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Multicompartment Polymeric Nanocarriers for Biomedical Applications.

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Multicompartment polymeric nanocarriers which mimic the compartmentalized architecture of living cells have received considerable research attention in the biomedical field. The advancement of synthetic polymeric chemistry has allowed multicompartment polymeric nanocarriers to be tailored for biomedical applications such as drug delivery, encapsulated catalysis and artificial cellular mimics. In this review, polymer based multicompartment nanocarriers (multicompartment micelles, multicompartment polymersomes and capsosomes) have been discussed. We have focused on studies over the last ten years which have investigated multicompartment materials for biomedical applications. The synthetic procedures and structural properties that impact the specific application are also discussed.

1. Introduction

Compartmentalization is a key structural feature of eukaryotic cells. Membrane bound organelles create distinct intracellular subcompartments within the cells to regulate a diverse range of cellular functions. Different biomolecules are spatially confined within these subcompartments to perform multiple biochemical reactions simultaneously without interfering with other cellular functions.[1] Inspired by this natural phenomena, recent studies have focused on designing multicompartment particulate carriers for biomedical applications because they can address multiple barriers to effective therapeutic delivery and thus improve efficacy.[2]

Multicompartment nanocarriers are complex multifunctional colloidal nanostructures containing two or more heterogenous compartments with distinct chemical and physical properties.[3] Multicompartment nanocarriers have been recognized as an emerging area of interest in biomedical applications. For example, multicompartment nanocarriers can be loaded with multiple therapeutics to provide combinational therapy by minimizing the side effects associated with high dosage of a single drug.[4] Multicompartment nanocarriers have also been investigated for designing artificial cell mimics, which could be used as a substitute for missing or lost cellular functions.[5,6]

Currently, there is a considerable progress in designing polymeric nanocarriers for therapeutic applications because their structural and functional properties can be easily tuned to achieve promising therapeutic efficacy.[7-11] Moreover, following the robustness and versatility of polymeric building blocks, compartmentalized polymeric nanocarriers including compartmentalized micelles, polymersomes and polymeric capsules with two or more compartments have gained substantial scientific interest in designing multifunctional drug delivery vehicles and cell mimics for encapsulated catalysis.[12,13]

To date, a range of synthetic approaches has been developed for the fabrication of multicompartment polymeric nanocarriers. Among them, self-assembly of block copolymers, layer-by-layer (LbL) assembly and emulsion-based polymerization are the most widely

employed fabrication techniques.[14-17] However, while synthesis of multicompartment materials with interesting properties is advanced, their design and application for biomedical applications is less developed. Therefore, further studies are required to fully develop the potential applications of these multicompartment polymeric nanocarriers. In this review, we highlight prominent examples of multicompartment polymeric nanocarriers utilized in biomedical applications such as drug delivery, encapsulated catalysis and synthesis of artificial cell mimics. Our overview focuses on three specific classes of multicompartment systems developed in the past ten years, including multicompartment micelles, multicompartment polymersomes and capsosomes by highlighting their assembly approaches, structural properties and potential for biomedical applications.

2. Multicompartment micelles

Polymeric micelles are formed via self-assembly of amphiphilic block copolymers to form stable colloidal nanoparticles with a hydrophobic core and hydrophilic shell architecture. The presence of the hydrophobic core allows space for encapsulation of hydrophobic molecules such as therapeutics and imaging agents through strong physical or chemical interactions.[18] Recently, polymeric micelles have generated significant interest as drug carriers due to the higher drug loading capacity of the hydrophobic core. They also have the ability to improve the pharmacokinetic properties of the drug by targeting drug delivery to the site of action with minimum cytotoxicity.[19]

In mid-1990s, Ringsdorf proposed multicompartment micelles consisting of a micro-phase separated multidomain hydrophobic core with each partition having distinct properties. Thereafter, various morphologies of multicompartment micelles have been reported, for example, core-shell-corona or onion-like micelles, raspberry-like, flower-like, hamburger-like and cylindrical micelles.[20-24]

Typically, the multicompartment morphologies of multicompartment micelles have been constructed either by solution self-assembly or polymerization induced self-assembly (PISA).[25] Block

copolymers such as linear ABC triblock terpolymers,[26,27] ABC miktoarm star terpolymers,[28-30] and AB/BC block copolymer blends [31,32] are commonly used in synthesis of multicompartment micelles via solution self-assembly process, in which A refers to a solvophilic block and B and C are two incompatible solvophobic blocks. Upon successful exchange of block selective solvents, these polymers undergo spontaneous self-assembly in order to produce multicompartment micelles with segregated solvophobic core containing B and C microdomains.[25] In addition, multicompartment micelles have been prepared by polymerization induced self-assembly (PISA) which involves chain extension of solvophilic macroinitiator with solvophobic block polymer using living radical polymerization technique, mostly via reversible addition-fragmentation chain transfer (RAFT) polymerization.[23,33,34]

Multicompartment micelles have shown potential applications in therapeutic delivery. The segregated core of multicompartment micelles have potential to facilitate the simultaneous storage and release of multiple incompatible payloads, such as hydrophobic chemotherapeutics, nucleic acids and photosensitizers, by minimizing the unwanted interactions between them.[35-37]

In 2014, Kataoka et al. developed three compartment core-shell-corona micelles to enhance photodynamic therapy (PDT) against A549 lung cancer cells in vitro and in vivo.[38] Micelles were prepared by self-assembly of ABC triblock terpolymer poly(butadiene)-*block*-poly(1-methyl-2-vinyl pyridinium methyl sulfate)-*block*-poly(methacrylic acid) (PB-*b*-P2VPq-*b*-PMA). During self-assembly, the core compartment of the micelles was loaded with hydrophobic photosensitizer porphyrazine derivative, which generates reactive oxygen species (ROS) upon irradiation for photodynamic therapy. Subsequently, interpolyelectrolyte complex formation of negatively charged PMA with positively charged poly(ethylene glycol)-*block*-poly(L-lysine) (PEG-*b*-PLL) diblock copolymer transformed negatively charged PMA corona into neutral corona of PEG to change the micellar structure into a compartmentalized "bottlebrush-on-sphere" morphology. These PEGylated multicompartment micelles were shown to inhibit A549 tumor cell growth effectively up to 20 days

upon irradiation. Notably, the authors reported the degree of PEGylation could dramatically affect the PDT efficacy due to the differences in cellular uptake and blood circulation properties of multicompartment PEGylated bottle brush micelles compared to the unPEGylated control particles.

In later work, Kataoka et al. reported multicompartment polyplex micelles for light-induced gene delivery.[39] The polyplex micelles were synthesized by sequential self-assembly of ABC triblock terpolymer poly(ethylene glycol)-*block*-poly{*N*-[*N*-(2-aminoethyl)-2-aminoethyl]aspartamide}-*block*-poly(L-lysine) (PEG-*b*-PAsp(DET)-*b*-PLL), plasmid DNA (pDNA) and a dendritic photosensitizer (PS) and, were composed of three-layered compartments, inner DNA-packed PLL core, intermediate PS-loaded PAsp(DET) layer and outer PEG corona. The light-induced gene delivery has a major challenge of delivering both pDNA and photosensitizer in a same carrier, because reactive oxygen species (ROS) generated by photoirradiated photosensitizer can inactivate pDNA molecules. Thus, in this work, photosensitizer and pDNA were accommodated within separate compartments of polyplex micelles to reduce the oxidative damage for pDNA. In vitro and in vivo studies revealed ROS generated by photoirradiated photosensitizer localized within endosomal/lysosomal membrane disrupted the membrane to induce change in cellular distribution of the pDNA. While, there is evidence that light is enhancing the ability of pDNA to transport into the cytosol, it is clearly not an efficient process as the Pearson's coefficient only changes from 0.45 to 0.36.

