Emerging Methods for the Fabrication of Polymer Capsules

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Abstract:

Hollow polymer capsules are attracting increasing research interest due to their potential application as drug delivery vectors, sensors, biomimetic nano- or multi-compartment reactors and catalysts. Thus, significant effort has been directed toward tuning their size, composition, morphology, and functionality to further their application. In this review, we provide an overview of emerging techniques for the fabrication of polymer capsules, encompassing: self-assembly, layer-by-layer assembly, single step polymer adsorption, bio-inspired assembly, surface polymerization, and ultrasound assembly. These techniques can be applied to prepare polymer capsules with diverse functionality and physicochemical properties, which may fulfill specific requirements in various areas. In addition, we critically evaluate the challenges associated with the application of polymer capsules in drug delivery systems.

Keywords: nanoparticles, polymer architecture, assembly, layer-by-layer, drug delivery, nanotechnology

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1. Introduction

Polymeric capsules, containers with a structure composed of a hollow core and a polymeric shell, have shown potential application as drug carriers, microreactors, sensors, and artificial organelles [1-3]. Generally, there are two approaches to produce polymer capsules; template-free and template-assisted techniques. The most commonly used methods are the self-assembly of block copolymers [4], which is a template-free method, and the layer-by-layer (LbL) technique [5], which makes use of a sacrificial template. In recent years, several alternative techniques have been developed to endow polymer capsules with novel and interesting properties. A plethora of different polymers can be used to tune the properties of polymeric capsules for a desired purpose. Besides (natural) biopolymers, well-established controlled polymerization and efficient post-polymerization functionalization techniques have become indispensable tools to synthesize polymers of tailored length, composition and functionality. Hence, it is now feasible to prepare polymer capsules of diverse size, composition, morphology, and properties.

In this review, we focus on the methodologies for the fabrication of polymer capsules via self-assembly and template-assisted approaches. We briefly highlight the application of polymer capsules as controlled drug and vaccine delivery vectors, and biomimetic microreactors. Emerging topics of interest, such as the assembly of capsules with different geometry (e.g., shape and size) to modulate biological responses are also discussed.

2. Methods for polymer capsule assembly

2.1. Self-assembly

Polymersomes are synthetic vesicles comprised of amphiphilic block copolymers, that is, polymers that consist of both hydrophilic and hydrophobic blocks, and can be considered the polymer analogue of liposomes [4]. Similarly, polymersomes are spherical structures with an aqueous core which is enclosed by a bilayer membrane. However polymersomes exhibit far greater mechanical stability than natural lipid membranes [4,6,7]. Consequently, they hold great potential to be used in drug or gene delivery, as they are able to encapsulate or load therapeutic molecules into their core and/or the membrane compartment. Most reported polymersomes are based on diblock copolymers and triblock terpolymers (Fig. 1). However, the desire to vary membrane conformation, increase functionality or mimic the asymmetric character of biological membranes has also led to the use of rather complex multiblock copolymers, or even blends of block copolymers.

Synthetic polymers offer almost infinite options to control the structural and physicochemical properties of membranes and vesicles. While there are already excellent reviews on polymersomes and their potential applications, we focus here on their preparation and highlight the most commonly used methods for the fabrication of polymersomes.

Polymersomes are formed *via* self-assembly or self-organization of block copolymers. The ratio of hydrophobic to hydrophilic block is therefore of great importance. Similar to surfactants, this ratio will dictate the self-assembly into either spherical or worm-like micelles or polymersomes [9]. There are numerous studies highlighting the diversity of block copolymer assembly, their intermediate structures and potential applications [1,8,10-22]. The most commonly used preparation methods for polymersomes are solvent-switching

techniques (solvent displacement) [23,24] and polymer rehydration techniques (solvent free approach) [6,25-27].

Solvent-switching generally describes the addition of a block selective solvent to a block copolymer solution in a good solvent for both blocks. This technique is not limited to, but mostly performed with, water as the selective solvent [16,28]. Whereas the hydrophilic parts of the polymer prefer contact with water, the hydrophobic parts tend to avoid and minimize contact with water and hence are attracted to each other [8]. This procedure is also referred to as 'phase inversion'. The addition of water can thereby be performed either slowly (drop-wise or during dialysis) or by a fast injection to the organic solution.

Based on the same principle, it is also possible to form polymersomes directly in water. This can be achieved by dissolving an amphiphilic block copolymer directly in water (for example under the aid of sonication [29] or detergent [30,31]). Stimuli-responsive bishydrophilic block copolymers dissolved in water are reported to form polymersomes under external stimuli, such as pH [18,32-35], temperature [28,35-38], or light [39]. An applied stimulus is then used to render one block hydrophobic, which subsequently triggers the self-assembly into polymersomes. Often, stimuli-responsiveness is reversible, which can be used to disassemble the polymersome again, leading to the release of encapsulated or loaded substances [40].

Another commonly used method to prepare polymersomes is the rehydration of thin films of amphiphilic block copolymers. The polymers are first dissolved in an organic solvent, followed by thin film formation via solvent evaporation. The subsequent addition of water results in rehydration of the film which, in turn, swells the polymer layers and forms protrusions that detach from the surface and close to form vesicles [8]. Polymersome formation occurs purely in water and is essentially solvent-free; however, it strongly depends

on the kinetics of the rehydration process. Faster rehydration, for example under the aid of vigorous mixing or sonication, leads to nanometer-sized vesicles.

Other methods used to produce polymersomes include oil-in-water (o/w) emulsions [40,41], water-in-oil-in-water (w/o/w) double emulsions [42-45], inkjets [46], microfluidic devices [44,47-49], and electroformation [4].

A common issue with non-templated techniques in general, and specifically with self-assembled polymersomes, is their polydispersity in size. Post-treatment, such as extrusion [50], sonication [26], or freeze-thaw cycles [26] have proved useful to homogenize polymersome size distributions. Recently, monodisperse polymer vesicles have also been produced via a template-directed approach combining photolithography and the rehydration technique [51]. By using monodispersed templates, around which the polymer coating is formed, the LbL approach produces monodisperse polymer capsules.

