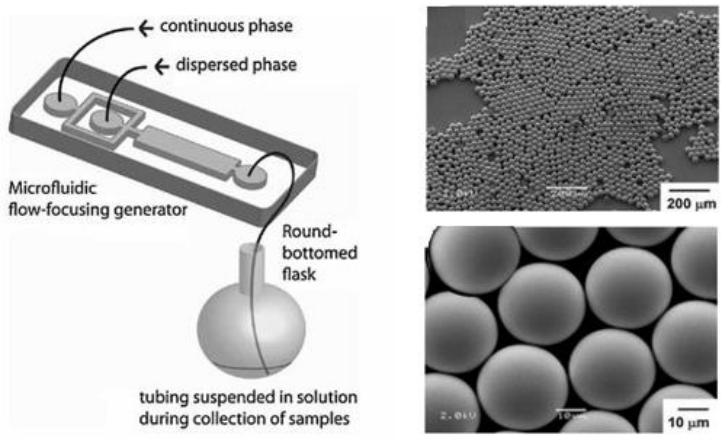

Fig. 11. Schematic of the microfluidic preparation of PLA microparticles. [78] Reprinted with permission from ref. 78. Copyright 2009, Wiley.

4.2 Polyanhydride-based drug-loaded microparticles

Polyanhydride, a promising material for manufacturing drug delivery device, has been extensively used. [79-82] These materials are hydrophobic, however, the hydrolysis of anhydride bond is especially fast, thereby producing surface-eroding devices. [82] In addition, the erosion duration can vary from weeks to months by adjusting the monomer composition or co-monomer ratio. [82] More importantly, these materials are biodegradable and non-toxic. [83, 84] Polyanhydride-based wafer which is composed of poly(1,3-bis-p-carboxyphenoxy propane-co-sebacic acid) has been approved by FDA and applied in delivering carmustine for the therapy of brain cancer.

Polyanhydride microcapsules can be produced by copolymerization of sebacic acid (SA) with 1, 3-bis (p-carboxyphenoxy) propane (CPP). [85] In addition, both hydrophobic compound curcumin and hydrophilic compound Brilliant Blue G (BBG) can be simultaneously encapsulated in the shell and core with a loading efficiency of 90% and 50%, respectively. These drug-loading microparticles exhibit stable release rate and both hydrophobic and hydrophilic drugs can be delivered simultaneously.

4.3 Poly(amino acid)s-based drug-loaded microparticles

Poly (amino acid)s are tissue components and their in *vivo* hydrolysis products are non-toxic oligopeptides or amino acids. Moreover, they can easily be functionalized and modified, thereby becoming a promising drug carrier. [86]

Among all poly (amino acid)s, poly (aspartic acid) and its derivatives emerge as focal points due to their easy synthesis and flexible functionalization comparing to harsh polymerization reactions of other poly (amino acid)s. Therefore, poly (aspartic acid)-based biopolymer has been extensively used in a variety of biomedical fields, such as injectable hydrogels and drug carriers. The amphiphilic poly(aspartic acid) derivative with both pH and redox sensitive property can be prepared via ring-opening reactions of polysuccinimide (PSI). [86] Drug or active substance can be encapsulated in the core of microcapsules via hydrophobic interaction. These drug-loaded microcapsules are able to keep their structural integrity in a neutral environment. When they are triggered upon the acid or reducing substances, the material decomposition starts and the drug release is achieved. Therefore, poly(aspartic acid) and its derivatives have become a promising carrier for drug release.

4.4 Other synthetic polymer-based drug-loaded microparticles

Polyphosphoesters, e.g. poly(phosphate)s, poly(phosphite)s and poly(phosphonate)s, is one type of the biopolymers with phosphate ester bonds in the backbone. [87] The research of this type of biopolymer began in the 1970s by Penczek and his colleagues. [88-90] It has several desirable features: (1) Biocompatibility. The final hydrolytic products of polyphosphoester, alcohol, phosphates and diols, are harmless and biocompatible. In addition, biocompatible polyphosphoester can be synthesized via nontoxic alcohol and diols. (2) Excellent physicochemical property. The flexible phosphate ester bond in the backbone makes it possible to reduce the glass transition temperature of the polymer, thereby providing good processing performance and solubility. (3) Versatility and flexibility. Both of the degradation and physicochemical properties can be tuned by changing the structure of backbone or side-chain. Therefore, this type of biopolymer is promising for various biomedical applications, especially for drug delivery and tissue engineering due to its favorable physicochemical characteristics.