In 2019, Stenzel and co-workers reported well-defined multicompartment patchy type micelles for simultaneous drug delivery.[40] They designed multicompartment micelles by the use of linear triblock ABC terpolymers, poly(glycosyloxyethyl methacrylate)-*block*-poly(benzyl acrylate)-*block*-poly(4-vinylpyridine) (PGlcEMA-*b*-PBzA-*b*-P4VP) through a stepwise solvent exchange process. A two-step hierarchical self-assembly procedure was followed to make patchy particles with two chemically different compartments. Firstly, polymer was dissolved in dimethylformamide (DMF) and then methanol (MeOH) was added to make spherical precursor micelles with hydrophobic PBzA core and PGlcEMA/P4VP corona. Secondly, precursor micelles were transformed into patchy particles by

removing DMF with dialysis against MeOH, where the pH sensitive P4VP block undergoes phase segregation to make patchy particles with P4VP core, PBzA patches and bioactive hydrophilic sugar corona, PGIcEMA. Subsequently, solvent exchange was done from MeOH to 10 mM phosphate buffer (PB, pH 7.4) to confirm that patchy particles are stable in physiological pH of the bloodstream.[41] A slight increase of particle size (approximately 30 nm) was observed by dynamic light scattering (DLS) analysis following the solvent exchange, without any morphological changes as revealed by transmission electron microscopy (TEM). After successful synthesis of patchy particles, they were able to demonstrate site-selective loading of patchy particles with two chemically distinct molecules, fluorescence active hydrophobic anticancer drug (Doxorubicin, DOX) within the non-polar PBzA patches and hydrophilic fluorescence active molecule (Cyanine 5, Cy5) within polar P4VP core compartments. Both cargoes were selectively co-loaded within separate compartments of patchy particles by following a similar two-step self-assembly procedure, except in this case methanolic DOX solution was used in first step to yield precursor micelles followed by methanolic Cy5 solution before dialysis against MeOH to yield patchy particles **(Figure 1)**. The authors reported toxicity with an IC_{50} value (inhibitory concentration which induce 50% cell death) of 15.3 μgmL^{-1} for DOX-loaded patchy particles which was equivalent to 0.69 μM free DOX and, no cytotoxicity was observed for blank patchy particles. While this sounds promising, it would have been useful to compare this value to a free DOX control. In addition, fluorescence resonance energy transfer (FRET) process was used to investigate the real-time drug release of patchy particles because both spectrally appropriate fluorophores (DOX and Cy5) are initially confined in separate compartments within a Förster distance of 1-10 nm. The initial FRET signal generated by DOX and Cy5 co-loaded patchy particles was shown to decrease over incubation time as co-loaded DOX and Cy5 were simultaneously released to MCF-7 breast cancer cells.

In addition to these examples of multicompartment micelles with segregated domains, multicompartment nanocarriers have been designed with micellar subcompartments. In 2017, Liu et

al. reported multicompartment hydrogels containing core-shell-corona micelles for synergistic delivery of chemotherapeutic drugs, hydrophilic gemcitabine (GCT) and hydrophobic doxorubicin (DOX).[42] Firstly, core-shell-corona micelles were synthesized by solution self-assembly of triblock copolymer poly(*N*-isopropylacrylamide)-*block*-poly(4-acryloylmorpholine)-*block*-poly(2-((((2-nitrobenzyl)oxy)carbonyl)amino)ethyl methacrylate) (PNIPAM-*b*-PNAM-*b*-PNBOC). Micelles were composed with hydrophobic photosensitive PNBOC cores, hydrophilic PNAM inner shells, and thermo-responsive PNIPAM corona. The multicompartment hydrogels comprising the micelles were formed when the temperature was higher than the critical gelation temperature of triblock polymer. Both GCT and DOX were simultaneously encapsulated within extramicellar hydrophilic phase of hydrogel and hydrophobic PNBOC micellar core respectively. Controlled release profiles of GCT and DOX were investigated according to the temperature variations and UV irradiation. It was demonstrated, at physiological temperature of 37 ^{0}C and without UV irradiation, less than 20% of GCT and 10% of DOX were released after 70 hour incubation time. However, after 10 min of UV irradiation, release rates of both GCT and DOX were increased remarkably due to the UV irradiation-induced gel-to-sol transition. This type of material is limited for in vivo applications due to limited penetration of UV light, however adaption of this strategy is clearly possible.

In 2019, Thurecht and co-workers designed a multicompartment oral drug delivery system for colorectal cancer to trigger the drug release at the targeted site by overcoming the therapeutic barriers within the gastrointestinal track (GIT).[43] Hydrophobic anticancer drug, curcumin-loaded micelles were prepared by self-assembly of hyaluronic acid (HA) modified poly(poly(ethylene glycol) monomethyl ether methacrylate)-*block*-poly(methyl methacrylate-*co*-cyanine 5 methacrylamide) (HA-PPEGMA-*b*-P(MMA-*co*-Cy5MA) and were then further encapsulated inside alginate microcapsules to prevent the unwanted side effects from the GIT environment **(Figure 2)**. It was found that the alginate microcapsule protects the drug-loaded HA micelles at acidic stomach environment (pH ~2), with limited drug release. However, both TEM and DLS results confirmed

microcapsules degraded at pH 6.8 (intestinal environment) Drug-loaded micelles were stable enough to retain curcumin and high level of in vitro drug release was observed in tumor microenvironment (pH 5). Remarkably, negligible amounts of drugs were released during the transit of GIT because the alginate capsule played a major role in protecting the drug-loaded micelles. This kind of compartmentalized drug delivery vehicles could have significant potential for the treatment of gastrointestinal track diseases, which are difficult to treat by conventional therapies due to poor vascularization and limited blood flow.

3. Multicompartment polymersomes

Polymersomes are a class of vesicles constructed from self-assembly of amphiphilic block copolymers consisting of an aqueous core compartment enclosed by a hydrophobic polymeric membrane.[44] These hollow spherical nanoparticles can offer several advantages for biological applications owing to their enhanced properties over liposomes such as higher mechanical strength, low membrane permeability, superior colloidal stability and easier functionalization with targeting moieties and stimuli responsive functional groups.[45-47] Moreover, polymersomes have ability to encapsulate both hydrophobic and hydrophilic cargoes within their membrane and aqueous core respectively, which make them useful for biomedical applications including drug delivery and constructing therapeutic nanoreactors to mimic biological cascade reactions.[44,48]

Different techniques have been extensively used in preparation of multicompartment polymersomes for biological applications, for example, multi-step self-assembly,[49] double emulsion via microfluidics,[50,51] and emulsion-centrifugation methods.[52,53]

