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Review

Microfluidic-Based Synthesis of Hydrogel Particles for Cell Microencapsulation and Cell-Based Drug Delivery

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Abstract: Encapsulation of cells in hydrogel particles has been demonstrated as an effective approach to deliver therapeutic agents. The properties of hydrogel particles, such as the chemical composition, size, porosity, and number of cells per particle, affect cellular functions and consequently play important roles for the cell-based drug delivery. Microfluidics has shown unparalleled advantages for the synthesis of polymer particles and been utilized to produce hydrogel particles with a well-defined size, shape and morphology. Most importantly, during the encapsulation process, microfluidics can control the number of cells per particle and the overall encapsulation efficiency. Therefore, microfluidics is becoming the powerful approach for cell microencapsulation and construction of cell-based drug delivery systems. In this article, I summarize and discuss microfluidic approaches that have been developed recently for the synthesis of hydrogel particles and encapsulation of cells. I will start by classifying different types of hydrogel material, including natural biopolymers and synthetic polymers that are used for cell encapsulation, and then focus on the current status and challenges of microfluidic-based approaches. Finally, applications of cell-containing hydrogel particles for cell-based drug delivery, particularly for cancer therapy, are discussed.

Keywords: microfluidics; cell microencapsulation; hydrogel particles; cell-based drug delivery

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1. Introduction

Cell microencapsulation is the technology that immobilizes cells in a physically isolated microenvironment, where cells can be protected from the outside environment and maintain their cellular functions. Materials that have been used to construct such microenvironments are commonly hydrogels due to their good biocompatibility [1]. In addition, because most hydrogels are porous, cells encapsulated in hydrogels can not only receive nutrients and oxygen from the outside environment but also are able to release continuously metabolic products and/or therapeutic agents to the outside [2]. Therefore, cells encapsulated in hydrogels can be treated as sources for sustainable drug release and have been used for cell-based drug delivery and therapy [3].

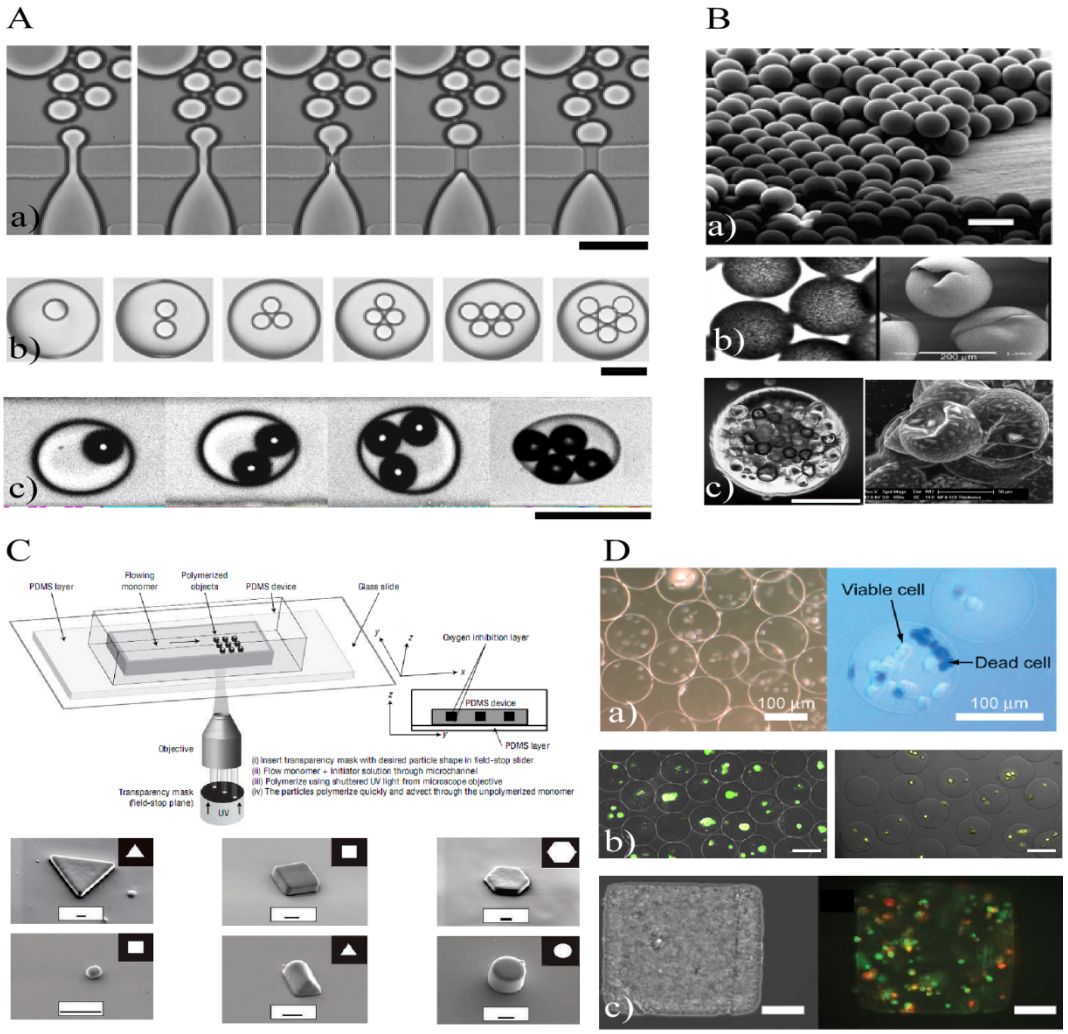
There are three key components in cell microencapsulation: cell lines that are encapsulated, types of hydrogel material, and the microencapsulation technology. Because different cell lines have their own unique pathological uses, the choice of cell lines is determined by specific applications before the encapsulation. Primary cells, stem cells, or genetically engineered cells are general resources for cell microencapsulation [4]. Once the cell line is chosen, types of the hydrogel material and the microencapsulation technology will determine the efficiency of encapsulation and the quality of a cell-based drug delivery system [4,5]. For example, natural and synthetic hydrogels have different viscosity, chemical composition, and methods of polymerization. Each of these properties of hydrogel will affect the encapsulation process and percentages of survived cells. Meanwhile, determining how to control the encapsulation process to achieve hydrogel particles with desired morphology, numbers of cells per particle, and spatial organizations of cells in the particle is also crucial for constructing effective cell-based drug delivery systems [5,6]. For example, the controlled size and morphology of the obtained microparticles, when encapsulated with cells and implanted as drug delivery vehicles, can minimize immune response and fibrosis formation [7]. In addition, small diameter and narrow size distributions of particles offers a sufficient and predictable diffusive mass transport, which is one of the key parameters in cell-based drug delivery [8].