2.2. LbL assembly

In the last 20 years, the layer-by-layer (LbL) technique has attracted significant interest in the fabrication of multilayer thin films [5,52-57]. Owing to its simplicity and versatility, researchers have reported numerous materials, templates and strategies which can be applied in LbL assembly. A distinct advantage of the LbL technique is the precise control over film properties, such as thickness and morphology that can be obtained. In general, LbL assembly is suitable for the fabrication of multilayer films on planar as well as particle supports. In recent years, research has been devoted to develop methods to overcome the issues accompanied by the transition to particle templates, such as mechanical stability and aggregation. The assembly of polymers onto (sacrificial) spherical substrates yields nano- and microcapsules after removal of the template [5,56,58]. The multilayer structure of these

capsules enables the combination of different properties in one system, mostly governed by the material. These systems can be rendered responsive to external stimuli or allow for the loading with different cargo [59,60], which are of interest for therapeutic delivery and microreactor applications [61-64].

In the following subchapters layering methods and driving forces for multilayer assembly on particle supports that have been reported to date are briefly summarized. Furthermore, selected examples of polymeric materials applied for LbL assembly are presented.

2.2.1. Different layering methods

In template-assisted assembly, films can be formed by either surface-confined polymerization (Chapter 2.5) or by depositing (multiple) layers by LbL assembly. In recent years, different techniques have been applied for the fabrication of LbL capsules, including centrifugation, filtration and electrophoretic approaches (Fig. 2). Depending on the type of polymers and templates, the technique has to be chosen carefully to allow for optimal particle coating.

Commonly, centrifugation of the particle suspension is employed to separate the free polymer from the coated particles (Fig. 2a). However, this process requires multiple centrifugation and washing steps, rendering it time-consuming and labor-intensive. Furthermore, it suffers from the necessity to sediment the coated particles, which can promote aggregation, especially for smaller-sized templates. In contrast, the sequential addition technique uses precise concentrations of the layering material to coat the particles in suspension [67]. Thus, there is no need for centrifugation; however, the precise control of all suspension components is rather complicated and does not prevent the formation of agglomerates. To overcome some of these drawbacks, other processes have been developed over the last decades. Membrane filtration (Fig. 2b) [58] as well as electrophoretic polymer assembly (EPA) (Fig. 2c) [66] represent continuous LbL processes. Both approaches allow the particles to remain suspended,

lowering their tendency to agglomerate. In the membrane filtration approach, template particles and polymer are suspended in a stirred tank to achieve layer deposition. Free polymer is subsequently separated from coated particles by applying a pressure differential across the filter membrane while adding a washing medium. Additional layer deposition was achieved by repeatedly adding oppositely charged polymer followed by a washing step. This approach is significant because it allows large quantities microcapsules from diverse templates to be produced using an automated technique. However, appropriate selection of filter material, to prevent polymer adsorption and filter obstruction, and the speed of filter cake formation, which leads to particle aggregation and damage, are critical factors that affect the process. Alternatively, the EPA method utilizes an agarose hydrogel to suspend template particles; layer deposition is then achieved by electrophoresis of polymers through the agarose gel. This technique allows a diverse size range of particles to be layered (down to 35 nm). However, successful layer deposition requires that the materials are mobile in electroosmotic flow, and separation of the coated particles from the immobilizing matrix requires the application of heat and centrifugation.

2.2.2. Assembly interactions

For the fabrication of LbL capsules, numerous interactions have been employed to date (Fig. 3) [68]. The alternate adsorption of materials through complementary interactions can be realized by three main strategies: (a) electrostatic interactions between oppositely charged polyelectrolytes [69], (b) hydrogen bonding of hydrogen bond donor and acceptor polymers [70-72], and (c) covalent bonding for direct multilayer build-up and stabilization of pre-formed films [73], respectively. Besides, other chemical and physical interactions have been used to assemble and/or stabilize multilayer films, including: (d) DNA hybridization [74-79], (e) stereocomplexation [80,81], (f) hydrophobic [82], and (g) host-guest interactions [83-85].

The variety of interactions which can be applied for the fabrication of multilayer films further demonstrates the enormous potential of LbL for the fabrication of smart delivery systems.

2.2.3. Templates and polymer building blocks

The choice of the template, as well as the polymer system are crucial factors for the fabrication of polymer capsules with distinct properties and, hence, applications. While the polymer system directly determines the properties of the capsules, template choice is of equal importance since the size and shape of the final capsules are mainly dependent on the templates. Suitable templates should be stable during LbL assembly and the process of template dissolution should not affect the structure and stability of the capsule shell. The most commonly used templates, along with their size range, shape, monodispersity, and the method of core removal are listed in Table 1.

As capsule wall materials, a large number of materials/polymers (Table 2) have been used. Due to this variety, capsules can be tailor-made for certain applications by simply choosing the designed material. Among the materials available, polyelectrolytes are the most frequently used polymers for the fabrication of LbL polymer capsules. In recent years, functional polymers that are able to form multilayers through different assembly interactions (Fig. 3) have been employed to assemble functional capsules. Particular attention has been focused on hydrogen bonding systems and efficient coupling reactions. For the former, hydrogen bonding donor (e.g. PMA, PGA) and acceptor (e.g. PVPON, PEG) polymers have been extensively studied because of their capability to form stable hydrogen bonding films. For covalent coupling of layers, polymers modified with, e.g., alkyne and azide groups as well as aldehyde/epoxy and amino groups, which are able to undergo copper-catalyzed azide-alkyne cycloadditions (CuAAC), imine formations and ring-opening reactions, respectively, have been studied. Current research has focused on using biomolecules, such as

carbohydrates and peptides, due to their biodegradability. A summary of selected polymer examples, and their associated interactions as described above, is provided in Table 2.