Multicompartment polymersomes have generated significant interest for constructing synthetic cellular mimics for multi-enzymatic cascade reactions. In particular, multicompartment polymersomes-in-polymersomes have been employed to conduct enzymatic cascade reactions by trapping the enzymes within a porous semi-permeable polymersome which allow diffusion of small

substrate molecules. Lecommandoux et al. prepared a polymersomes-in-polymersomes nanoreactor to mimic a natural multistep enzymatic pathway.[54] Two enzymes *Candida antarctica* lipase B (CalB) and alcohol dehydrogenase (ADH) were encapsulated in porous semi-permeable poly(styrene)-*block*-poly(3-(isocyano-L-alanyl-amino-ethyl)-thiophene) (PS-*b*-PIAT) polymersomes. Together with cytosolic enzyme phenylacetone monooxygenase (PAMO) and reaction reagents, they were further encapsulated inside lumen of poly(butadiene)-*block*-poly(ethylene oxide) (PB-*b*-PEO) polymersomes via a emulsion-centrifugation approach.[53] These enzymes encapsulated within separate compartments were able to perform a cascade reaction; transformation of profluorescent substrate, 7-((4-oxopentyl) oxy)-3*H*-phenoxazin-3-one to fluorescent resorufin. The profluorescent substrate first underwent oxidation by the PAMO enzyme with the use of nicotinamide adenine dinucleotide phosphate (NADPH) as the cofactor to yield an ester, which was then hydrolyzed by CalB to yield a primary alcohol followed by alcohol dehydrogenase (ADH) oxidation and beta-elimination to yield resorufin as the product **(Figure 3)**. The successful reaction demonstrated encapsulated enzymes could carry out cascade reaction similar to that of free enzymes as small reaction intermediates could diffuse across the semi-permeable polymersome membranes. Furthermore, CalB was then replaced by a protease enzyme alcalase, which degrade other enzymes present in the solution. Due to the compartmentalization of alcalase within PS-*b*-PIAT polymersomes, enzyme degradation was suppressed, thus allowing the successful substrate conversion.

In addition, protein transport channels have been employed in non-permeable polymersome membranes to enhance the selective transportation of molecules within multicompartment polymersomes systems. Nallani and co-workers formed a polymersomes-in-polymersomes architecture by encapsulating non-permeable poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA) polymersomes within semi-permeable poly(styrene)-*block*-poly(L-isocyanoalanine(2-thiophen-3-yl-ethyl) amide) polymersomes.[55] In addition, an outer membrane protein channel (OmPF) was incorporated in the non-permeable

(PMOXA-*b*-PDMS-*b*-PMOXA) polymersome membranes. Bienzymatic cascade reaction was performed by selective encapsulation of two enzymes, horseradish peroxidase (HRP) and glucose oxidase (GOx) within inner and outer compartments respectively. In this cascade reaction, GOx loaded within the outer compartment catalyzed the oxidation of β-D-glucose into D-gluconolactone and hydrogen peroxide (H_2O_2). Afterwards, the OmPF channel facilitated the diffusion of H_2O_2 into the inner compartment to act as a co-substrate for the HRP catalyzed conversion of Amplex red to the fluorescent end product resorufin. The authors demonstrated higher rate of Amplex red conversion for multicompartment polymersomes with the OmPF channel than without suggesting that OmPF facilitated the diffusion of H_2O_2 through the non-permeable polymersome membrane.

In living cells, biochemical reactions occur within stimuli-responsive natural compartments called organelles, by giving them the opportunity to control their metabolic processes in response to external chemical stimuli (e.g.; redox state, pH, chemical potential). Thus, when designing multicompartment carriers for synthetic cell mimics, it is important to use the external environments of the system in order to tune the internal metabolic reactions such as enzymatic reactions. In particular, Palivan et al. developed environmentally responsive multicompartment polymersomes to induce enzymatic reactions in response to external stimuli.[56] The multicompartment architecture was based on a giant polymersome loaded with subcompartments of reduction-responsive nanoassemblies. The material was self-assembled by the film rehydration method using a mixture of amphiphilic block copolymers poly(2-methyl-2-oxazoline)-*block*-poly(dimethylsiloxane))-*block*-poly(2-methyl-2-oxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA) and PDMS-*b*-heparin in the presence of enzymes and pre-formed nanoassemblies loaded with enzymatic substrates. Redox potential was used as the external stimuli signal to trigger the enzymatic conversion, and redox agent dithiothreitol (DTT) was added in order to induce a redox environment within the lumen of giant polymersome. It was demonstrated that DTT passively diffused across the membrane of the giant polymersome to induce the disintegration of reduction-sensitive

nanoassemblies and release the substrate molecules to react with the enzyme molecules and trigger the enzymatic reaction. This type of signal responsive multicompartment assemblies would have potential as cell-inspired responsive systems for applications in biosensing and catalysis.

4. Capsosomes

Multicompartment polymeric capsules containing liposomal subcompartments are known as capsosomes. They are dual component multicompartment assemblies primarily formed via layer-by-layer (LbL) assembly which involves sequential deposition of complementary materials onto a template surface. In this case it is the sequential deposition of polymers and liposomes onto a silica particle template. A polymer precursor layer is first adsorbed to the silica template, followed by deposition of repeated layers of liposomes and polymer separation layers until attaching desired number of liposomal layers. The polymer carrier capsule is then formed by sequential deposition of interacting polymer layers and removal of silica template yields colloidally stable capsosomes.[6,57]

These multicompartment systems are of interest as they combine physicochemical properties of both liposomes and polymer capsules while minimizing some limitations associated with each system. Liposomes are natural lipid carriers which have potential to encapsulate variety of small therapeutic cargoes including enzymes,[58] nucleic acids,[59,60] anticancer drugs,[61-63] proteins,[64,65] and imaging agents.[66,67] However, they can be prone to leakage. Therefore, the combination of structural integrity and semi-permeable nature of polymer capsules make capsosomes attractive for biological applications.[5,68]

Capsosomes have been widely applied in designing synthetic cell mimics to perform enzymatic cascade reactions. Recently, Stadler and co-workers constructed poly(dopamine) (PDA) based capsosomes by encapsulating three different enzymes in distinctive liposomal compartments to demonstrate their ability to perform parallel enzymatic reactions towards the detection of uric acid.[69] The assembly procedure followed for capsosome synthesis is shown in **figure 4**. The authors

assembled capsosomes by using the LbL technique, in which first a polymer precursor layer, based on a combination of poly(L-lysine) (PLL) and poly(methacrylic acid-*co*-cholesteryl methacrylate) (P(MA-*co*-CMA)) was adsorbed onto the silica particle template followed by the deposition of enzyme-loaded liposomes. Then, another polymer separation layer (P(MA-*co*-CMA)) was adsorbed on to the liposomes before deposition of another liposome layer. Finally, the assembly was capped with another P(MA-*co*-CMA) layer before adding a PDA polymeric shell via single step self-polymerization. The silica core was removed by dissolution using a buffered hydrofluoric acid (HF) (pH~5) to obtain intact, monodisperse capsosomes. Fluorescence spectroscopy revealed that liposomes are homogenously distributed within the PDA shell. The potential of capsosomes to carry out multiple enzymatic reactions was confirmed by using the uric acid detection reaction method. Different liposomal compartments were encapsulated with equal amounts of three different enzymes, uricase (UR), horseradish peroxide (HRP) and ascorbate oxidase (AO). The encapsulated uricase catalyzed the conversion of uric acid to allantoin, H_2O_2 and carbon dioxide followed by HRP converting Amplex ultra red reagent to the fluorescence product, resorufin with the use of H_2O_2. They compared enzymatic conversion of uric acid in the presence and absence of AO by comparing the resorufin fluorescence intensity, approximately three times higher fluorescence intensity (over 3 hour time period) was observed in the presence of AO. The results demonstrate that capsosomes are efficient in performing multiple enzymatic reactions in parallel thus making them attractive for the development of artificial organelles.