Polyphosphazenes (PPZs) [91] are a kind of organic–inorganic hybrid polymers, which consist of alternating single and double P–N bonds, with two organic side groups attached to each phosphorus atom. The PPZs have excellent biocompatibility, good water dispersibility, and adjustable chemical properties with low toxicity of degradation products. Nanomaterials prepared by PPZs for cancer treatment have become one of its important applications, for example, doxorubicin–polyphosphazene conjugating

hydrogels, doxorubicin-loaded polymeric micelles based on amphiphilic polyphosphazenes with polymer as side groups, controlled release of doxorubicin from thermosensitive poly (organophosphazene) hydrogels, and pH-sensitive drug delivery system with polyphosphazene.

In addition, polycaprolactone, polyvalerolactone, polydecalactone, polyethylene glycol oxalate, poly 3-hydroxybutyrate, poly 3-hydroxyl Valerate, poly-β-malic acid, etc. have also been applied to drug sustained-release systems.

Above all, the comparison of various synthetic polymer-based microparticles is as follows (**Tab. 2**):

Tab. 2. Synthetic polymer-based microparticles

Synthetic polymer	Microfluidic device	Microparticle structure	Curing method	Encapsulated drug	Results	Ref.
polylactic acid	flow-focusing	microsphere	solvent evaporation	bupivacaine (a local anesthetic)	reduced burst release	78
polyanhydride	dual-coaxial	core shell	solvent evaporation	brilliant blue G	reduced burst release	85
poly(amino acid)s	flow-focusing	microsphere	lyophilize	DOX hydrochloride	pH and redox responsive	86
polyphosphoesters	flow-focusing	microsphere	solvent evaporation	FITC-BSA	reduced burst release	87

5 Stimulus-responsive microparticles

Environment-responsive microencapsulation systems have been extensively discussed because of their ability to release drug upon selective external stimulation, thereby allowing desired drug delivery. [92] The development and progress of functional materials allows the utilization of a variety of external stimuli to prepare time-controlled and targeted drug release systems. So far, commonly used stimuli include mechanical strength, biomolecules, pH, temperature, magnetic/electric field, ionic strength and light. These stimuli can flexibly and effectively trigger changes in the chemical or physical properties of drug-loaded microcapsules, thus achieving controlled and desired drug release. For instance, the microencapsulation system can switch freely between “on” and “off” in response to the magnetic field or light. In addition, the microencapsulation system can also flexibly transform between shrink and swell upon pH-dependent protonation/deprotonation or temperature change-induced phase transition. Furthermore, pressure-induced volume phase transition, ultrasound-triggered cavitation of microbubbles,

and redox-induced destabilization have all proven the universality and potential of environment-sensing systems in drug delivery.

5.1 pH-responsive drug-loaded microparticles

Different tissues and organs have great pH differences (cytosol (7.4), Golgi apparatus (6.4), endosomes (5.5–6), lysosomes (4.5–5)). [93] Apparent pH discrepancy also exists between non-healing and healing woods. For example, non-healing woods show alkaline while healing woods show acidic. In addition, normal tissues exhibit a higher pH comparing with that in tumors, a phenomenon known as "Warburg effect". The reason for this is that the proliferation of tumors exceeds the blood supply, resulting in insufficient supply of nutrients and oxygen to cancerous cells. Therefore, lactic acid is formed in cancerous cells through glycolysis instead of oxidative phosphorylation. These pH differences between organs and tissues have promoted the development and synthesis of pH-responsive drug-loaded microparticles.

pH-responsive nanomaterials have proven their applications in multiple bio-related fields, such as cell recognition devices, controllable release surfaces, pH sensors and diagnostics. [94-97] A novel pH-responsive "Janus particle" has been generated, which can undergo morphological alterations in response to pH changes (**Fig. 12**). [98]