2.3. Single-step adsorption of polymers to assemble polymer capsules

Despite significant progress in preparing LbL capsules using templated assembly, due to precise control over the size, composition, wall thickness and functionalities, LbL assembly typically requires multiple polymer adsorption steps, which can be time and material consuming. An alternative route to prepare polymer capsules in a minimum of steps is to exploit a surface-mediated, single-step deposition of polymer onto sacrificial templates.

2.3.1. Mesoporous silica-templated capsules

Mesoporous particles with large surface areas are able to entrap materials for the preparation of nanostructured materials [148-150], since adsorption is a surface driven phenomenon. Recently, a general and facile approach has been reported for the fabrication of polymer capsules via the single-step adsorption of polymers in solid core/mesoporous shell (SC/MS) silica particle templates, followed by cross-linking of the polymer chains, and subsequent removal of the templates (Fig. 4) [151]. This approach proves its versatility in generating single-component capsules of synthetic polyelectrolytes (i.e., PAH), polypeptides (i.e., PLL and PGA), and polypeptide-drug conjugates (PGA-Dox). This approach offers several distinct advantages. First, it eliminates the need for multiple polymers and/or multiple polymer adsorption steps. Secondly, this method combines the versatility and benefits of the solid core particles (high stability and monodispersity) and the high loading of mesoporous shells to prepare thick-walled polymer capsules with controlled drug payload. In this technique, the size and thickness of the capsules can be controlled by the diameter of the solid core and shell thickness of the SC/MS template, respectively [152]. However, the wall thickness of the capsules is also influenced by the molecular weight of the polymers, due to

molecular weight-dependant infiltration of polymers into the mesopores [151], which demonstrates size matching between the mesopores and polymers is critical, as small pore sizes will exclude larger molecules [153-155].

2.3.2. Bromoisobutyramide-mediated assembly

Film fabrication based upon the non-covalent interaction between various biopolymers and bromoisobutyramide (BrIBAM) moieties has been recently reported by Mertz *et al.* [156]. In this method, biopolymers were adsorbed to the surface of BrIBAM-functionalized templates, which produced biopolymer capsules following template removal (Fig. 5a). The versatility of the technique was demonstrated by forming capsules using a range of biopolymers, including the enzymes alkaline phosphatase (AP), horseradish peroxidase (HRP) and lysozyme (LYS), the antibody immunoglobulin G (IgG), the hormone insulin (INS), polypeptide poly-L-lysine (PLL), single- and double-stranded DNA (DNA_{ss} and DNA_{ds}), and the polysaccharide dextran (DEX) (Fig. 5b-i).

It was proposed that the adsorption of protein to the template and stabilization of the film was due to non-covalent halogen bonding between BrIBAM groups and the biopolymers [156], analogous to previously observed interactions between proteins and DNA with various bromoamide compounds [157].

Due to the moderate mechanical stability of the protein capsules fabricated using a single adsorption step, two techniques were devised to improve the robustness of BrIBAM-mediated capsules. Cross-linking of the core-shell particles with an amine reactive cross-linker, followed by core removal produced stable capsules with improved colloidal stability in comparison to non-cross-linked BrIBAM capsules [158]. Importantly, the cross-linking process did not comprise the catalytic activity of two capsule systems fabricated from enzymes, and allowed the fabrication of sub-micron sized protein capsules, which could not

be achieved using a non-cross-linked BrIBAM adsorption process. Alternatively, mechanically stable capsules could be obtained by refunctionalization of a single layer core-shell particle with BrIBAM groups. Following this, additional film deposition was achieved by repeated protein adsorption and BrIBAM refunctionalization [128]. When the sequential adsorption process utilized the non-brominated IBAM, reduced layer buildup was observed compared to the BrIBAM case, supporting the conclusion that film assembly was due to a combined halogen and hydrogen bonding network between BrIBAM groups and protein chains.

2.3.3. Polyrotaxane capsules

Due to their novel material properties, the assembly of polymer nanostructures from supramolecular building blocks, in particular polymeric rotaxanes or polyrotaxanes (PRXs), is an emerging field of research. PRXs are macromolecules that consist of a non-covalent, mechanically interlocked structure of cyclic molecules threading a linear backbone axis, analogous to beads on a necklace [159-161]. Among PRX materials, cyclodextrin (CD) and PEG-derived PRXs possess several advantages compared to alternative systems, due to the low inherent cytotoxicity of CDs and PEG, as well as low cost and commercial availability.

The dynamic nature of this threading/dethreading requires that the free PEG chain ends are stoppered with bulky blocking groups to produce stable PRX molecules [160,161]. Through the use of stimuli-responsive blocking groups e.g., redox or pH, PRXs can be engineered with degradable properties [162-164]. The non-covalent nature of the material imparts several favorable properties. For example, variation of the molecular weight of the axial component and threading degree allows the persistence length and rigidity of the PRXs to be tuned [165,166]. Moreover the non-covalent interaction between the cyclic and axial components allows both rotational and longitudinal movement of the cyclic component on the molecule axis. Importantly, this mobility results in improved multivalent binding interactions, due to

the ability of the binding ligand to adopt a more favorable binding conformation, in comparison to covalent macromolecular building blocks [160,161]. These unique and interesting properties have led to increasing research interest into PRX building blocks.

The presence of multiple hydroxyl groups around the CD torus allows the PRXs to be readily functionalized. Using this approach, Dam and Caruso synthesized polyanion and polycation PRXs by functionalization of the α CD hydroxyl groups with carboxyl and amino moieties respectively. These PRX polyelectrolytes were subsequently used to fabricate PRX films [162] and capsules [164] using the LbL technique. Due to the presence of a disulfide containing blocking group at the chain ends, incubation of the PRX films with the intracellular reducing agent glutathione (GSH) resulted in unblocking of the chain end, causing dethreading of the α CDs and subsequent film degradation into α CD and PEG components.