Microfluidics combines both the advantages of the synthesis of polymer particles and the control of the process of microencapsulation, and therefore provides a promising route to encapsulate cells in hydrogel particles. Microfluidics controls fluids at the microscale and can generate micrometer diameter droplets and bubbles with a narrow size distribution and controlled hierarchical structures (Figure 1A) [9–13]. Most importantly, micro-size polymer particles including hydrogel particles can be synthesized using droplets generated by microfluidic approaches as templates [14–17]. A wide range of polymer particles, such as solid, porous, and hollow particles, have been synthesized using this approach and have a well-defined structure and morphology (Figure 1B) [18–21]. Meanwhile, microfluidic-based stop-flow lithography has also been developed for the synthesis of polymer particles with desired shapes (Figure 1C) [22–25].

In addition, microfluidics can control the process of encapsulation by adjusting flow parameters and/or using a proper geometry of microfluidic channels. It has been shown that cells can be encapsulated in droplets and/or hydrogel particles with a high efficiency of encapsulation by adjusting the flow rate ratio between continuous and disperse phases or using the stop-flow lithographic technique (Figure 1D) [26–28]. Encapsulation of exact single cells per droplets with a high yield has also been demonstrated by employing high aspect ratios of microfluidic channels [29].

In the article, I start from the basic understanding of hydrogel materials in terms of chemical structures and methods of polymerization. Then I review recent microfluidic approaches for the synthesis of hydrogel particles and discuss their advantages for the encapsulation of cells. Finally, I present applications of cell-encapsulated hydrogel particles for cancer therapy.

Figure 1. (**A**) Obtained a) monodisperse droplets [12], b) droplet(s)-in-droplet [10] and c) bubble(s)-in-droplet [13] by using microfluidic approaches. Scale bar: 200 μm. (**B**) Synthesized a) poly(tri(propylene glycol) diacrylate) particles [21], b) particles with rigid spherical shells [19], and c) porous polyacrylamide particles (left) and hollow silica particles (right) [20] by using droplets generated in microfluidic devices as templates. Scale bars are 100 μm, 200 μm, and 100 μm for a), b) and c), respectively. (**C**) Synthesis of polymer particles with different shapes using the stop-flow lithography [25]. Above shows the experimental setup and below is the SEM image of poly(ethylene glycol)(400) diacrylate particles with different shapes. Scale bar: 10 μm. (**D**) Microfluidic encapsulation of cells in a) alignate [28], b) agarose [27], and c) poly(ethylene glycol) diacrylate particles [26]. Particles in a) and b) are synthesized using droplets as templates and contain Jurkat cells and murine embryonic stem cells, respectively. Particles in c) are synthesized via the stop-flow lithography and contain NIH-3T3 fibroblast cells. Scale bar: 100 μm.



2. Natural and Synthetic Hydrogels

2.1. Introduction

Hydrogels are polymers that are water-swellable but not water-soluble. Because they contain a high volume fraction of water, display tissue-like elastic properties, and are biocompatible, hydrogels are so far the most attractive biomaterials for encapsulation of cells and tissue engineering [30,31].

Hydrogels can be either obtained from natural resources (natural hydrogels) or synthesized in a laboratory (synthetic hydrogels) [32]. Natural hydrogels are proteins, e.g., collagen, gelatin, and fibrin, and polysaccharides, e.g., alginate, hyaluronic acid, chitosan and agarose, which are extracted from mammalian or non-mammalian cells. Most natural hydrogels form networks via ionic or physical interactions under physiologically relevant conditions. Synthetic hydrogels, e.g., poly(ethylene glycol), poly(acrylic acid), and poly(vinyl alcohol), are hydrophilic polymers and synthesized covalently by radical chain polymerization or step-growth polymerization reaction in a laboratory.

The choice of different hydrogel precursors and methods of polymerization affects the microenvironment of a hydrogel matrix and consequently influences cellular functions, such as the growth, migration, and differentiation of encapsulated cells. Natural hydrogels have the innate features of the extracellular matrix and can interact directly with cells and promote cellular functions, whereas synthetic hydrogels have little cell-gel interactions. On the other hand, however, most natural hydrogels have poor mechanical properties, such as stiffness, and are difficult to be modified to tune their structures. Synthetic hydrogels can be designed to have fine-tuned material properties and produced in large quantities. Therefore, it is important to understand the differences in gel structures and properties, precursor molecules, and crosslink methods regarding natural *vs.* synthetic hydrogels. Table 1 shows the summary of features of different natural and synthetic hydrogels.

2.2. Natural Hydrogel

2.2.1. Protein-Based Hydrogel

Collagen. Collagen, a major component of skin and bone, is one of the most abundant proteins in the human body [33]. There are 29 types of collagen that have been characterized to date and all of them have a distinct triple helix structure at the molecular level. These triple helix structures can aggregate to form a microfibril, which then associates with its neighboring microfibrils to form a collagen fiber and collagen hydrogel [34].

Although there are many types of collagen, e.g., type I, II, III, V XI, only a few of them can be used to fabricate collagen-based biomaterials. Collagen type I is one of the most studied materials for tissue-engineering and has a character of low immunogenicity. In addition, collagen-based biomaterials contain specific peptide sequences that can be recognized by cell receptors and thus can enhance cell-matrix interactions and promote cellular functions [35].

Non-cross-linked collagen, however, has a poor mechanical property and low resistance to the enzymatic degradation. Various cross-link methods, therefore, have been developed to improve the stability and mechanical properties of collagen, including chemical (glutaraldehyde and water-soluble

carbodiimide) [36], physical (UV irradiation and dehydrothermal) [37], and enzymatic [38] cross-linking approaches.