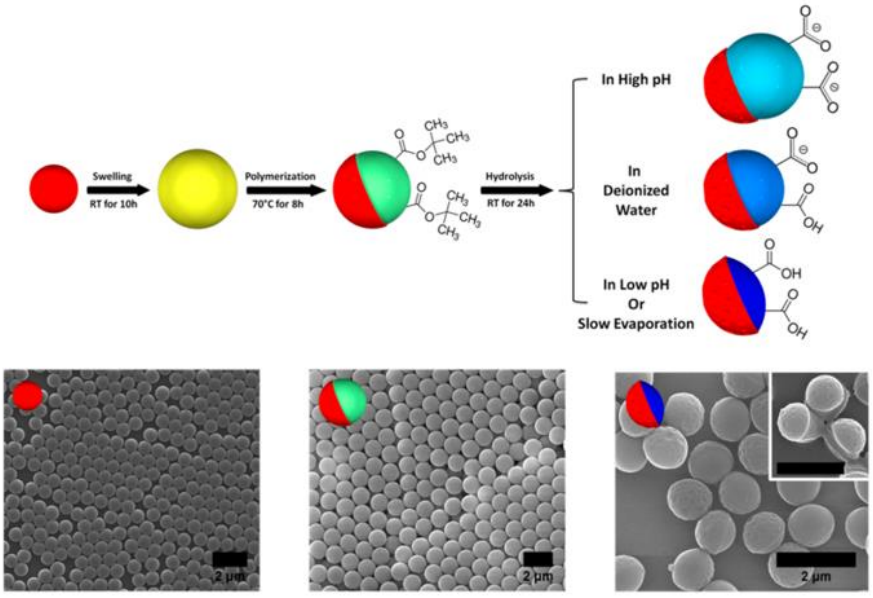

Fig. 12. Schematic illustration for the synthesis of amphiphilic Janus particles by seeded emulsion polymerization followed by acid hydrolysis. [98] Reprinted with permission from ref. 98. Copyright 2014, American Chemical Society.

During the past several years, the novel pH-responsive nanomaterials have made great progress and development in the diagnosis and therapy of a number of serious diseases, for example, infections and

fatal malignancies. Such nanomaterials have also demonstrated strong antitumor effects, low toxicity of chemotherapy and targeted delivery and release of peptides, nucleic acids and proteins, etc. [99, 100] pH-responsive microparticles are promising because of its potency in tumor inhibition and oral bioavailability. In addition, these microparticles make it possible to release the drugs to tumor cells selectively and effectively, while producing only insignificant toxicity or side effects to normal cells.

5.2 Thermo-responsive drug-loaded microparticles

Extensive research has been carried out on temperature, one of the most typical stimuli, to design a controlled drug delivery system with desired release profile. The high temperature of inflamed sites and tumors is internal stimulus that can be utilized to activate thermo-sensitive microparticles, while the external temperature change can also provide the opportunity to trigger sustained release of bioactive substances. What makes these thermo-sensitive microparticles more remarkable is that the microparticles can be injected into the pathological tissue in liquid form without surgery. [101] The ideal and desired microparticles would release bioactive only at the specific position and right timing, while maintain its original load capability with no leakage in other tissues.

The most widely used temperature-sensitive polymers for the manufacturing of environment-sensing microparticles are those whose structures can respond to temperature change, thereby altering between shrink and swell states. As a matter of fact, the solubility of polymers varies significantly with temperature. Once the surrounding temperature reached a critical value, the temperature-induced obvious structural transformation will lead to the release of bioactivities or drugs to the targeted issue. Such a critical temperature is characterized by lower critical solution temperature (LCST) or upper critical solution temperature (UCST). With the ingenious utilization of critical temperature, the bioactivities can be encapsulated within the polymers simply at ambient temperature, and released to the targeted issue with the temperature approaching to LCST or UCST (**Fig. 13**). [102, 103]