An alternative approach to LbL assembly, is the use of a radial assembly technique pioneered by Wu and Li, in which the PRX orientation is ideally directed away from the substrate, analogous to substrate tethered polymer brushes [167]. In this example, gold nanoparticles (AuNPs) were PEG functionalized, then subsequently threaded with α CD. Following blocking of the free PEG chain end with 2,4,6-trinitrobenzene sulfonic acid (TNBS), and intermolecular covalent crosslinking between the α CD toroids, PRX nanocapsules were obtained following AuNP etching (Fig. 6). Alternatively, Dam and Caruso demonstrated the radial assembly of PRX capsules using pre-synthesized α CD/PEG PRXs [163]. Alkyne end-functional PRXs were grafted to the surface of azido-functional silica particles. Following cross-linking of the PRX shell with a disulfide cross-linker and silica etching, hollow PRX capsules were obtained. Use of a disulfide containing cross-linker and blocking group allowed degradation of the capsules to free PEG and α CD upon exposure to GSH.

In combination with their readily tunable properties, the non-covalent nature of PRX building blocks, which engenders them with unique physicochemical properties, is likely to see increasing application in the assembly of polymer nano- and microcapsules for next generation applications.

2.4. Bio-inspired polymer capsules

Inspired by the adhesive properties of mussel proteins, polydopamine (PDA) films can be coated on a wide range of planar substrates via covalent polymerization and non-covalent self-assembly in typically alkaline solution [168-170]. Based on this mussel-inspired catechol chemistry, PDA capsules have been prepared via single-step assembly of PDA films on silica particles, followed by template removal [171,172]. The obtained PDA capsules showed negligible toxicity. Similarly, polystyrene and CaCO_3 have been used as templates for PDA capsule preparation, which can be removed by organic solvents and EDTA, respectively [172,173]. The pH-dependant encapsulation and release of small molecules (e.g. methyl orange, alizarin red, and Rhodamine 6G) was observed [174]. Methyl orange and alizarin red were selectively encapsulated at low pH, whereas Rhodamine 6G was encapsulated at high pH, due to the electrostatic interaction between capsules and dyes. In addition, different enzymes (i.e., α -amylase, β -amylase and glucosidase) have been immobilized via physical encapsulation in the hollow capsule core, *in situ* entrapment within the capsule wall, and chemical attachment on the outer surface of capsules, respectively [175]. Monodisperse PDA capsules can also be obtained using dimethyldiethoxysilane (DMDES) oil-in-water emulsion droplets (Fig. 7a and b) [176]. These templates are removed with ethanol. The size and wall thickness of the PDA capsules can be easily tuned. Functional components, such as magnetic Fe_3O_4 nanoparticles, fluorescent quantum dots (CdSe/CdS), or an anticancer drug (thiocoraline), have been successfully encapsulated in PDA capsules. The cargo was preloaded into the emulsion droplets.

Due to the reactivity of PDA films based on Michael addition or Schiff base formation, DOX has been conjugated to thiolated poly(methacrylic acid) (PMA_{SH}) with a pH-cleavable hydrazone bond and subsequently immobilized onto PDA capsules [177]. This approach takes advantage of the facile PDA coating to form capsules and the acid-labile groups in the polymer-conjugate for sustained pH-induced drug release. The obtained DOX-loaded PDA capsules showed improved reduction in cell viability of HeLa cancer cells, compared with free DOX under the same assay conditions. As PDA capsules are resistant to (bio)degradation, enzymatically degradable PGA was modified with dopamine and used for the continuous assembly of biodegradable capsules [178]. This approach allows for protease triggered cargo release while maintaining the one-step assembly procedure. The degradation kinetics in the presence of protease was correlated with the dopamine content of the polymers. Due to the simple approach and low cytotoxicity of PDA, the PDA capsules are expected to find widespread application in the generation of new particulate delivery systems.

2.5. Surface and Interfacial Polymerization Methods

In recent years, the use of surface-initiated polymerization (SIP) or ‘grafting from’ processes have emerged as facile and flexible routes to prepare hollow polymer capsules of diverse functionalities and properties. Controlled radical polymerization techniques such as Atom Transfer Radical Polymerization (ATRP) [179-183] and Reversible Addition-Fragmentation Chain Transfer (RAFT) [184-188] polymerization are amongst the most widely utilized techniques to prepare polymer capsules via SIP. Similar to polymer chain growth in solution, the SIP of polymer brushes proceeds via the chain growth polymerization of monomer units from an initiating group. However, in the case of SIP, the initiating group is anchored to the chosen substrate, forming surface attached polymer brushes. In comparison to ‘grafting to’

processes, which utilize physi- or chemisorption of polymer chains to a substrate to produce thin polymer films, SIP affords higher brush densities and hence thicker films [189].

2.5.1. Grafting from hard templates

The use of hard templates has several advantages, namely the fine control over the shape, size and monodispersity of template particles. Using polymer brushes polymerized from particulate templates, hollow capsules can be achieved via two main routes. If the grafted brush is hydrophobic, core removal and dispersion in aqueous solutions will produce free-standing capsules. However, due to the non-cross-linked nature, destabilization of the capsules occurs when the capsules are dispersed in a good solvent for the grafted polymer [190]. A more versatile approach to fabricate capsules is *via* cross-linking of grafted polymer brushes, either through the copolymerization of a cross-linking monomer, or via post-polymerization cross-linking reactions. Depending on the end application and desired properties, various cross-linking strategies can be used, for example UV cross-linking of poly(styrene)-grafted silica particles [191], or UV cross-linkable comonomers [192]. An interesting non-covalent cross-linking technique was recently demonstrated by Vamvakaki and coworkers, which demonstrates the first example of visible light-induced capsule degradation [193]. Using SI-ATRP, copolymer brushes containing a photosensitive spiropyran (SP)-containing monomer were grafted from SiO₂ particles. Subsequent UV irradiation of the core-shell particles caused photoisomerization of the SP groups from the SP to merocyanine (MC) form, cross-linking the shell *via* MC π - π stacks. Degradation of the capsules was achieved by visible light irradiation, which caused isomerization of the MC groups back to the SP form, thus disrupting the π - π stacking and capsule cross-linking.