Many of the currently reported multicompartment architectures have only been investigated in simulated cellular conditions and thus intracellular activity of these materials is not well understood. In 2016, Hosta-Rigau and co-workers established the capacity of capsosomes to perform multiple enzymatic reactions concurrently inside cells.[70] Multicompartment capsosomes were fabricated with bovine serum albumin (BSA) stabilized gold nanoclusters (BSA-AuNCs) to ensure the capsosomes uptake by macrophage cells. The capsosomes were assembled via LbL assembly with

initial deposition of polymer layer PLL on to a silica particle followed by deposition of three layers of BSA-AuNCs/ PLL and then two layers of enzyme-loaded liposomes with alternating adsorption of poly(methacrylic acid) (PMA)/ PLL separation layers. Finally, silica core was removed using a buffered hydrofluoric acid (HF) (pH~5) after creating the polymeric shell by alternating deposition of five layers of poly(*N*-vinyl pyrrolidone) (PVP) and thiol-containing PMA (PMA_{SH}) **(Figure 5)**. Successful deposition of polymers and liposomes were confirmed by measuring the zeta potential values after each coating. Multiple microscopic techniques confirmed the unique structural properties of the assembled capsosomes including homogeneous distribution of BSA-AuNCs and liposomes within the carrier capsule to yield stable, intact and monodisperse capsosomes. Differential interference contrast images (DIC) proved shape of capsosomes remained unchanged after being internalized into cells. More importantly, the authors reported selective loading of two different enzyme-loaded liposomes as the inner and outer layer of the capsosome architecture; trypsin (TRP)-loaded and HRP-loaded liposomes (L_{TRP} and L_{HRP}) respectively. They confirmed intracellular enzymatic conversion of two reactions in parallel where TRP and HRP acted together on their respective substrates BA-Rho-110 and Amplex red respectively, to give their corresponding fluorescence products, MA-Rho-110 and resorufin.

Many biological reactions take place in a tandem manner in which intermediates of one reaction are essential to carry out the next reaction. Thus, in a later study, the capsosomes outlined above were entrapped with two other enzymes (GOx and HRP) which can act in a cascade manner to perform a two-step enzymatic reaction.[71] Liposomes loaded with GOx and HRP were selectively entrapped inside the capsosome as the inner and outer layer respectively to conduct the cascade reaction, conversion of glucose to resorufin. The GOx catalyzed the conversion of glucose into D-gluconolactone and H_2O_2 and then, HRP together with H_2O_2 catalyzed the conversion of Amplex red to resorufin within the intracellular macrophage environment.

Besides the application of capsosomes as cell mimics, Caruso et al. reported use of capsosomes for long-term delivery of protein therapeutics.[72] Many protein therapeutics undergo deterioration in the protease rich environment of the gastrointestinal tract, before reaching the targeted site. Liposomes have been widely considered in protein delivery, but unstable chemical and mechanical properties of liposomes limit long-term delivery of protein therapeutics.[73] In this work, proteins were encapsulated within liposomal compartments of capsosomes to improve their prolonged delivery. The capsosomes were assembled by LbL method. A PLL precursor layer was first deposited onto a silica particle template, followed by alternative deposition of five layers of protein-loaded liposomes and P(MA-*co*-CMA) polymer separation layers. Then, the polymeric shell was assembled by adsorption of alternating layers of poly(*N*-vinyl pyrrolidone) (PVP) and thiol functionalized PMA layers. Three different lipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) were used in fabrication of three types of liposomes: (i) zwitterionic DMPC/DPPC, (ii) zwitterionic DPPC, and (iii) negatively charged DMPC/DPPC/DOPS. Loading capacity and release profile of two types of proteins, lysozyme (a model protein) and brain-derived neurotrophic factor (BDNF) (physiologically relevant neurotrophin protein therapeutic), were investigated for capsosomes with different liposomal compositions. The highest protein-loading capacity was observed for capsosomes with anionic DMPC/DPPC/DOPS liposomes. This was due to the efficient adsorption of negatively charged liposomes to the cationic poly(L-lysine) membrane and electrostatic interactions between the encapsulated positively charged proteins at physiological conditions (pH 7.4) and negatively charged liposomal membrane.[74] In vitro studies proved these capsosomes can release proteins up to 80 days depending on the lipid composition of encapsulated liposomes.

5. Conclusion

Multicompartment polymeric carriers which resemble the architecture of living cells have potential to be applied in future therapeutic applications. In this review, we have provided an overview of the multicompartment polymeric nanocarriers reported in the past ten years, based on three specific classes; micelles, polymersomes and capsosomes. We have also limited the discussion to systems that have demonstrated potential for biomedical applications such as drug delivery, encapsulated catalysis and synthetic cellular mimics.

Multicompartment micelles with distinct compartments have enabled them to encapsulate different types of therapeutics within the carrier to provide controlled simultaneous drug release, thus opening up the possibility of combination therapy. More importantly, compartmentalization has provided protection for the therapeutics from harsh physiological conditions by minimizing the unwanted side reactions. In contrast, multicompartment polymersomes and capsosomes have shown potential to perform multiple enzymatic cascade reactions simultaneously, thus making them attractive as artificial cell organelles.

However, most of the recent studies are based on proof-of-concept illustration of particular applications, predominately in vitro. There is no clear evidence showing these nanocarriers are efficient in treatment of variety of chronic diseases, cancers and biofilm related infections. Therefore, future studies should be performed in order to understand in vivo performance of these multicompartment nanocarriers. Understanding the impact of the multicompartment structure in an *in vivo* setting is essential to ensure their performance for real clinical applications. Understanding the cell uptake and mechanisms of therapeutic delivery of these complex nanocarriers should also be investigated. The synthesis of these materials involves some complex elements and thus another issue for translation is the reproducible production of these materials at large scale. This will not be possible with all systems. Although there are challenges associated with these multicompartment polymeric

nanocarriers, we expect the currently reported studies will provide a significant contribution to the progression of these technologies towards advanced therapeutic applications.

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References

[1] T. Trantidou, M. Friddin, Y. Elani, N. J. Brooks, R. V. Law, J. M. Seddon, O. Ces, *ACS Nano* **2017**, *11*, 6549.

[2] A. C. Misra, J. Lahann, *Adv. Healthcare Mater.* **2018**, *7*, 1701319.

[3] J. Yoon, K. J. Lee, J. Lahann, *J. Mater. Chem.* **2011**, *21*, 8502.

[4] W. Wang, H. Song, J. Zhang, P. Li, C. Li, C. Wang, D. Kong, Q. Zhao, *J. Controlled Release* **2015**, *203*, 57.

[5] R. Chandrawati, M. P. Van Koeverden, H. Lomas, F. Caruso, *J. Phys. Chem. Lett.* **2011**, *2*, 2639.

[6] R. Chandrawati, F. Caruso, *Langmuir* **2012**, *28*, 13798.

[7] X. Feng, F. Lv, L. Liu, H. Tang, C. Xing, Q. Yang, S. Wang, *ACS Appl. Mater. Interfaces* **2010**, *2*, 2429.

[8] M. Elsabahy, K. L. Wooley, *Chem. Soc. Rev.* **2012**, *41*, 2545.

[9] J. Nicolas, S. Mura, D. Brambilla, N. Mackiewicz, P. Couvreur, *Chem. Soc. Rev.* **2013**, *42*, 1147.

[10] A. Kowalczuk, R. Trzcinska, B. Trzebicka, A. H. Müller, A. Dworak, C. B. Tsvetanov, *Prog. Polym. Sci.* **2014**, *39*, 43.

[11] G. Gunkel-Grabole, S. Sigg, M. Lomora, S. Lörcher, C. G. Palivan, W. P. Meier, *Biomater. Sci.* **2015**, *3*, 25.

[12] W. Scarano, P. De Souza, M. H. Stenzel, *Biomater. Sci.* **2015**, *3*, 163.

[13] M. Marguet, C. Bonduelle, S. Lecommandoux, *Chem. Soc. Rev.* **2013**, *42*, 512.