Gelatin. Gelatin is derived from collagen and has similar advantages as collagen as a biomaterial for tissue engineering. Depending on how collagen is treated before the extraction process, two types of gelatin can be obtained [39]. Alkaline treatments of collagen generate a greater proportion of carboxyl groups on the gelatin, and thus produce a negatively charged gelatin with a lower isoelectric point. Gelatin obtained by acidic treatments of collagen, on the other hand, has no significant changes of the charges and has a similar isoelectric point as collagen.

Gelatin has been used to form the polyion complexation with biomolecules and can act as drug delivery carriers [40,41]. However, gelatin is soluble in water at the physiological temperature. Therefore, chemical or physical treatments to crosslink gelatin are necessary to maintain its stability and mechanical properties.

Fibrin. Fibrin is a fibrous protein that can form mesh networks over a wound site and plays crucial roles in the blood clotting and wound healing [42]. Its precursor, fibrinogen, is a plasma glycoprotein with two sets of three polypeptide chains (Aα, Bβ, and γ). These polypeptide chains can self-associate or crosslink to form an insoluble fibrin under specific conditions. For example, when fibrinogen is exposed to thrombin, two polypeptide chains of fibrinogen (Aα and Bβ) will change their conformation and expose polymerization sites, inducing the self-association of polypeptide chains among fibrinogen molecules and forming fibrin [43]. The other method to polymerize fibrinogen is adding chemical cross-linkers (e.g., blood coagulation factor XIIIa or genipin) that act on the third polypeptide chain (γ) and form fibrin [44].

Fibrin gels have a high seeding efficiency, a uniform cell distribution, and a distinct adhesion capability, and therefore have been used widely in tissue engineering [45]. However, as a natural hydrogel, fibrin also needs improvement of its low mechanical stiffness.

Elastin-like polypeptides. Because elastin is an insoluble extracellular matrix protein, elastin hydrogels are made generally from the soluble forms of elastin, such as tropoelastin and α-elastin. Elastin-like polypeptides, composed of repeats of a pentapeptide motif VPGXG where X can be any amino acid except proline [46], are artificial polypeptides derived from elastin by mimicking the amino acid sequences of the hydrophobic domain of tropoelastin. Therefore, elastin-like polypeptides, like tropoelastin, have an intrinsic ability for self-organization and formation of gels.

Elastin-like polypeptides have unique temperature responsive properties and can exhibit a temperature-dependent phase transition: they are soluble in water when the temperature is below its critical solution temperature but become insoluble at a higher temperature [47]. This process is reversal and can be fine-tuned by changing the composition and distribution of the X residue, the molecular weight of ELPs, and solution factors such as pH and ionic strength. In addition, ELPs can be obtained by the genetically-encodable synthesis [48], which provides a precise control over the molecular weight and sequence, and consequently the critical solution temperature. Thus, ELPs have a high level of control over the polymer design for specific applications [49,50].

Table 1. Summary of hydrogel materials.

Types of hydrogel material	Precursor and gelation mechanism	Crosslink method	Advantages for cell encapsulation	Disadvantages for cell encapsulation
Protein-based hydrogel				
Collagen	The precursor contains a characteristic triple helix structure; gel is formed by aggregation of the triple helix structures (microfibril) under chemical, physical, or enzymatic treatments	Using glutaraldehyde, water-soluble carbodiimide, UV irradiation, dehydrothermal, or enzymatic method to crosslink precursors	Collagen hydrogel offers direct cell-matrix interactions	Poor mechanical property
Gelatin	It is derived from collagen and is negatively or positively charged depending on the treatment method	Using glutaraldehyde, water-soluble carbodiimide, UV irradiation, dehydrothermal, or ionic interactions to crosslink precursors	Geltatin hydrogel also offers direct cell-matrix interactions	Soluble in water; the chemical or physical crosslinking is required to form stable gels
Fibrin	The precursor, fibrinogen, has three polypeptide chains (Aα, Bβ, and γ), which can self-associate or crosslink to form fibrin gels	Using thrombin, blood coagulation factor XIIIa , or genipin to crosslink fibrinogen	Fibrin offers a high seeding efficiency, a uniform cell distribution, and a distinct adhesion capability	Low mechanical stiffness
Elastin-like polypeptides (ELPs)	A repeating pentapeptide motif, Val-Pro-Gly-Xaa-Gly, is the characteristic of ELPs; gelation is achieved via a temperature -dependent phase transition of the polymer	Increase the temperature to be above its critical solution temperature	The critical solution temperature can be varied by changing the composition and distribution of the X residue and the molecular weight of ELPs.	Biosynthesis of ELPs is commonly needed

Table 1. *Cont.*

Types of hydrogel material	Precursor and gelation mechanism	Crosslink method	Advantages for cell encapsulation	Disadvantages for cell encapsulation
Polysaccharide-based hydrogel				
Alginate	(1-4)-linked β-L-guluronic (G block) and α-D-mannuronic acids (M block); gelation is achieved via ionic interactions between G blocks and divalent cations	Adding divalent cations, e.g., calcium ions, to an alginate solution	Gelation occurs rapidly under mild conditions	There is a diffusion issue associated with the gelation process
Hyaluronic acid (HA)	β-(1-4)-linked glucuronic acid and β-(1-3)-linked N-acetyl-D-glucos-amine; gelation is achieved by modifying chemically the functional groups on HA	Crosslink via thiol-modified HA, dihydrazide-modified HA, aldehyde-modified HA, or click reactions.	Biocompatible	Soluble in water; the chemical modification is required to form polymer gels
Chitosan	β-(1-4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose; gel forms via ionic interactions or chemical modifications of the amine groups on chitosan	Ionic interactions between positively charged chitosan and negatively charged polyelectrolyte; chemically crosslink the amine groups	Chitosan is biocompatible and made from abundant renewable resources	The chemical modification may introduce toxicity to the polymer gel
Agarose	The precursor is a combination of several types of sugar residues; it forms gels by aggregating single or double helices at a decreased temperature	Decrease the temperature to its critical solution temperature	Biocompatible	The gelation temperature needs to be adjusted to be physiologically relevant
Poly(ethylene glycol) (PEG)-based hydrogel				
PEG	Poly(ethylene glycol) diacrylate (PEG-DA); the radical polymerization	Crosslink by expose PEG-DA solutions to a UV irradiation	Bioinert, well-tuned structure-function relations; convenient incorporation of bioactive and biodegradable groups; suitable for micro fabrication	Incorporation of bioactive and biodegradable groups is necessary; radicals and heavy metal ions for polymerization may induce cytotoxicity
	PEG with acrylate and thiol end groups; the Michael addition reaction	Crosslink by mixing the solution of PEG with acrylate groups with the solution of PEG with thiol end groups at the room temperature		
	PEG with azide and alkyne end groups; the click chemistry	Crosslink by mixing the solution of PEG with azide groups with the solution of PEG with alkyne end groups in the presence of Cu^{2+}		