The use of small molecule cross-linkers is also attractive, because additional stimuli responsive functionalities can be incorporated into the capsules, and allows tuning of capsule properties by varying the cross-linker size and architecture. For example, using active esters

such as maleic anhydride, cross-linking can be achieved using bifunctional nucleophiles such as diamines [194]. Recently, Voit and coworkers synthesized dual-responsive polymer capsules via SI-RAFT polymerization of pH-responsive (*N,N*-diethylaminoethyl methacrylate) (DEAEMA) with a thiol reactive comonomer (PDSM) (Fig. 8) [195]. By varying the length of the dithiol cross-linker, the capsule permeability and swelling behavior in response to pH was tuned. In combination, these results demonstrate the tremendous versatility of controlled radical SIP polymerization techniques to produce hollow polymer capsules of diverse functionality and properties.

2.5.2. Continuous Assembly of Polymers

Recently, Qiao and Caruso developed a process, termed Continuous Assembly of Polymers (CAP), to assemble thin cross-linked (bio)polymer films and capsules [196]. Similar to ‘grafting from’ syntheses, CAP proceeds via a controlled chain-growth polymerization of polymerizable species from a surface bound initiating site. However, in contrast to ‘grafting from’ syntheses, which use monomers or small cross-linking species to produce surface confined brush films, CAP uses preformed polymer chains, termed macrocross-linkers, functionalized with multiple pendant polymerizable groups, to produce cross-linked films in one step (Fig. 9a). The use of pre-functionalized macrocross-linkers allows surface confined assembly of densely cross-linked (bio)polymer films not accessible via ‘grafting from’ approaches, for example polysaccharides and step-growth polymers [196]. Moreover, the CAP process is extremely versatile because it allows a range of controlled polymerization techniques such as ring opening metathesis polymerization (ROMP) [196-198], ATRP [199] and photoiniferter-mediated polymerization [200] to be utilized to drive CAP film assembly, to afford surface-confined films of diverse morphology and structure on both planar and particle templates.

Of particular interest in biomedical applications is the fabrication of capsules via ATRP-mediated CAP (CAP_{ATRP}) [199]. Through judicious selection of reaction conditions, ATRP may be conducted under aqueous conditions in the presence of biomolecules such as proteins and enzymes [201,202], with minimal effect on enzymatic functionality [203,204]. This is likely to facilitate the application of CAP_{ATRP} for the facile assembly of advanced drug and enzyme delivery vectors.

2.5.3. Soft template polymerization methods

In addition to hard templates, soft templates have also been used for the preparation of polymer capsules via various polymerization methods. Recent studies have demonstrated that seeded radical copolymerization can be used to prepare polymer capsules using thermosensitive spheres (PNIPAM) as templates, which can be removed in water via dialysis below the lower critical solution temperature [205-208]. Copolymerization in liposomal bilayers has resulted in polymer nanocapsules with uniform nanopores, which have been used for the selective encapsulation and release of cargos [209-211]. Further, the use of emulsion templates for surface confined polymerizations has emerged as a versatile method to fabricate polymer nano- and microcapsules. As the literature of conventional miniemulsion techniques [212] has already been reviewed extensively [213], we concentrate here exclusively on techniques that confine polymerization or cross-linking to an emulsion interface.

Interfacial cross-linking is extremely versatile, as a range of cross-linking chemistries, and polymer functionalities can be incorporated. An early example is the work of Breitenkamp and Emrick [214]. In this case, an oil-in-water emulsion was stabilized using an amphiphilic graft copolymer, containing grafted hydrophilic PEG chains and a double bond-containing backbone. Addition of a biscyclooctene cross-linker and ruthenium catalyst caused cross-linking by ring-opening cross metathesis between the cyclooctene and backbone double bond groups. Bernard and coworkers formed glyconanocapsules by the interfacial step-growth

polymerization of a bisazido sugar and hydrophobic alkyne [215]. Another interesting technique is the work of Lu and coworkers [216], which demonstrates the use of metal mediated cross-linking to synthesize functional capsules [216-219]. In one example, stabilization of an oil-in-water emulsion was achieved using a polymeric metallosurfactant, which served as a Prussian blue precursor. Addition of pyrrole, followed by iron(III), caused metal coordination polymerization of the surfactant periphery (termed miniemulsion periphery polymerization, MEPP) in addition to oxidative polymerization of pyrrole contained in the oil-core [217].

An alternative approach to surface confined cross-linking reactions is SIP from emulsion droplets stabilized by reactive polymer surfactants. This process is simply a ‘grafting from’ synthesis, which is conducted from the functionalized interface of an emulsion droplet instead of a functionalized hard template. An exemplary example is the work of Matyjaszewski and coworkers, who utilized surface-initiated miniemulsion ATRP to synthesize polymer nanocapsules [220,221]. Stabilization of the dispersed monomer phase was achieved using amphiphilic diblock copolymers, containing ATRP active end groups in the hydrophobic block. Incorporation of stimuli-responsive cross-linking comonomers (redox sensitive disulfide or acid sensitive acetal) allowed degradable polymer nanocapsules to be formed [221]. A novel extension of this work, termed inverse miniemulsion periphery RAFT polymerization (IMEPP) was recently reported by Utama and coworkers (Fig. 10) [222,223].

While the previously described miniemulsion techniques utilize a hydrophobic phase as template to achieve inward shell growth, the IMEPP technique uses an aqueous droplet as the template particle to achieve outward chain growth from the droplet surface. Consequently, the IMEPP technique allows proteins to be encapsulated inside the polymer capsule with no adverse effects on protein function, in contrast to methods which use a potentially denaturing organic phase as the template [222]. Due to their widespread application, modifications to

conventional emulsion polymerization techniques are likely to find increasing application in the fabrication of hollow polymer capsules. However, a significant hurdle in comparison to hard-templated techniques, is the polydispersity of the templating emulsion droplets and hence size distribution of the capsules.