[14] A. O. Moughton, M. A. Hillmyer, T. P. Lodge, *Macromolecules* **2012**, *45*, 2.

[15] X. Huang, B. Voit, *Polym. Chem.* **2013**, *4*, 435.

[16] J. Du, R. K. O'Reilly, *Chem. Soc. Rev.* **2011**, *40*, 2402.

[17] S. M. Jin, J. Jeon, M. K. Park, G. H. Kim, E. Lee, *Macromol. Rapid Commun.* **2018**, *39*, 1700545.

[18] Z. Ahmad, A. Shah, M. Siddiq, H.-B. Kraatz, *RSC Adv.* **2014**, *4*, 17028.

[19] S. Biswas, P. Kumari, P. M. Lakhani, B. Ghosh, *Eur. J. Pharm. Sci.* **2016**, *83*, 184.

[20] A. M. Telford, B. Hawkett, C. Such, C. Neto, *Chem. Mater.* **2013**, *25*, 3472.

[21] A. Dag, J. Zhao, M. H. Stenzel, *ACS Macro Lett.* **2015**, *4*, 579.

[22] M. Huo, Y. Zhang, M. Zeng, L. Liu, Y. Wei, J. Yuan, *Macromolecules* **2017**, *50*, 8192.

[23] S. Li, H. Nie, S. Gu, Z. Han, G. Han, W. Zhang, *ACS Macro Lett.* **2019**, *8*, 783.

[24] X. Zhao, P. Liu, *ACS Appl. Mater. Interfaces* **2015**, *7*, 166.

[25] J. Huang, Y. Guo, S. Gu, G. Han, W. Duan, C. Gao, W. Zhang, *Polym. Chem.* **2019**, *10*, 3426.

[26] W. Zhang, H. Bao, J. He, X. Dong, *Colloid. Polym. Sci.* **2015**, *293*, 3013.

[27] A. Piloni, A. Walther, M. H. Stenzel, *Polym. Chem.* **2018**, *9*, 4132.

[28] A. Hanisch, A. H. Gröschel, M. Förtsch, M. Drechsler, H. Jinnai, T. M. Ruhland, F. H. Schacher, A. H. Müller, *ACS Nano* **2013**, *7*, 4030.

[29] A. O. Moughton, T. Sagawa, L. Yin, T. P. Lodge, M. A. Hillmyer, *ACS Omega* **2016**, *1*, 1027.

[30] F. Xu, D. Wu, Y. Huang, H. Wei, Y. Gao, X. Feng, D. Yan, Y. Mai, *ACS Macro Lett.* **2017**, *6*, 426.

[31] L. Cheng, X. Lin, F. Wang, B. Liu, J. Zhou, J. Li, W. Li, *Macromolecules* **2013**, *46*, 8644.

[32] X. Liu, Y. Hou, X. Tang, Q. Wu, C. Wu, J. Yi, G. Zhang, *RSC Adv.* **2016**, *6*, 5312.

[33] S. Li, X. He, Q. Li, P. Shi, W. Zhang, *ACS Macro Lett.* **2014**, *3*, 916.

[34] M. Huo, M. Zeng, D. Li, L. Liu, Y. Wei, J. Yuan, *Macromolecules* **2017**, *50*, 8212.

[35] T. P. Lodge, A. Rasdal, Z. Li, M. A. Hillmyer, *J. Am. Chem. Soc.* **2005**, *127*, 17608.

[36] A. C. Rinkenauer, A. Schallon, U. Günther, M. Wagner, E. Betthausen, U. S. Schubert, F. H. Schacher, *ACS Nano* **2013**, *7*, 9621.

[37] M. Wu, Y. Zhu, W. Jiang, *Angew. Chem. Int. Ed.* **2018**, *57*, 3578.

[38] C. V. Synatschke, T. Nomoto, H. Cabral, M. Förtsch, K. Toh, Y. Matsumoto, K. Miyazaki, A. Hanisch, F. H. Schacher, A. Kishimura, N. Nishiyama, A. H. E. Müller, K. Kataoka, *ACS Nano* **2014**, *8*, 1161.

[39] T. Nomoto, S. Fukushima, M. Kumagai, K. Machitani, Y. Matsumoto, M. Oba, K. Miyata, K. Osada, N. Nishiyama, K. Kataoka, *Nat. Commun.* **2014**, *5*, 1.

[40] C. K. Wong, F. Chen, A. Walther, M. H. Stenzel, *Angew. Chem.* **2019**, *131*, 7413.

[41] G. K. Such, Y. Yan, A. P. Johnston, S. T. Gunawan, F. Caruso, *Adv. Mater.* **2015**, *27*, 2278.

[42] C. Wang, G. Zhang, G. Liu, J. Hu, S. Liu, *J. Controlled Release* **2017**, *259*, 149.

[43] D. T. Akhter, J. D. Simpson, N. L. Fletcher, Z. H. Houston, A. V. Fuchs, C. A. Bell, K. J. Thurecht, *Adv. Ther.* **2019**, 1900171.

[44] F. Meng, Z. Zhong, *J. Phys. Chem. Lett.* **2011**, *2*, 1533.

[45] F. Liu, V. Kozlovskaya, S. Medipelli, B. Xue, F. Ahmad, M. Saeed, D. Cropek, E. Kharlampieva, *Chem. Mater.* **2015**, *27*, 7945.

[46] Z. Pang, H. Gao, Y. Yu, L. Guo, J. Chen, S. Pan, J. Ren, Z. Wen, X. Jiang, *Bioconjugate Chem.* **2011**, *22*, 1171.

[47] L. Wang, G. Liu, X. Wang, J. Hu, G. Zhang, S. Liu, *Macromolecules* **2015**, *48*, 7262.

[48] S. Iqbal, M. Blenner, A. Alexander-Bryant, J. Larsen, *Biomacromolecules* **2020**, *21*, 1327.

[49] Z. Fu, M. A. Ochsner, H.-P. M. de Hoog, N. Tomczak, M. Nallani, *Chem. Commun.* **2011**, *47*, 2862.

[50] S.-H. Kim, H. C. Shum, J. W. Kim, J.-C. Cho, D. A. Weitz, *J. Am. Chem. Soc.* **2011**, *133*, 15165.

[51] H. C. Shum, Y. j. Zhao, S. H. Kim, D. A. Weitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 1648.

[52] M. Marguet, L. Edembe, S. Lecommandoux, *Angew. Chem.* **2012**, *124*, 1199.

[53] M. Marguet, O. Sandre, S. Lecommandoux, *Langmuir* **2012**, *28*, 2035.

[54] R. J. Peters, M. Marguet, S. Marais, M. W. Fraaije, J. C. Van Hest, S. Lecommandoux, *Angew. Chem. Int. Ed.* **2014**, *53*, 146.

[55] W. Siti, H.-P. M. de Hoog, O. Fischer, W. Y. Shan, N. Tomczak, M. Nallani, B. Liedberg, *J. Mater. Chem. B* **2014**, *2*, 2733.

[56] S. Thamboo, A. Najer, A. Belluati, C. von Planta, D. Wu, I. Craciun, W. Meier, C. G. Palivan, *Adv. Funct. Mater.* **2019**, *29*, 1904267.

[57] L. Schoonen, J. C. van Hest, *Adv. Mater.* **2016**, *28*, 1109.

[58] B. Chatin, M. Mével, J. Devallière, L. Dallet, T. Haudebourg, P. Peuziat, T. Colombani, M. Berchel, O. Lambert, A. Edelman, *Mol. Ther. Nucleic. Acids* **2015**, *4*, e244.