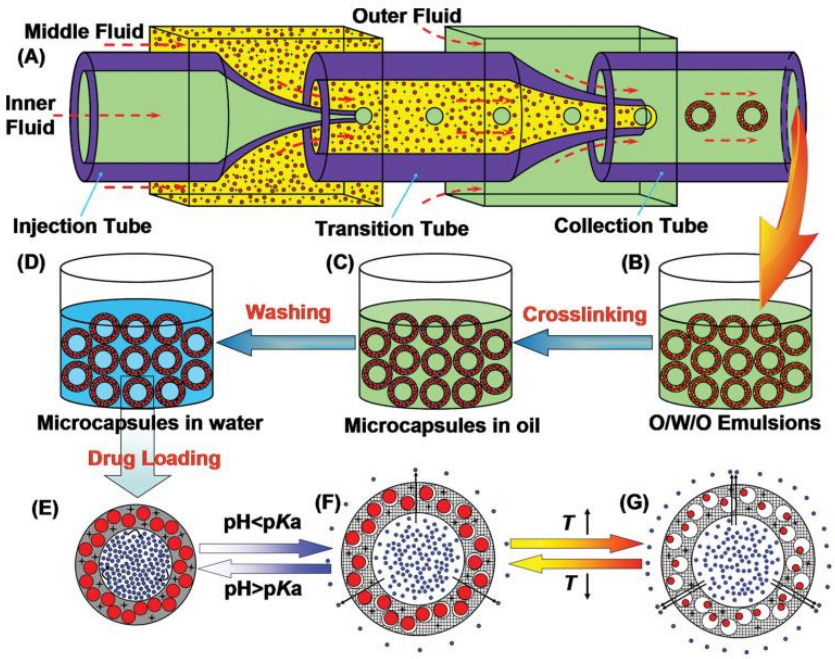

Fig. 13. Schematic illustration of fabrication process and controlled-release mechanism of temperature-sensitive hydrogels. [102]

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PNIPAAm and its modified forms have caused extensive attention in the application of temperature-sensitive hydrogels. This is mainly because its proper phase transition temperature is approximately 32 ℃, which is close to the body temperature. [104,105] Such temperature-sensitive hydrogels usually can act in synergy with other proteins or peptides to achieve better tissues targeting and sustained release. [106] Certainly, PNIPAAm also has apparent defect and limitation. That is the LCST of this polymer is below the body temperature. Consequently, the drug may begin to be released before it reaches its target location once PNIPAAm is injected into the human body. To resolve this issue, Fu et al. increases the swelling ratio and LCST by copolymerizing PNIPAAm with hydrophilic polymer, AAm. It has been revealed that the LCST of the copolymer is raised from 32 ℃ to 41 ℃ when the proportion of AAm is above 5.5%. [107]

5.3 Magnetic-responsive drug-loaded microparticles

Magnetic force, which has been known as one of the most promising external stimulus, is also widely used in drug release system since no physical interaction is needed comparing with ultrasound stimulus or light irradiation. Similarly, magnetic-responsive microparticles are capable of rapidly responding to magnetic flux, making the alternating magnetic field (AMF) an effective tool to activate drug-loaded particles in the goal of realizing sustained and targeted release. [108] Many research is

underway for magnetic-responsive microparticles applied as carrier materials because of its non-contact, easy functionalization, and magnetic driving nature (**Fig. 14**). [109, 110] In 1960, Freeman et al. first applied magnetic field as the external stimuli in biomedical targeting and drug delivery. [111] Comparing with ferromagnetic microparticles, those single domain magnetic particles whose superparamagnetic are higher than blocking temperature could potentially be used in drug release, since any dipole attraction is rarely found in the magnetic field. Thus, these magnetic microparticles feature uniform dispersion and high stability in polymeric matrix.

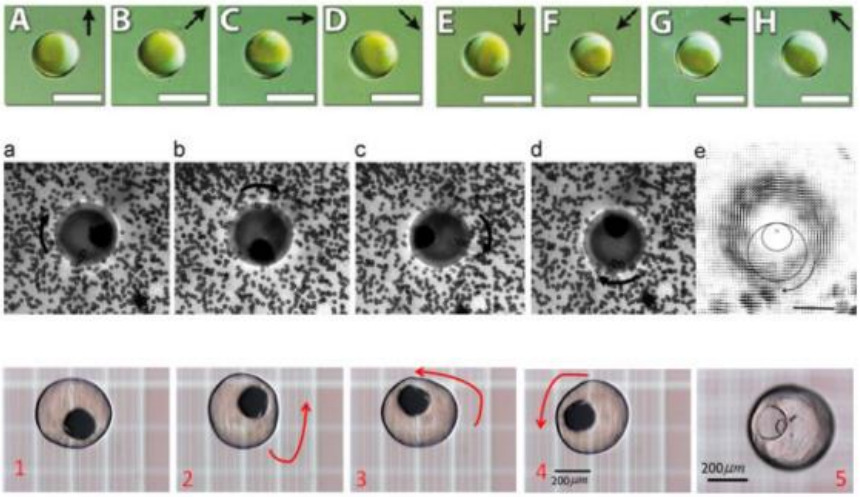

Fig. 14. Schematic illustration of anisotropic magnetic polymer microparticles under a rotating magnetic field. [109] Reprinted with permission from ref. 109. Copyright 2013, The Royal Society of Chemistry.