2.2.2. Polysaccharide-Based Hydrogel

Alginate. Alginate is a linear anionic polysaccharide composed of two uronic acid monomers: the (1-4)-linked β-L-guluronic (G block) and α-D-mannuronic acids (M block) [51]. These monomers are connected by glycosidic bonds and appear as either homopolymeric blocks (G or M blocks) or alternative blocks (MG blocks) in alginate. When cross-linked during the gel formation, G blocks provide a more rigid gel network whereas M and MG blocks produce a gel with more elasticity. The intrinsic composition of M/G ratio in alginate determines the gel's mechanical property.

Alginate forms hydrogel when its chains get contact with divalent cations, mainly due to the interaction between G blocks and divalent cations [52]. The gelation process can occur rapidly under mild conditions. Moreover, divalent cations with a high binding constant with alginate can enhance the strength of the hydrogel, which provides another way to tune the mechanical properties of the alginate hydrogel. Alginate hydrogel has been used widely as a biomaterial for encapsulation of cells [31,53].

Hyaluronic acid. Hyaluronic acid (HA) is also a linear anionic polysaccharide and consists of repeating disaccharides of β-(1-4)-linked glucuronic acid and β-(1-3)-linked *N*-acetyl-D-glucosamine [54]. HA is the major component in the extracellular matrix and plays important roles in supporting connective tissues, joint lubrication, cell signaling, and wound healing [55]. However, HA is soluble in aqueous solution in its native form and has a poor mechanical property. Chemical modification of HA to form cross-linked networks is thus necessary. The target functional groups on HA are normally the carboxylic acid group of glucuronic acid, the hydroxyl groups, and the *N*-acetyl group. Methods for chemical modification of HA have been reviewed recently [56].

Chitosan. Chitosan is a linear cationic copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose. It is derived from chitin, a long chain polymer of *N*-acetylglucosamine found in many places throughout the natural world, by hydrolyzing the amino acetyl groups [57]. Chitosan is soluble in dilute acidic solutions.

Due to the presence of amine groups, chitosan is positively charged at acidic solutions and can form a gel network, e.g., ionic complexes or polyelectrolyte complexes, via ionic interactions [58]. In addition, disulfide-crosslinked chitosan hydrogels and cross-linking chemically the amine groups of chitosan with reactive groups in cross-linkers have been demonstrated [59,60]. Because chitosan can be made from abundant renewable resources and modified physically and chemically, it has been used widely for tissue engineering, drug delivery, and wound-healing applications [61,62].

Agarose. Agarose is a polysaccharide extracted from the cellular walls of agarophyte seaweed. Agarose solution forms gels when the temperature is below its gelling temperature (15–30 °C) and the process is thermally reversible . The molecular composition of agarose is a combination of several types of sugar residues [63]. The proposed gel structure is an aggregation of single or double helices of polysaccharide chains. Agarose has been utilized commonly for cell culture and tissue engineering [64].

2.3. Synthetic Hydrogel

Unlike most natural hydrogels that are extracted from natural resources and have intrinsic material properties that are difficult to modify, synthetic hydrogels are synthesized based on the well-established polymer chemistry and are convenient to scale up and to be modified. The structure-function relations in synthetic hydrogels, therefore, can be studied systematically. Moreover, bioactive peptides and functional groups, such as Arg-Gly-Asp (RGD), a cell adhesion peptide sequence, can be incorporated into synthetic hydrogels to mimic the extracellular matrix and direct cell-matrix interactions [65]. Thus, a promising future of synthetic hydrogels is the research of a system that can incorporate the features of natural hydrogels into synthetic hydrogels and possesses the advantages of each [66,67]. For example, RGD peptides were covalently grafted to poly(ethylene glycol) diacrylate (PEG-DA, MW 8000) and human foreskin fibroblasts could attach to and spread on the crosslinked RGD-PEGDA gel. No cell adhesion was observed on unmodified gels [68]. Indeed, a number of synthetic hydrogels, e.g., poly(ethylene glycol), poly(acrylic acid), poly(ethylene oxide), poly(vinyl alcohol), poly (*N*-isopropyl acrylamide) and their derivatives have been developed successfully for tissue engineering and encapsulation of cells [32,69,70].

Because synthetic hydrogels have common features in terms of their synthetic approaches and can be obtained by using classical polymerization methods, here I have taken one of the most utilized synthetic hydrogels, poly(ethylene glycol) (PEG), as an example to introduce the properties and synthetic approaches of synthetic hydrogels.

PEG-based hydrogel has a property of low protein absorption. Thus, PEG is bioinert and has reduced immune and inflammatory responses. By fine-tuning the versatile PEG polymer chemistry, e.g., incorporation of copolymers, its network structure can be modified conveniently [71]. Chemical and physical cues, such as degradable linkages and cell adhesion groups, can also be incorporated to mimic critical aspects of the natural hydrogel matrix [72,73]. Most importantly, PEG hydrogels can be processed using photolithographic or microfluidic approaches [74,75], which enable the gradient patterning of gel properties and facilitates the fabrication of microarchitectures that can potentially mimic the key aspects of tissue architecture.