[59] S. Lee, H. Koo, J. H. Na, K. E. Lee, S. Y. Jeong, K. Choi, S. H. Kim, I. C. Kwon, K. Kim, *ACS Nano* **2014**, *8*, 4257.

[60] H. K. Dhaliwal, Y. Fan, J. Kim, M. M. Amiji, *Mol. Pharm.* **2020**.

[61] S. R. Paliwal, R. Paliwal, H. C. Pal, A. K. Saxena, P. R. Sharma, P. N. Gupta, G. P. Agrawal, S. P. Vyas, *Mol. Pharm.* **2012**, *9*, 176.

[62] S. A. Shein, I. I. Kuznetsov, T. O. Abakumova, P. S. Chelushkin, P. A. Melnikov, A. A. Korchagina, D. A. Bychkov, I. F. Seregina, M. A. Bolshov, A. V. Kabanov, *Mol. Pharm.* **2016**, *13*, 3712.

[63] M. Emamzadeh, M. Emamzadeh, G. Pasparakis, *ACS Appl. Bio Mater.* **2019**, *2*, 1298.

[64] S. R. Sarker, R. Hokama, S. Takeoka, *Mol. Pharm.* **2014**, *11*, 164.

[65] S. Kube, N. Hersch, E. Naumovska, T. Gensch, J. Hendriks, A. Franzen, L. Landvogt, J.-P. Siebrasse, U. Kubitscheck, B. Hoffmann, *Langmuir* **2017**, *33*, 1051.

[66] N. Mitchell, T. L. Kalber, M. S. Cooper, K. Sunassee, S. L. Chalker, K. P. Shaw, K. L. Ordidge, A. Badar, S. M. Janes, P. J. Blower, *Biomaterials* **2013**, *34*, 1179.

[67] J. Malinge, B. Géraudie, P. Savel, V. Nataf, A. Prignon, C. Provost, Y. Zhang, P. Ou, K. Kerrou, J.-N. Talbot, *Mol. Pharm.* **2017**, *14*, 406.

[68] R. Chandrawati, P. D. Odermatt, S.-F. Chong, A. D. Price, B. Stadler, F. Caruso, *Nano Lett.* **2011**, *11*, 4958.

[69] L. Hosta-Rigau, M. J. York-Duran, Y. Zhang, K. N. Goldie, B. Städler, *ACS Appl. Mater. Interfaces* **2014**, *6*, 12771.

[70] M. Godoy-Gallardo, C. Labay, M. M. Jansman, P. K. Ek, L. Hosta-Rigau, *Adv. Healthcare Mater.* **2017**, *6*, 1601190.

[71] M. Godoy-Gallardo, C. d. Labay, V. D. Trikalitis, P. J. Kempen, J. B. Larsen, T. L. Andresen, L. Hosta-Rigau, *ACS Appl. Mater. Interfaces* **2017**, *9*, 15907.

[72] J. W. Maina, J. J. Richardson, R. Chandrawati, K. Kempe, M. P. Van Koeverden, F. Caruso, *Langmuir* **2015**, *31*, 7776.

[73] D. S. Pisal, M. P. Kosloski, S. V. Balu-Iyer, *J. Pharm. Sci.* **2010**, *99*, 2557.

[74] R. Chandrawati, L. Hosta-Rigau, D. Vanderstraaten, S. A. Lokuliyana, B. Stadler, F. Albericio, F. Caruso, *ACS Nano* **2010**, *4*, 1351.

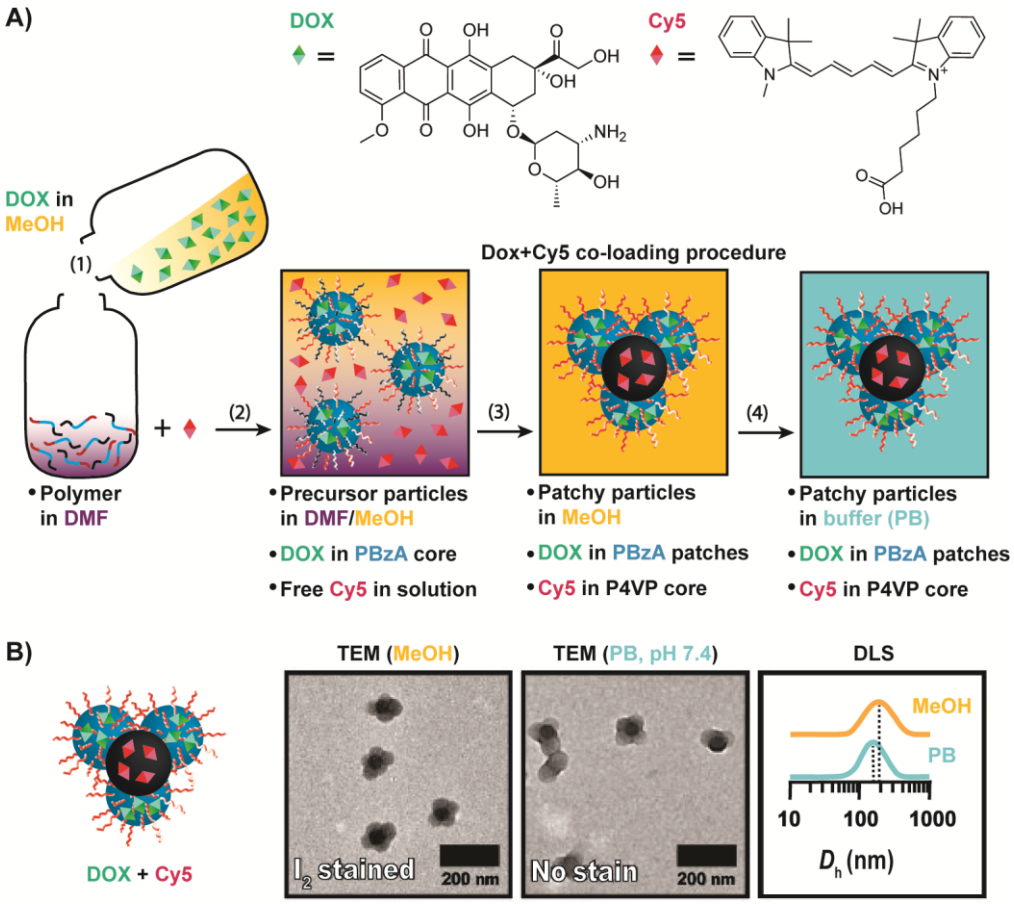

Figure 1. (A) Site selective co-loading procedure of patchy particles with DOX and Cy5 within PBzA patches and P4VP core involved four major steps; (1) Addition of methanolic DOX solution to polymer (PGlcEMA-*b*-PBzA-*b*-P4VP)/DMF solution to yield precursor particles; (2) Addition of methanolic Cy5 solution; (3) Dialysis to remove DMF to yield patchy particles with DOX encapsulated in PBzA patches and Cy5 encapsulated in P4VP core; (4) Dialysis against phosphate buffer (PB) to remove methanol and to transfer particles into an aqueous media. (B) TEM and DLS results showing the unchanged patchy morphology of particles in both MeOH and PB after drug encapsulation. Reproduced with permission.[40] Copyright 2019, Wiley-VCH.

