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REVIEW

Self-assembled architectures with multiple aqueous compartments

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A vital organizational feature of living cells is that of compartmentalization. This allows cells to run concurrently incompatible metabolic processes and to regulate these processes by selective transmembrane transport. Although strategies that effectively mimic cell function in simple architectures have been researched extensively, soft matter systems with membranes that delineate distinct and multiple aqueous environments have only recently caught attention. We highlight a range of multi-compartmentalized soft matter systems including vesosomes, capsosomes, polymersomes, double emulsions, and their combinations, and demonstrate that the unique properties of the multi-compartmentalized architectures have the potential to add value to application areas such as drug-delivery and multi-enzyme biosynthesis.

Introduction

The eukaryotic cell is a complex organization of multiple lipidic compartments, or organelles, within the larger compartment of the cytosol. This multicompartmentalization allows the cell to separate in time and space the functional processes governing its survival.[1] Examples of membrane-delineated compartments include lysosomes, where the pH is markedly lower than in the rest of the cell, and peroxysomes, where specific harsh enzymes reside, which when freely residing in the cytosol would interfere with cellular metabolic pathways. Compartmentalization is not limited to lipid containers. Numerous smaller scale containers exist, which exert functions such as assisting in protein folding (chaperones), iron metabolism (ferritins) or protein degradation (proteasomes).[2]

The past decades have seen a steady stream of reports on simple, predominantly lipidic, model systems that aimed at understanding complex compartmentalized systems created

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during the evolution.[3–7] These reports included, among others, studies on simple enzymatic reactions as well as on complex *in vitro* translation and transcription processes.[8–10] In contrast, there are comparatively fewer examples in the literature of self-standing, artificially created multi-compartmentalized architectures. However, as we will demonstrate in this review, multicompartmentalized self-assembled soft matter systems represent an important class of architectures with unique structure and function, which may be exploited for novel applications in drug delivery and as nano-sized reactors.[11–13]

The successful development of synthetic drug carriers for use in humans requires systems that effectively encapsulate drugs and release their load selectively to targeted cells in the body, and at the same time evade being captured by the immune system. During the past 10 years nanotechnology and biotechnology have allowed the emergence of ever more complex novel drug delivery systems where delivery of multiple drugs and imaging contrast agents are combined with *in situ* drug synthesis and stimulus responsive drug release, all combined into a single particulate carrier.[13–15] As the complexity of these drug delivery systems increases multi-compartment architectures are no longer made of one material but rather sequester diverse compounds and compartmentalization provides here a basic design strategy.[16] In addition to that, they will be beneficial to the developing research field of nano-reactors, where an ultimate goal is to streamline the batch synthesis of complex chemical compounds into cascade processes, eliminating the need for intermediate purification steps and minimizing waste products. Indeed, this process resembles that of natural cells. Therefore design of multi-compartments seems to be also a prerequisite for the construction of an artificial living cell.[17]

In this review, we provide an overview of the major advances in the field of artificial compartmentalized systems and highlight selected structures and fabrication methods (see Table 1). We define multi-compartmentalized soft matter systems as artificial, self-standing micro- and nanostructures, which have well-defined boundaries and delineated, distinct and multiple aqueous environments, *i.e.*, compartments, obtained by self-assembly of synthetic or biological materials, and which are able to encapsulate molecular cargo independently and selectively in each of the compartments. Regarding the fabrication material we start the review with lipid systems, which have traditionally been the material of choice for creating artificial micro- and nano-compartments.[18] We then continue with the more recent developments in polymer based architectures; polymers attracted much attention as compartment membrane materials due to their structural wealth and superior mechanical stability.[19–21] Bi-continuous self-assembled multi-compartment structures formed by liquid crystalline mono-glyceride lipids are excluded from this review.[22] We will also not review pericentric or acentric structures made of polymeric particles, multi-component micelles or colloidosomes.[23–26]

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Lipid multicompartments

Liposomes are vesicles consisting of an aqueous core enclosed in one or more phospholipid bilayers formed by self-assembly.[27] In nature, they form the primary building block of the plasma membrane and organelles, and a wealth of literature exists on their chemistry, structure, fabrication, and applications. Major advantages of liposomes as artificial liquid containers are their ease of fabrication, low cost, and inherent biocompatibility. When provided with a PEG-coating they have been shown to accumulate at tumour sites because of the enhanced permeability and retention effect (EPR-effect). Once at the site, encapsulated cytotoxic drugs are passively released, and as a result the drug exerts its effect preferentially at the target site, while its toxic effect on normal cells is reduced.[28] A common problem associated with applying lipid vesicles in biological systems is the premature and uncontrolled release of internalized species, which is thought to result from enzymatic degradation through lipases or through protein insertion into the lipid bilayer.[29] For instance, the half-life of the release of calcein from osmotically stabilized egg lecithin liposomes decreases from 202.5 h in buffer to 5.4 h in serum (at 22 °C).[30] Although liposome formulations have been optimized in terms of stability and leakage, for multiple drug/liposome formulations the release kinetics are much higher compared to the model therapeutic example of encapsulated doxorubicin (Doxil®) preventing the accumulation of the drugs at the target site.[31,32]