PEG-based hydrogel is synthesized commonly via (1) the radical polymerization, e.g., redox or photo-initiated polymerization of vinyl groups on PEG macromers; (2) the Michael addition chemistry where PEG macromers with thiol groups react PEG macromers with α,β-unsaturated carbonyl groups; (3) the click chemistry where PEG macromers with azide groups react PEG macromers with alkyne groups in the presence of Cu^{2+} as a catalyst; and (4) the enzyme-catalyzed reaction [76]. Because the radical polymerization is fast but the step-growth polymerization, e.g., the Michael addition chemistry, can fine-tune the structure of the gel's network, a mix-mode polymerization approach, such as the thiol-acrylate photopolymerization, has been developed to take advantages of the chain-growth and step-growth mechanisms [77]. The structure of the gel's network formed by poly(ethylene glycol)-poly(lactic acid)-diacrylate (PEG-PLA-DA), for example, is different substantially when there is a presence of a tetrathiol monomer. The network structure is dependent significantly on the relative concentrations of the thiol group to the acrylate group.

3. Microfluidic-Based Synthesis of Hydrogel Particles for Cell Microencapsulation

Encapsulation of cells into hydrogel particles has been achieved by using extrusion methods [78], emulsions [79], interfacial polymerization [80], and complex coacervation [81]. Conventional methods, however, produce large hydrogel particles (millimeter or hundreds of micrometer diameter) and have a less control over the size distribution of particles. A recent advance in microfluidics and microlithography provides an effective route to control the generation of droplets and particles, including the size/size distribution and the shape of particles [21,25]. Therefore, microfluidics has emerged as one of the promising approaches for the synthesis of hydrogel particles and cell microencapsulation. In this section, I will introduce the controlled encapsulation of cells in droplets and droplet-based synthesis of hydrogel particles using microfluidic approaches. Hydrogel particles synthesized by the stop-flow lithography and the synthesis of hydrogel microfibers for cell microencapsulation will also be discussed.

3.1. Microfluidic-Controlled Encapsulation of Cells in Emulsion Droplets

Microfluidics has been demonstrated for the encapsulation of droplets or bubbles inside another droplet (Figure 1A). The encapsulation is accomplished by tuning wetting properties of the surface of microfluidic channels, the surface tension and viscosity ratio, and the flow rate ratio between the disperse and the continuous liquid phase. Using microfluidic approaches, the number and size of encapsulated droplets or bubbles can be controlled precisely. For example, a droplet-in-droplet double emulsion and a droplet-in-droplet-in-droplet triple emulsion with different numbers and sizes of encapsulated droplets can be produced using glass capillary microfluidic devices [10]. Gas bubble(s) can also be encapsulated in a droplet using similar approaches in polydimethylsiloxane (PDMS) microfluidic channels [13].

Microfluidics also shows the ability to encapsulate cells in droplets if a cell-containing solution is used as the disperse phase. A high efficiency of encapsulation can be achieved by choosing the size of droplets based on the cell density [27]. For example, if the cell density is 10^6 cells per milliliter in a cell suspension, every cell will occupy a volume of the solution of 10^{-12} cubic meter assuming cells are well dispersed in the solution. In order to achieve 100 % encapsulation, which means every droplet contains at least one cell, the least volume of droplets should be 10^{-12} cubic meter, which equals a droplet with a radius of 100 μm. Because the size of droplets can be controlled conveniently by adjusting the flow rates in a microfluidic setup, cells can be encapsulated with a high efficiency of encapsulation. For example, Kumachev et al. reported a microfluidic approach where embryonic stem cells (8×10^6 cell/mL) can be encapsulated in agarose microgels (precursor droplets with a mean diameter of 110 μm) at an encapsulation efficiency of 98.5% [27].

Encapsulation of exact one cell per droplet with a high yield, however, is challenging because the encapsulation is a random process and has its intrinsic limitation: it follows the Poisson statistics. It will require a very low average cell density (number of cells per volume) to minimize the probability of having more than one cells per droplet. This is a very inefficient process because there will be a significant amount of droplets containing no cells at all.

Microfluidics, however, can beat the Poisson limitation in terms of the encapsulation of single cells per droplets (Figure 2A). Using polymer particles as a model system, it is found that when particles are close-packed in a microfluidic channel, they can form regular orders and generate a periodic flow of particles [82]. By matching the periodicity of the drop formation to the particle flow using microfluidics, desired number of particles, such as single particles in each droplet can be achieved with a high efficiency. Because the particles used in the study are deformable, like most cells, this approach provides a potential method for the controlled encapsulation of cells in drops. In fact, encapsulation of single cells in a droplet with a high yield has been achieved by using high aspect-ratio microfluidic channels [29]. High aspect-ratio PDMS channels restrict cells to certain streamlines inside the channel and generate two trains of cells with a precisely staggered longitudinal spacing. This self-ordering phenomenon is utilized to generate drops containing exactly one cell when applied in a flow-focusing microfluidic device.

Figure 2. (**A**) (Above) Close-packed ordering of deformable polyacrylamide particles and droplet-triggering in microfluidic channels to control the encapsulation of particles in droplets [82]. Scale bar: 75 μm. (Middle) Encapsulation of single polystyrene (DI = 9.9 μm) beads in water droplets using polydimethylsiloxane (PDMS) microfluidic channels with high aspect ratios [29]. Note that in this microfluidic design, single beads can be encapsulated precisely into single droplets with a high yield. Scale bar: 100 μm. (Below) Encapsulation single hybridoma cells in water droplets using the same device as shown in the middle image. Scale bar: 100 μm; (**B**) Synthesis of alginate particles using droplets as templates in microfluidic channels [28]. The droplets contain an alginate solution and $CaCO_3$ nanoparticles. The acetic acid in the oil phase diffuses into the droplets and triggers the release of calcium ions, which leads to the gelation of the droplets to form alginate particles. Note that the incorporation of $CaCO_3$ nanoparticles allows the internal gelation of alginate droplets and avoids the generation of undesired shapes of particles; (**C**) Experimental setup for the encapsulation of cells into poly(ethylene glycol) diacrylate particles with desired shapes using the stop-flow lithography [26]; (**D**) Microfluidic setup to generate alginate hollow microfibers [102]. In the experiment, a three-layer of coaxial flow is generated along the channel. The core and out-layer fluids are the calcium chloride solution and the middle layer fluid is an alginate solution. The calcium ions in the core and out-layer fluids diffuse into the alginate solution in the middle layer and solidify the middle layer to form hollow fibers.