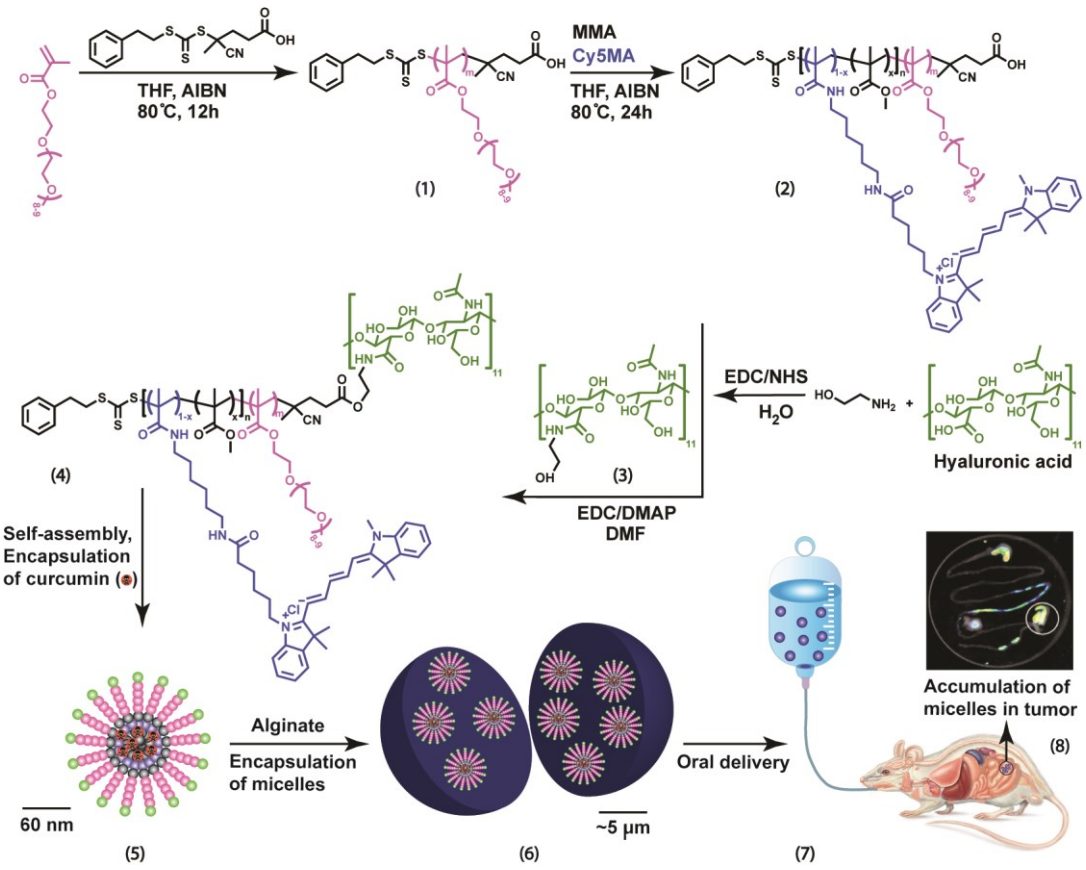

Figure 2. A schematic representation of synthetic scheme for the formation of HA-PPEGMA-*b*-P(MMA-*co*-Cy5MA) block copolymer (1-4), synthesis of curcumin-loaded micelles via self-assembly (5), encapsulation of micelles into alginate microcapsules (6), oral delivery for the site-specific drug release at tumor cells (7,8). Reproduced with permission.[43] Copyright 2019, Wiley-VCH.

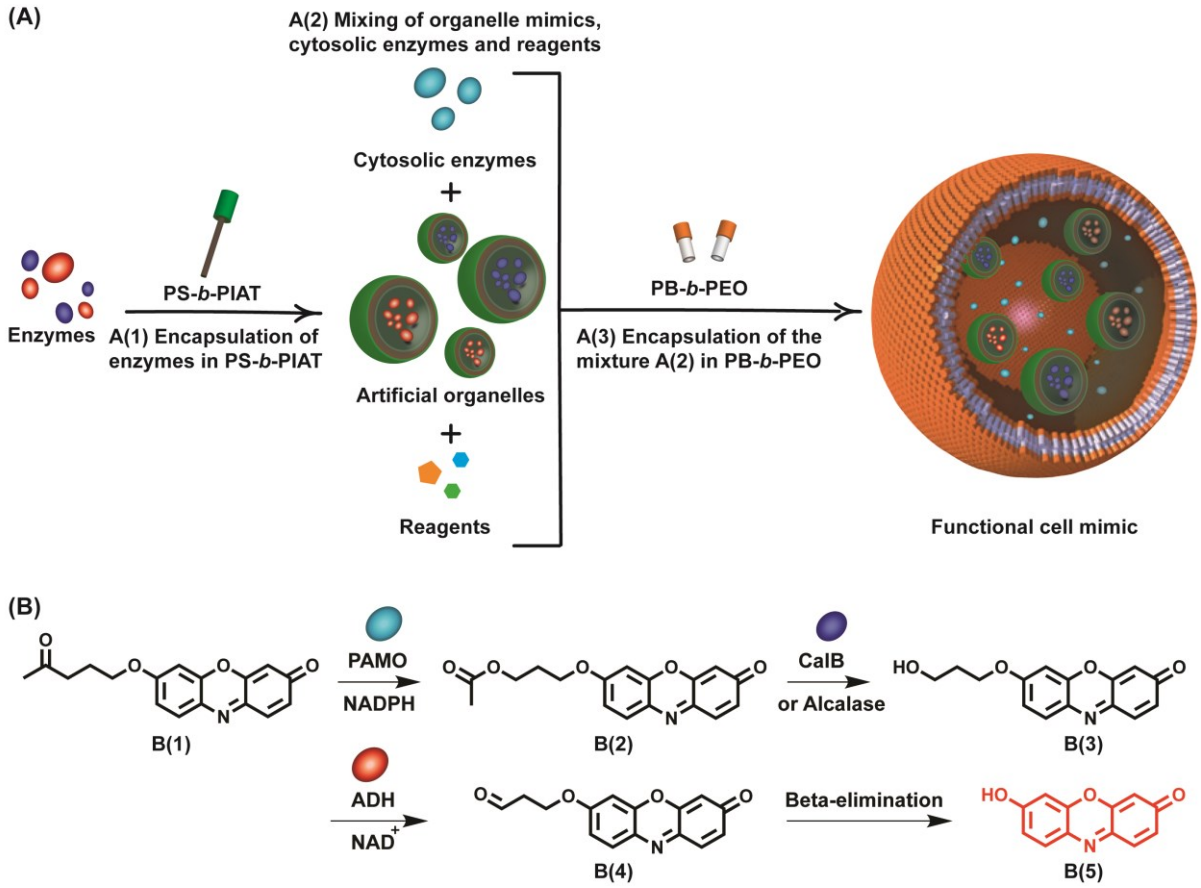

Figure 3. (A) Schematic representation of polymersomes-in-polymersomes nanoreactor which creates functional cell mimic to perform enzymatic cascade reaction shown in (B). Reproduced with permission.[54] Copyright 2014, Wiley-VCH.