2.6. Ultrasonic assembly of polymer capsules

Microcapsules/microbubbles can be obtained *via* a sonochemical route [224]. Ashokkumar and coworkers reported the synthesis of stable air-filled LYS microcapsules in aqueous solution via emulsification followed by protein cross-linking under high-intensity ultrasound (Fig. 11a) [225]. A key factor to prepare stable air-filled LYS microcapsules is the efficient cross-linking between protein clusters at the air/water interface. To achieve this, LYS was denatured by DL-dithiothreitol to expose free thiol groups for protein cross-linking. In addition, the hydrophobic nature of the proteins is important to provide foaming properties, which is one of the requirements to produce air-filled microcapsules. After incubation with phosphate buffer or salt solution, the air inside of the microcapsules can be removed, resulting in hollow capsules. In a subsequent study, the efficiency of formation, size distribution and morphology of these microcapsules were controlled by manipulating the sonication time and power [226]. An increase in the sonication time and power led to the formation of larger microcapsules with a broader size distribution. The microcapsule wall thickness was found to decrease with an increase in the sonication power and time.

In addition to proteins, this ultrasonic technique can also be applied to thiol-containing synthetic macromolecules (i.e., PMA_{SH}) for the preparation of polymer capsules [227]. The wall thickness was controlled by the degree of cross-linking, which in turn was determined by the thiol content in the polymers. However, this technique usually results in micrometer-sized capsules with broad size distributions. To synthesize stable and relatively monodisperse

nano- and microcapsules, a flow-through (FT) sonication technique was developed [228]. LYS capsules were prepared by pumping a partially denatured LYS solution through a FT horn at a defined flow rate and acoustic power (Fig. 11b). The size of the capsules was controlled by the active cavitation zone. The obtained capsules showed echogenic properties and a high loading capacity of oligonucleotides. In addition, large quantities of uniform capsules can be generated at a relatively low cost using the FT method.

3. Applications

3.1. Biomimetic microreactors

Polymer capsules in the nano- to micrometer size regime are important for a range of different applications, including catalysis, sensing, and the encapsulation and release of various substances [229]. More specifically, significant research interest is being devoted toward the use of polymer capsules as scaffolds and compartments in the fabrication of nano- and microreactor systems [2,3,230-232]. This research focus is inspired by the observation that biological cells use hierarchical, subcompartmentalized architectures to achieve precise control over a multitude of cascade reactions. These systems may be utilized to perform biomimetic spatially confined synthesis, or as a step toward artificial organelles and cells designed to reproduce lost metabolic function. Ideally, compartmentalization of enzymatic reactions has several major outcomes: protection of fragile enzymes from the exterior environment; spatial and temporal regulation of enzymatic reactions by controlling the local availability of competing substrates; and finally, increased local substrate and enzyme concentrations leading to improved reaction rates.

By variation of the polymer capsule structure, such as chemical functionality or membrane density, a diverse range of nano- and microreactor systems can be produced. For example, Vriezema *et. al.* fabricated a three enzyme cascade nanoreactor system using the coil-rod

diblock copolymer poly(styrene)-*b*-poly(L-isocyanoalanine(2-thiophen-3-yl-ethyl)amine) (PS-PIAT) [233], and demonstrated that enzyme substrates and products could freely diffuse through the semipermeable membrane walls [234]. In contrast, Palivan and coworkers showed that incorporation of channel proteins into the membrane of poly(2-methyloxazoline)-*b*-poly(dimethylsiloxane)-*b*-poly(2-methyloxazoline) (PMOx-PDMS-PMOx) polymersomes was necessary to allow diffusion of enzyme substrates across the hydrophobic membrane [235]. Kroeger and coworkers also presented a polymersome model system to demonstrate particle incorporation by artificial membranes [236]. These examples highlight the critical importance of material structure on functionality and performance.

Alternatively, by incorporating a range of polymer capsule architectures into one reactor system, the physicochemical properties of one system, for example low stability, may be overcome by the other polymer capsule component. Caruso and coworkers have developed capsosomes systems, which consist of LbL polymer multi-layer capsules incorporating liposome subcompartments [140-147,230,237]. In this case, the multilayer shell provides mechanical stability and permeability while the liposomes allow encapsulation and protection of enzymes and small cargoes. This diversity of material structure and polymer capsule systems has led to them becoming widely used in nano- and microreactor assembly.

3.2. Drug and vaccine delivery

Due to their readily tuned size, chemistry and functionality, another major potential application of polymer capsules is in controlled drug and gene delivery [1,64,238,239]. Effective drug delivery vectors have several key requirements [240-244]: low toxicity and optional degradability; high loading capacity; triggered release mechanisms, such as pH-, enzyme-, or redox-triggered; low immunogenicity; and targeting groups to direct the vector to designated sites. Drug and therapeutic molecule loading can be achieved by several routes, including: co-assembly of polymer-drug conjugates [119,151,245]; drug post-loading by

temporarily altering capsule permeability [246-251]; pre-loading of therapeutics to functional templates [176,252-257]; or incorporation into specific domains of the capsule [142,258,259]. Controlled drug release can be achieved using a range of external and biological stimuli [59,238,260,261], including pH [110,262], redox potential [263,264], and enzymatic reactivity [113,151,265]. In addition to control over biocompatibility, drug encapsulation, and triggered cargo release, stealth and targeting are of paramount importance for advanced delivery systems. PEGylation of polymer capsules is typically used to effectively prevent non-specific adsorption and cellular uptake [266-268]. Furthermore, targeting of specific cell types using specific targeting ligands, such as antibodies [269-272], may improve the effectiveness of drug accumulation and aid minimizing harmful off-target side effects.

Polymer capsules have also been used as carriers for antigens and adjuvants to promote adaptive responses in immune cells [273,274]. Recently, Caruso, Kent and coworkers reported the use of PMA_{SH} capsules for the delivery of antigens (i.e., ovalbumin and KP9 oligopeptides) to professional antigen presenting cells, causing subsequent activation of T cells [275-277]. De Geest and coworkers utilized dextran sulfate/poly(L-arginine) capsules as antigen delivery vehicles to investigate the intracellular uptake, processing, and cross-presentation of encapsulated antigens *in vitro* and *in vivo* [278,279]. In a subsequent study, it was found that ovalbumin-loaded capsules functionalized with oligonucleotides (i.e., CpG) were superior in priming antibody responses and IFN- γ -secreting Th1 and CTL responses [280]. For all the aforementioned vaccine delivery studies, polymer capsule fabrication was achieved using LbL assembly.