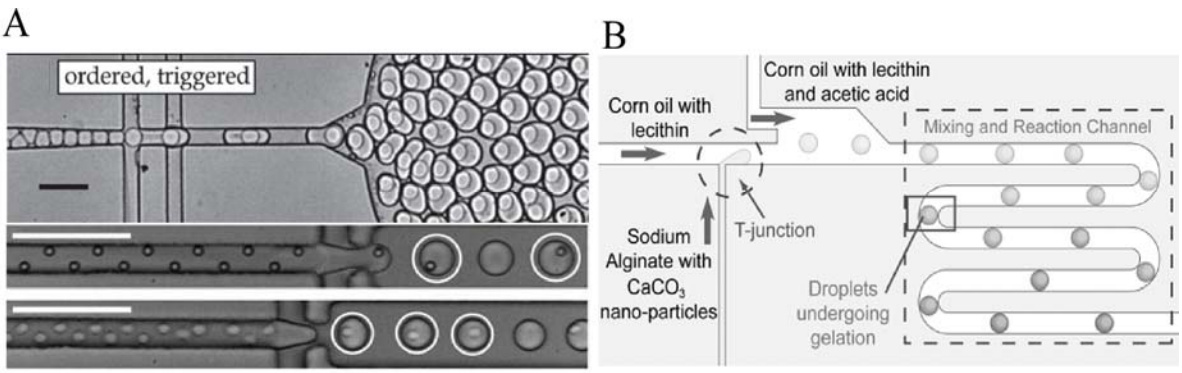
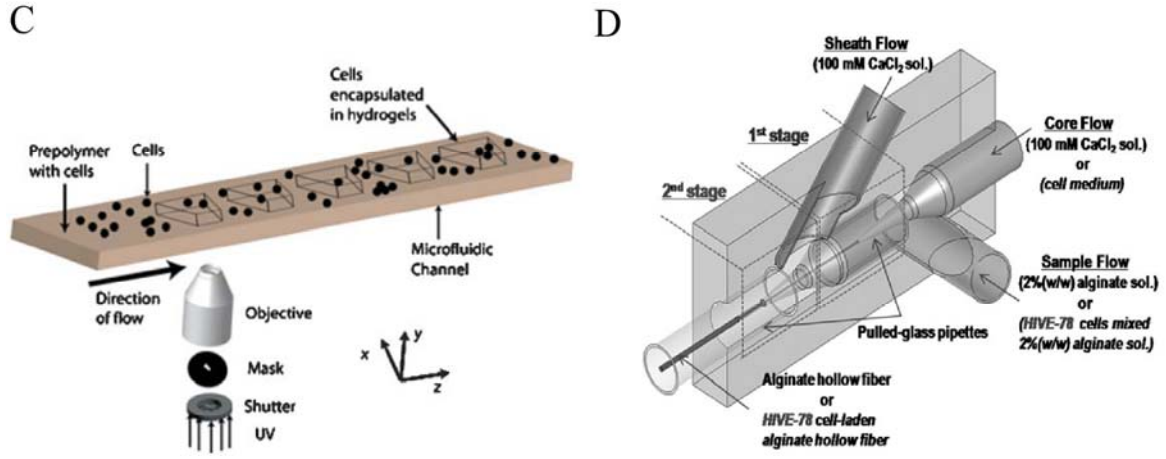


Figure 2. *Cont.*



3.2. Synthesis of Hydrogel Particles Using Microdroplets as Templates

Droplets generated by microfluidic approaches have been used as templates for synthesis of polymer particles, including hydrogel particles [15,18]. The approach has several advantages: (1) Because the droplets are monodisperse and the size of droplets can be controlled by changing the flow rate ratio, particles of different size can be obtained. Meanwhile, the obtained particles have a narrow size distribution [12]. (2) Core-shell, hollow, and porous particles can be obtained using multiple emulsion droplets, such as droplet-in-droplet and/or bubble-in-droplet [10]. (3) Particles with different shapes, such as disk-shape particles, can be synthesized by polymerizing droplets in a confined geometry inside the channel [83]. (4) Janus particles can be obtained by polymerizing droplets containing two polymerizable solutions before a diffusion-induced mixing occurs [84].

The advantageous synthesis of polymer particles using microfluidics provides a useful platform for cell microencapsulation. For example, embryonic stem cells have been encapsulated in monodisperse agarose hydrogel microbeads, which are synthesized by gelling cell-containing droplets in a continuous oil phase in microfluidics (Figure 1D) [27]. Moreover, by varying the concentration of agarose in a droplet, agarose hydrogel particles with different elasticities can be produced continuously. This approach offers a convenient method to synthesize hydrogel particles with a varied mechanical property, which can be used to study cellular responses of encapsulated cells to the mechanical property of their microenvironment. Meanwhile, the example also demonstrates a common strategy used in microfluidics for the generation of cell-containing hydrogel particles: cell suspensions with a proper cell density and natural or synthetic hydrogel precursors are injected into microfluidic devices as the disperse phase. Cell-containing droplets are then generated in a continuous oil phase in the microfluidic channel. Solidifying chemically or physically the cell-containing droplets produces gel particles with encapsulated cells. A variety of cell-containing hydrogel particles, including agarose, alginate, gelatin, and PEG particles has been produced based on this strategy.

Encapsulation of cells in alginate particles has been studied intensively and shown promising applications for cell-based drug delivery and therapy [5,85–89]. The synthesis is achieved via an external or internal gelation of cell-containing alginate drops using divalent ions, such as calcium ions [90,91]. External gelation is conducted by forming cell-containing alginate droplets in the air and

then delivering the droplets to a reservoir solution containing calcium ions. Calcium ions in the reservoir diffuse to the surface of alginate droplets and crosslink alginate polymer chains to form a membrane, which hinders further diffusion of calcium ions to the interior of alginate droplets. The external gelation method, therefore, results in alginate particles with a highly cross-linked surface but less cross-linked core. For the internal gelation, alginate droplets containing cells and insoluble calcium salts are generated in an oil phase. The oil phase is then acidified, which triggers the release of calcium ions from the insoluble salts. The released calcium ions crosslink the alginate droplets, which leads to the formation of alginate particles. Alginate particles produced by the internal gelation method have a homogenous but less condensed matrix structure.