5.4 Electrical-responsive drug-loaded microparticles

Electrical-responsive microparticles are capable of rapidly responding to the variation of electrical field (**Fig. 15**). [112-114] Therefore, electrical field can be utilized to activate drug-loaded particles to realize pulsed, sustained or on-demand release. The electro-responsive drug release can be performed via several platforms, for example, electro-responsive compound loaded nanostructures, electro-responsive nanostructures and the combination of electro-responsive materials with other stimuli-responsive vehicles such as temperature or magnetic. Introducing the polyelectrolyte with a couple of ionizable groups makes it possible to respond to electrical field through swelling or shrinkage of polymers. [115] For example, Ying et al. successfully synthesized electro-responsive nanoparticles loaded with antiepileptic drugs for sustained and targeted release. Due to the existence of polyelectrolyte poly (4-vinylbenzene sulfonate) (PSS), the ionization degree in the structure was enhanced in the presence of external electrical field. The particle diameter and swelling rate were controlled by external electric field. Furthermore,

triggered and increased drug release in vitro were demonstrated and its potential application in epilepsy treatment was shown. [116]

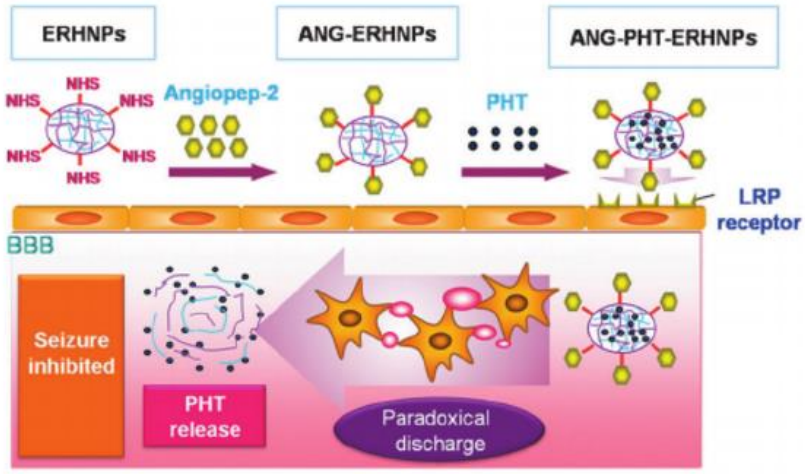

Fig. 15. Schematic illustration of electro-responsive hydrogel nanoparticles. [116] Reprinted with permission from ref. 116.

6 Conclusion

In this paper, the progress of microfluidic technique and biodegradable microparticles in the application of drug release has been systematically summarized, which is very important for both scientific research and industrial development. The commonly used structures of microchannel and methods to generate biodegradable microparticles with droplet microfluidics has been introduced. The microparticles generated by droplet microfluidics allow high controllability in size, structure and uniformity, and high uniformity of microparticles guarantees a consistent drug release profile. [9] In addition, two kinds of biopolymers for preparing biodegradable microparticles, natural biopolymers and synthetic biopolymers, are also reviewed in detail. The synergistic combination of droplet microfluidics with biodegradable materials makes it possible to encapsulate actives/drugs inside microparticles effectively and controllably. These biodegradable microparticles with diverse structures and integrated functions have made great progress in biomedical fields especially in drug delivery. Environment-sensing microencapsulation systems are also quickly developed due to their sustained release upon external stimuli. However, there is still a long way to go before these microparticles can be utilized in practical application, and much work is remained to be done to solve the critical issues.

1) There are two key issues in the fabrication of microfluidic device: high cost and weak robustness. Soft-lithography allows the preparation of PDMS-based microfluidic device with high accuracy and

reproducibility. However, the manufacture of template on silicon wafer is still very expensive. These problems can be avoided effectively in capillary-based microfluidic devices, but such capillary-based devices need to be aligned and assembled manually, thereby leading to poor reproducibility.

2) Another issue restricted the development of microfluidic device is small-scale production of biomedical microparticles. Although parallelization of multiple droplet generators has been developed to achieve mass production and scale up of microparticles, this parallelization device is mostly applied in laboratory. The complicated device design and surface wettability treatment have limited their commercial production.

3) Only several biopolymers have been approved by FDA and applied in biomedical targeting and drug release. Therefore, it is essential to develop more types of biopolymer with high safety, controlled release and specific targeting in biomedical applications.

4) The characterization and evaluation of biomedical microparticles is still in vitro stage, and much more research needs to be done on evaluating the safety and release performance of microparticles in vivo.

In the future, the development of cross discipline between chemistry, chemical engineering, biology and computer science may open the door to a new world. The rapid rise of artificial intelligence can bring breakthroughs to device design, drug screening and toxicity testing, and it will facilitate biopolymer-based microparticles to be utilized in practical application and address various medical issues.

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