4. Future perspectives

Until now, most approaches to modulate the biological response and activity of polymer capsules have been chemistry-based; however, successful utilization of these systems *in vivo*

is likely to require a multifaceted approach. Variation of capsule size, geometry and mechanical properties to modulate their biological interactions is an emerging area of study. A significant impetus to synthesize non-spherical capsules has been the recent findings that show particle geometry can have a dramatic influence upon biological interactions [281]. Recently, Shimoni *et al.* produced PMA_{SH} hydrogel capsules on different aspect ratio silica rod templates using LbL assembly, and subsequently examined their shape-dependant cellular uptake [282]. Instead of LbL adsorption of polymers, it is also possible to polymerize around templates and produce different-shaped polymeric capsules, such as nanocubes or nanoplates [283].

Because precise control of both the size and shape of capsules using non-templated and soft templated methods often is challenging, fabrication of geometrically- and size-diverse capsules is dependent on the availability of well-defined template particles. However, conditions for removal of many of the monodisperse templates of controlled morphology that have been used to date need to be tuned to the polymer materials and intended applications to avoid any influence on polymer/material chemistry, encapsulated cargo functionality, and/or tethered receptors. The continued development of alternative templates of controlled size and morphology, which require only mild etching conditions, is important to broaden the application of polymer capsules in biomedical and other diverse applications. The mechanical behavior of soft materials is known to dramatically influence their biological interaction; therefore, elucidation of the chemical and structural parameters which govern material mechanical properties is also of significant importance [281]. Although fabrication of the next-generation of functional polymer capsules will be facilitated by the development of alternative (bio)polymer systems with multifunctionality, tunable degradability and cargo loading properties, their introduction must be accompanied by structure-property relationships which define the link between system chemistry and mechanics. Additionally,

new chemistries which are introduced should be robust and facile, to facilitate utilization of scalable fabrication techniques which will ultimately be essential for the widespread application of polymer capsules. One such promising example is the work of Ejima *et. al.*, who utilized the complexation between metal ions and natural polyphenols to produce thin films and capsules [284]. In this case, film deposition was achieved on a range of planar and particulate templates of varying chemistries. Furthermore, the metal-polyphenol films are formed extremely rapidly and are degradable under acidic conditions. Alternatively, in cases where submicron-sized spherical capsules are desired, the use of self-assembly or emulsion-templated polymerization techniques may also prove highly versatile.

Although significant hurdles to widespread biological applications remain, our understanding of the importance of capsule properties, such as composition, size, shape and mechanics is rapidly increasing. These advances, coupled with the development of robust and facile techniques to fabricate polymer capsules, are creating new opportunities for the application of polymer capsules in biomimetic catalysis, drug delivery and other diverse fields.

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Tables

Table 1. Templates used for the fabrication of polymer capsules. Adapted with permission from Ref. [86]. Copyright 2004 WILEY-VCH Verlag GmbH & Co. KGaA.

Template	Size (μm)	Shape	Monodispersity	Core Removal	Ref
Melamine formaldehyde	0.3 - 12	Spherical	Very high	HCl (0.1 M)	[56]
Polystyrene	0.1 - 10	Spherical	Very high	THF, DMF	[87]
SiO ₂ (solid/porous)	0.03 - 100	Spherical, different aspect ratios	High – very high	HF/NH ₄ F	[88,89]
CaCO ₃ /MnCO ₃	2 - 10	Spherical	Medium	EDTA	[90-92]
Red blood cell	4 - 8	Discocytes	High	NaClO (pH ≈ 12)	[93,94]
Emulsion	0.3 - 100	Spherical	Low	Organic solvent	[95,96]
Bubble	1 - 20	Spherical	Low	N/A	[97]

Table 2. Selected polymers and their respective modifications applied for the fabrication of LbL capsules (a: electrostatic, b: hydrogen-bonding, c: covalent, d: DNA hybridization, e: stereocomplexation, f: hydrophobic interactions, g: host-guest interactions).

	Polymer	Modification (chemistry)	Interaction	Reference
	synthetic polymers			
01	poly(styrene sulfonate) sodium salt (PSS)		a	[56]
02	poly(allylamine) (PAH)		a	[56]
		(glutaraldehyde)	c	[98]
		β -cyclodextrin/ferrocene	g	[83]
03	poly(diallyldimethylammonium) chloride (PDADMAC)		a	[99]
04	poly(ethyleneimine) (PEI)		a	[100]
05	Nafion/Fe ³⁺		a	[101]
06	poly(4-vinylpyridine) (P4VP)		a	[100,102]
07	poly(meth)acrylic acid (PMA/PAA)		a	[103]
		cysteamine (disulfide)	b	[104,105]
		PDS ^a (disulfide)	b	[106,107]
		alkyne/azide (CuAAC)	b, c	[108]
		alkene (thiol-ene)	b	[109]
		azobenzene	g	[84]
08	poly(2-diisopropylaminoethyl methacrylate) (PDPA)	alkyne (CuAAC)	b	[110]
09	poly(<i>N</i> -vinylpyrrolidone) (PVPON)	alkyne (CuAAC)	b	[111,112]
10	poly(hydroxy-propylmethacrylamide) (PHPMA)	dimethylaminoethyl (hydrolytically cleavable linker)	a	[113]
		oligonucleotide	d	[79]
11	poly(<i>N</i> -isopropylacrylamide) (PNIPAM)	alkyne, azide (CuAAC)	c	[114]
12	poly(diethylene glycol methacrylate- <i>r</i> -oligoethylene glycol methacrylate) (P(DEGMA- <i>r</i> -OEGMA))	alkyne (CuAAC)	b	[115]
13	poly(methyl methacrylate) (PMMA)	isotactic/syndiotactic	e	[81]
14	poly(glycidyl methacrylate) (PGMA)	(ring-opening)	c	[116]
15	NDR/MPR ^b	aryl diazonium (azo coupling)	c	[117]
16	poly(ferrocenylsilane) (PFS)	sulfonate/ethyl dimethyl ammonium	a	[118]