Conventional emulsion techniques, such as the dripping-extrusion method, generate large alginate particles (DI = 1500–5000 μm) [88]. In addition, most conventional approaches use external gelation methods and have a less control of the size, the size distribution, and the morphology of obtained particles [92,93]. In fact, deformed alginate particles with tails are often obtained using the dripping technique [94]. In order to produce spherical-shape particles using the dripping technique, a wide range of parameters have to be optimized.

Microfluidic approaches, on the other hand, can overcome the limit set by conventional methods and generate cell-containing alginate particles in a well-controlled manner. One strategy is injecting simultaneously a cell suspension, an alginate solution, and a solution contains soluble calcium salts from three independent inlets (channels) of a microfluidic device [95]. All the solutions meet each other at a junction where a fourth channel that contains a continuous oil phase is introduced. Aqueous droplets containing cells, alginate, and calcium ions are then generated in the continuous oil phase and gelled to form alginate particles. Cell-containing alginate particles produced in this method have a well-defined size and morphology. However, cautions have to be paid to the control of the flow rates of different solutions to ensure that all the solutions arrive at the junction at the same time. In this scenario, the concentration of alginate and calcium ions will be the same for all droplets and crosslinking the cell-containing droplets occurs only after the generation of droplets. Another internal gelation approach is using $CaCO_3$ nanoparticles in aqueous droplets as the source of calcium ions (Figure 2B) [28]. When acetic acid is added in the continuous oil phase, it diffuses into the droplets and reacts with $CaCO_3$ nanoparticles to release calcium ions, and consequently the droplets is gelled to form alginate particles. This approach avoids the use of insoluble $CaCO_3$ powders and ensures the homogeneous distribution of calcium salts in the droplets, and, therefore, provides a reliable method to encapsulate cells in alginate particles. Other microfluidic approaches, such as the fusion of drops containing alginate and cells with drops containing calcium ions to produce Janus alginate particles [96] and to control the shape of alginate particles [97] have also been demonstrated.

3.3. Synthesis of Hydrogel Particles Using the Stop-Flow Lithography

Stop-flow lithography is another type of microfluidic approach to synthesize polymer particles and to encapsulate cells. In contrast to the droplet-based methods, there is no disperse phase in the stop-flow lithography and only a single phase photocrosslinkable prepolymer solution is used. The prepolymer solution containing cells flows through a microfluidic channel and is polymerized in the channel upon a UV irradiation through a microscope objective (Figure 2C) [26].

One of the most distinct advantages of the synthesis of particles using the stop-flow lithography is that it can generate particles with a wide range of desired shapes by simply using a proper photo mask between the UV light source and the microscope objective [23–25]. For example, particles with a shape of triangle, square, and hexagon, and posts with circular, triangular and square cross-sections have been demonstrated using the stop-flow lithography (Figure 1C) [25]. The shapes of obtained particles show a good fidelity to the designed mask. In addition, the stop-flow lithography has several advantages in terms of cell microencapsulation: (1) there is no high shear stress in the process of encapsulation, which minimizes the chance of mechanical lysis of cells; (2) the obtained cell-containing particles is free of oil as there is no oil phase involved; (3) stop-flow lithography does not require a short polymerization time and thus a high concentration of photoinitiator or prepolymer can be avoided. As a proof-of-principle, NIH-3T3 fibroblast cells have been encapsulated in poly(ethylene glycol) diacrylate (PEGDA) particles using the stop-flow lithography (Figure 1D) [26]. The synthetic process and cell viability have been studied systematically by changing the UV exposure time, concentrations of PEGDA and photoinitiator, and the shape of particles. Under optimized conditions, cell's viability is up to 68%. Although the system is still needed for further optimization, the stop-flow lithography can generate cell-loaded particles continuously and have a fine control of the shape and size of particles, it is therefore a promising approach for the microencapsulation of cells in particles.

4. Microfluidic-Based Synthesis of Hydrogel Fibers for Cell Microencapsulation

Fibrous materials can guide the growth, alignment, and migration of encapsulated cells, and therefore to promote aggregations of cells in a certain manner that can enhance the cellular activity and function [98–100]. In addition, cell-containing microfibers can be woven to obtain three-dimensional scaffolds, such as cell sheets [101], or used to generate tubular structures (hollow fibers) for vascular tissue engineering [102]. Therefore, fibrous materials are highly desired for tissue engineering and cell-based therapy.

Many methods, such as electrospinning [103], hydrodynamic spinning [104], and melting extrusion [105], have been demonstrated for the generation of polymer fibers. These approaches, however, have limitations on the control of the morphology, dimension, or direction of alignment of obtained fibers.

Microfluidics shows advantages for the synthesis of microfibers due to its simplicity and the ability to control the size and structure of microfibers [106]. The synthesis of microfibers using microfluidic approaches is accomplished by polymerizing the coaxial flow of a polymerizable liquid in a microfluidic channel. Because multiphase fluids in microfluidic channels can co-flow with each other and form laminar streams as long as the interfacial energy is low, when a core fluid (a polymerizable liquid) and a sheath fluid (a non-polymerizable liquid) are injected into a microfluidic device, a co-flow is generated in the main channel and microfibers can be obtained by polymerizing the core fluid physically or chemically.

Both glass capillary and PDMS microfluidic devices have been developed to synthesize cell-containing hydrogel microfibers using natural hydrogels such as alginate [107], chitosan [108], chitosan-alginate [100], and synthetic hydrogels such as poly(lactic-co-glycolic acid) (PLGA) [99] and

polyurethane [109]. For example, HepG2 cells are encapsulated in chitosan microfibers produced by a glass capillary microfluidic device [100]. The cells aggregate as spheroid-like structures in the fiber and their hepatic functions in terms of albumin secretion and urea synthesis are found to be higher than the cells that are cultured on uncoated polystyrene dishes. Meanwhile, because multiphase streams in glass capillary microfluidic channels are co-axial and can form core-shell jests, hollow microfibers can be fabricated if the core fluid is non-polymerizable but the shell fluid is polymerizable [102,110]. Indeed, alginate hollow fibers are fabricated by using a glass capillary microfluidic setup where the core, shell, and sheath fluid are $CaCl_2$, alginate, and $CaCl_2$ solution, respectively (Figure 2D) [102]. The alginate hollow fibers are then used to encapsulate endothelial cells (HIVE-78) and co-culture with smooth muscle cells ((HIVS-125) in agar-gelatin-fibronectin hydrogels to construct three-dimensional microvascularized structures. The results show a good cell viability and the formation of three-dimensional vessels. Moreover, additional microchannel-like approaches, such as microfabricated nozzles (filters) [111] and coaxial triple cylinder [112], have also been demonstrated to produce solid and hollow hydrogel microfibers for encapsulation of cells.