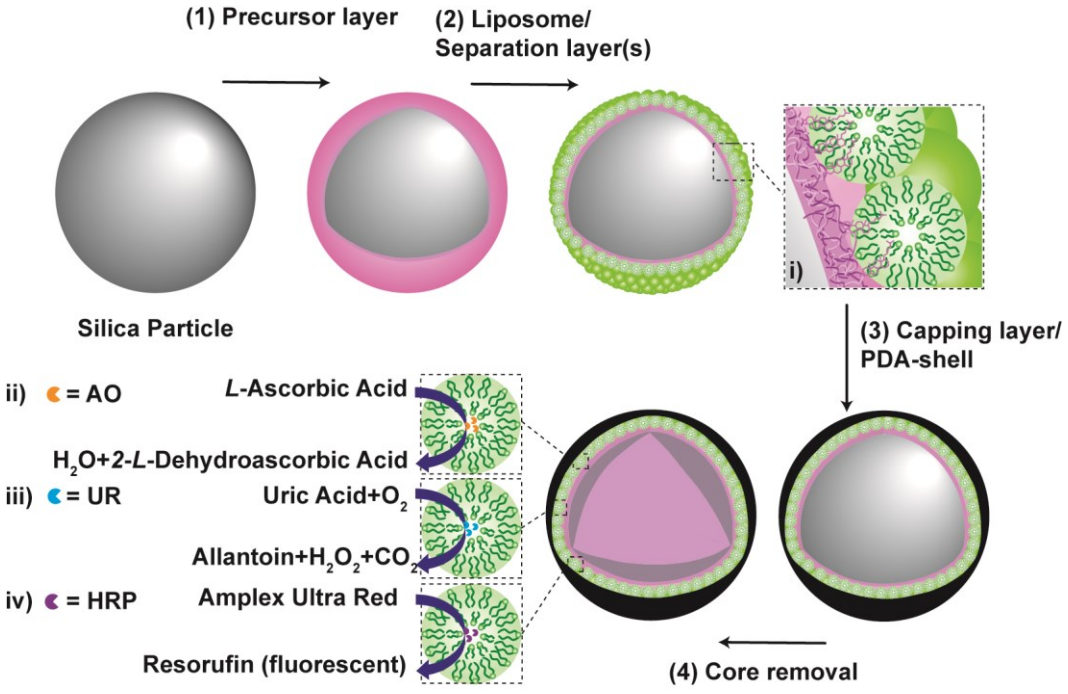

Figure 4. Detailed explanation of capsosome assembly. (1) Adsorption of precursor polymer layer on silica particles, followed by (2) deposition of liposomes and separation layers. (i) Liposomes are non-covalently attached to precursor

and separational layers without rupturing the structure. (3) Next, assembly is capped with P(MA-*co*-CMA) and polymeric PDA shell is assembled by self-polymerization of dopamine. (4) Core removal to obtain capsosomes with three different enzyme-loaded liposomes (ii, iii, and iv). Reproduced with permission.[69] Copyright 2014, American Chemical Society.

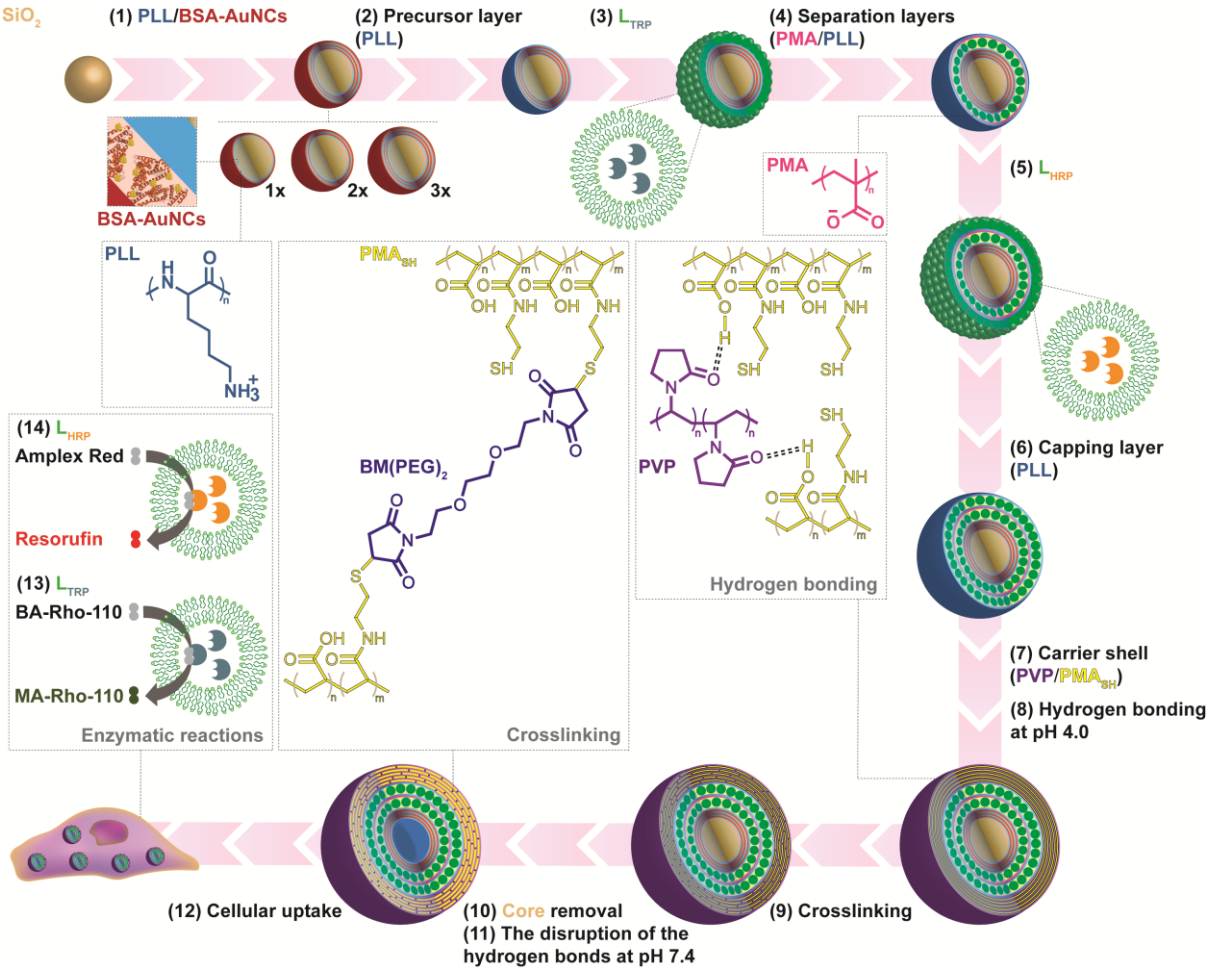

Figure 5. The schematic illustration of capsosomes assembly. Three bilayers of PLL and BSA-AuNCs are deposited onto SiO_2 core particles (1). The assembly is coated with precursor layer (PLL) (2), followed by the adsorption of TRP-loaded liposomes (L_{TRP}) (3), separation layers (PMA/PLL) (4), HRP-loaded liposomes (L_{HRP}) (5), and assembly is capped with PLL layer (6). The polymeric carrier shell is assembled by sequential deposition of five bilayers of PVP and PMA_{SH} (7), which interact via hydrogen bonding at pH 4.0 (8). The thiols of the carrier shell are crosslinked by thiol-maleimide chemistry by means of 1,8-bismaleimido-diethyleneglycol ($(BM(PEG)_2)$ (9). After removal of SiO_2 core (10), hydrogen bonds between PVP and PMA_{SH} are disrupted at pH 7.4 (11). Following the internalization of capsosomes by a macrophage cell line (12), TRP and HRP loaded within separate liposomal compartments (L_{TRP} and L_{HRP}) conduct two enzymatic reactions simultaneously (13 and 14). Reproduced with permission.[70] Copyright 2016, Wiley-VCH.

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Multicompartment polymeric nanocarriers have significant potential in biomedical applications such as drug delivery and synthesis of artificial cell mimics. In this review some of the most promising multicompartment polymeric nanocarriers used in biomedical applications are outlined depending on three specific classes: multicompartment micelles, multicompartment polymersomes and capsosomes.