polypeptides, proteins, and DNA				
17	poly-L-glutamic acid (PGA)	alkyne, azide (CuAAC)	b	[119,120]
		alkyne, azide (CuAAC)	c	[121]
18	poly-L-aspartic acid		a	[122]
		adamantane	g	[85]
19	poly-L-lysine (PLL)	(imine formation)	a	[123]
		(carbodiimide chemistry)	c	[124]
		(CuAAC)	c	[121]
20	poly-L-arginine		a	[113]
21	proteins	albumin, protamine	a	[125-127]
		bromoisobutyramide (BrIBAM)	b	[128]
		(glutaraldehyde)	c	[129]
22	silk proteins	PGA/PLL	a	[130,131]
		(physical cross-links, β-sheets)	f	[82]
23	oligonucleotides		d	[74-79]
Polysaccharides				
24	dextran	sulfate	a	[132-134]
		alkyne, azide	c	[135]
		(CuAAC, carbonate linker)		
		carboxymethyl/α-cyclodextrin	g	[84]
		dialdehyde/β-cyclodextrin	g	[85]
		(hydrolytically cleavable linker)		
25	chitosan		a	[136]
		quaternized	a	[137]
		sulfate	a	[138]
26	hyaluronic acid	alkylated	a	[137]
27	alginate		a	[125]
		dialdehyde	c	[139]
		(imine formation)		
Other				
28	liposomes	(capsosomes)	a, f	[140-147]

^a Pyridine dithioethylamine

^b *N*-methyl-2-nitro-diphenylamine-4-diazo resin/*m*-methylphenol-formaldehyde resin

Figure Captions

Fig. 1. (a) Schematic diagram of the structure of a polymersome in water, showing the hydrophobic membrane (red) and hydrophilic corona (blue). Polymersome membrane conformation formed by self-assembly of (b) AB diblock and (c) ABA triblock copolymers, and (d) ABC triblock terpolymers. Adapted with permission from Ref. [8]. Copyright 2009 The Royal Society of Chemistry.

Fig. 2. Schematic representation of LbL assembly of polymer capsules with different approaches: (a) Centrifugation; (b) Filtration; and (c) Electrophoretic polymer assembly. Adapted with permission from Ref. [66]. Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA.

Fig. 3. Different polymer interactions for the assembly of multilayer capsules (centre). Interactions clockwise from left: (a) electrostatic; (b) hydrogen bonding; (c) covalent bonding; (d) DNA hybridization; (e) stereocomplexation; (f) hydrophobic; and (g) host–guest interactions.

Fig. 4. (a) Schematic representation of the preparation of single-component polymer capsules by using SC/MS particles as templates. (b) SEM image of PAH nanocapsules. (c) TEM image of PLL nanocapsules. (d) TEM and (e) fluorescence microscopy images of PGA-Dox nanocapsules. Adapted with permission from Ref. [151]. Copyright 2008 American Chemical Society.

Fig. 5. (a) Single-step adsorption of a biopolymer (e.g., polypeptide, protein, nucleic acid or polysaccharide) from aqueous solution onto BrIBAM-modified silica templates followed by template removal yields the formation of free-standing biopolymer capsules. Fluorescence microscopy images of (b) AP, (c) HRP, (d) LYS, (e) IgG, (f) INS, (g) PLL, (h) DNA_{ds}, and (i) DEX capsules. RITC- or FITC-labeling results in red and green fluorescence, respectively.

Scale bars are 5 μm . Adapted with permission from Ref. [156]. Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA.

Fig. 6. Assembly of PRX nanocapsules by radial assembly from PEG functionalized AuNPs. Crosslinking was achieved by activation of the αCD hydroxyl groups followed by reaction with PEI. Adapted with permission from Ref. [167]. Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA.

Fig. 7. (a) Schematic representation of the encapsulation of hydrophobic species in PDA capsules using DMDES emulsion templates. (b) An enlarged schematic of a loaded PDA capsule. Adapted with permission from Ref. [176]. Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA.

Fig. 8. Schematic for the assembly of dual-responsive (pH and redox) polymer capsules via SI-RAFT polymerization from aminated silica templates. Reproduced with permission from Ref. [195]. Copyright 2012 American Chemical Society.

Fig. 9. (a) Schematic of film formation via the CAP_{ROMP} process on a planar substrate. Surface initiator functionalization is achieved using catalyst **1** followed by the CAP_{ROMP} reaction with either **P1**, **P2** or **P3** macrocross-linkers. **X'** represents the interlayer spacing. (b) Schematic of capsule formation via CAP_{ROMP} . Increased layer build-up was achieved by reinitiation of the polymer film with catalyst **1** followed by a CAP_{ROMP} assembly step. This process was repeated until the desired film thickness was achieved followed by core removal. Adapted with permission from Ref. [196]. Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA.

Fig. 10. Schematic of hollow protein-loaded polymer capsule assembly by IMEPP using poly(HPMA-*b*-MMA) as the surface-active RAFT agent. Reproduced with permission from Ref. [222]. Copyright 2012 The Royal Society of Chemistry.

Fig. 11. (a) Proposed mechanism for the formation of disulfide cross-linked air-filled polymer microcapsules. (1) Emulsification of the amphiphilic polymer nanoaggregates induced by high energy ultrasound. (2) Diffusion of the polymer nanoaggregates to the interface. (3) Disulfide bridge formation between polymer nanoaggregates adsorbed at the air/water interfaces. Adapted with permission from Ref. [225]. Copyright 2008 American Chemical Society. (b) Schematic representation of the formation of microcapsules using the flow through sonication cell. Adapted with permission from Ref. [228]. Copyright 2012 American Chemical Society.

Figures

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