5. Applications of Encapsulated Cells in Hydrogel for Cancer Therapy

Microencapsulation of cells in hydrogels provides cells a physically isolated but semipermeable microenvironment, which not only prevents cells from the immune response and mechanical stresses originated from the host but also ensures the proper influx of nutrients and oxygen and outflows of bioactive molecules or drugs. Therefore, hydrogels with encapsulated cells find applications as a cell-based drug delivery vehicle for the treatment of a wide range of diseases, such as diabetes [113], bone and cartilage defects [114], cancer [115], and heart [116] and neurological [117]diseases. Further details of the application of cell microencapsulation are summarized in recent reviews [5,31,89].

Cells that are encapsulated in hydrogel commonly need to be engineered specifically for a particular drug delivery purpose. For cancer therapy, cells are engineered to be able to either produce cytokines for the anti-tumor immunotherapy, release anti-angiogenic molecules for the anti-angiogenic therapy, or over-express enzymes that can activate chemotherapeutic agents or prodrugs for the gene-directed enzyme prodrug therapy [5,118]. Because cancer is of great interest for both fundamental and clinical research, it is important to understand the strategies that have been developed for cancer therapy using cell-loaded hydrogels [118,119].

As mentioned abover, there are generally three strategies that have been developed to suppress the growth of tumor cells: the anti-tumor immunotherapy, the anti-angiogenic therapy, and the gene-directed enzyme prodrug therapy. Cells that are used for the cell-based drug delivery for cancer treatments are normally bioengineered according to these three approaches. For instance, human erythroleukemia K562 cells engineered genetically to produce granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine that can induce potent, specific and long-lasting anti-tumor immunity in mice, have been encapsulated in hollow fibers of polyethersulfone as a system for the anti-tumor immunotherapy [120]. The encapsulated cells show sustainable release of GM-CSF for 25 days and preserved cell survival when implanted in mice. On the other hand, HEK293 cells engineered genetically to produce anti-angiogenic agents, such as angiostatin, are encapsulated in alginate particles for the anti-angiogenic therapy [121]. The results show a relatively stable production

of angiostatin up to 11 weeks. This study also demonstrates that the size, structure, and morphology of alginate particles and the cell density affect the cell viability and the production of angiostatin. Meanwhile, research has shown a strategy that combines both the anti-tumor immunotherapy and the anti-angiogenic therapy into one treatment [122]. It is achieved by engineering mouse myoblasts that can deliver both angiostatin and an interleukin 2 fusion protein in a B16 mouse melanoma model. The results show an improved survival and delayed tumor growth. The combing approach also can protect the host from the adverse side effects of single treatments.

Encapsulation of cells that can over-express enzymes, such as the cytochrome P450 enzyme, to activate chemotherapeutic agents or prodrugs, such as ifosfamide, for the gene-directed enzyme prodrug therapy is one of the most encouraging approaches for cancer therapy [123]. The first demonstration of this method is using genetically modified epithelial cells but later the results can also be reproduced using HEK293 cells [124]. In addition, not only ifosfamide but also cyclophosphamide and related agents can be activated by the cytochrome P450 enzyme. Therefore, this strategy opens a range of opportunity to treat tumors. . In fact, NovaCaps®, the encapsulated cell therapy product based on this strategy, have been tested in a Phase I/II clinical trial in patients with pancreatic cancer and the results are promising [125].

Last, encapsulation of stem cells has also been demonstrated for cancer therapy [126]. Unlike nonautologous cells that can evoke an immune response from the host, which may cause damages to the encapsulating matrix, stem cells are hypoimmunogenic and can be bioengineered to release therapeutic agents. It is therefore believed that the encapsulation of stem cells for cancer therapy will overcome the shortcomings of current approaches. For example, human mesenchymal stem cells (hMSC) have been modified genetically to produce hemopexin-like protein and encapsulated in alginate-poly-l-lysine microcapsules for the anti-angiogenic therapy in a model of human glioblastoma [127]. The results show that, when hMSC-loaded microcapsules are injected locally to the area adjacent to glioblastoma tumors, significant volume reduction of tumors (87%) is observed 22 days post treatment. Meanwhile, comparing to encapsulated HEK293 cells, the encapsulated MSCs reduce the immunogenic responses significantly.

6. Conclusion and Outlook

Microfluidic-based synthesis of hydrogel materials has advantages for the control of the size/size distribution, the structure, and the morphology of micro-particles and micro-fibers. In addition, microfluidics can control precisely the encapsulation of cells in hydrogel particles. The combined ability of microfluidics in the particle synthesis and cell encapsulation makes microfluidics a promising approach for the controlled encapsulation of cells in particles and construction of effective cell-based drug delivery systems.

Challenges, however, still exist. For example, overgrown cells in the encapsulating particles will leak out of the particle and trigger immune responses. How to control overgrew cells in the matrix is important. Second, most of cell-containing particles are synthesized based on the solidification of cell-containing aqueous droplets in a water-immiscible liquid, such as oil. Although most of the studies show that the oil phase can be removed by simply centrifuging the particles, how to get rid of the oil completely from particles, particularly from soft particles, is a current challenge. Moreover, during the

process of encapsulation of cells in droplets in microfluidic channels, cells possibly experience high shear stresses, which will damage the cell. Development of mild conditions for cell microencapsulation in microfluidics is thus preferred. In this case, the stop-flow lithography is a promising approach [26]. Finally, the control of spatial arrangements of multiple cells in an encapsulating hydrogel particle is of interest for tissue engineering and the co-delivery of therapeutic agents. There is some degree of success on this topic using microfluidic approaches but no systematic study has been conducted yet [128]. Also, there is a lack of approach to detect *in vivo* the mobility of particles and time-dependent particle distributions after the cell-loaded particles are injected into the body. Future research in this direction will be of great interest for clinical applications.

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