Encapsulation of vesicles inside larger liposomes was already observed for most of the commonly employed vesicle preparation protocols. Multi-compartmentalization in lipid vesicles can proceed *via* processes known to occur in biological systems, especially those processes that result in dynamic morphological changes to the membrane. For example, Okumura *et al.* showed that electro-formed, giant, cell-sized (20 μm) lipid membrane vesicles, when incubated in dilute buffer or glucose solutions self-transform *via* invagination to an oligovesicular system where an inner vesicle engulfs part of the surrounding solution.[33] In this way a two-compartment vesicle system containing different cargos in different compartments could be obtained. The process exhibited however a relatively low yield of maximum 20% and was strongly dependent on the nature of the surrounding medium and on the process dynamics; it was therefore hard to control.[34]

Table 1 Multicompartmentalized soft matter systems

Key structural features	D_o[a]/μm	D_i[b]/nm	Internalized compartments	Ref.
Lipid vesicles inside lipid vesicles; vesosomes	0.1–10	50–250	~50 to 100[c]	35,37,41 and 42
Giant unilamellar vesicles containing phase-separated (responsive) material	10–50	$0.7D_0$[d]–5000	2	43,45 and 51
Polymer multicore capsules; continuous preparation in microfluidic devices	100–500	50 000	1–20	48 and 51
Polymer LbL capsules inside polymer LbL capsules	5–150	300–3000	2–60	55,57,60 and 67
Lipid vesicles inside polymer capsules; capsosomes	5	50	~800 (per layer) to 160 000[e]	69,75 and 76
Polymersomes in polymersomes or non-spherical polymersome multicompartments	0.5–15	0.05–5	2–3	95,96 and 98

[a] Diameter of the outer compartment. [b] Diameter of the inner compartment(s). [c] Estimated from TEM images. [d] Shrinking ratio at temperature above the LCST of PNIPAM. [e] Estimated from fluorescence measurements.

To obtain stable, compartmentalized lipid vesicles, in which unilamellar lipid vesicles are encapsulated inside bigger vesicles, resulting in multiple non-nested compartments, one has to design a process which does not disrupt other vesicles in solution or does not change the conformation and function of biological molecules that need to be compartmentalized. Such a method was reported by Zasadzinski and co-workers who prepared multicompartmentalized lipid vesicles by spontaneous encapsulation, and who coined the term *vesosomes* for such architectures.[35] The encapsulation proceeded *via* pre-formation of dioleoylphosphatidylserine lipid cochleate cylinders in the presence of Ca^{2+} and their subsequent unrolling by addition of ethylenediaminetetraacetic acid (EDTA). In the presence of separately prepared vesicle aggregates vesosomes were produced.[36] The process was shown to be versatile and could be used to encapsulate nanoscale and microscale objects inside the bilayer compartments. TEM images clearly showed that a single unrolled bilayer was able to fully encapsulate vesicle aggregates without causing damage to the encapsulated structures (Fig. 1a). The encapsulants and encapsulating bilayer could be tuned with respect to their permeation rates, rigidity and phase transition temperature (or melting temperature, T_m). Since the formation of the encapsulated vesicles is decoupled from the encapsulation process, the encapsulated vesicles can be made of chemically distinct lipids exhibiting different physicochemical properties, allowing for a truly complex multi-compartmentalized system.

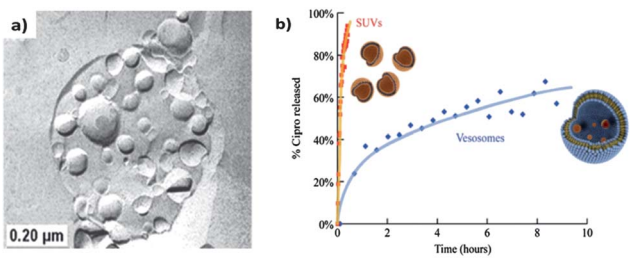

Fig. 1 (a) TEM image of a vesosome formed by extruding through a 400 nm pore size filter. The internal 50 nm vesicles were unaffected by extrusion and remained encapsulated throughout the process. Vesosomes were produced *via* the 'interdigitated bilayer sheet' method. Reprinted with permission from ref. 37. Copyright (2002) American Chemical Society. (b) Ciprofloxacin release profiles obtained for unilamellar liposomes and vesosomes at 37 °C in 50% calf serum. Ciprofloxacin release from liposomes is complete within 30 min, while the half-life in vesosomes is equal to ~6 h. Sizes of the lipid architectures are not to scale.[38] Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Vesosomes were also produced by taking advantage of the interdigitated bilayer phase formation, a process which occurs upon addition of ethanol to neutral saturated phosphatidylcholine phospholipids below their transition temperature.[37,39] At temperatures below the T_m the lipid–ethanol sheets are rigid and flat due to intercalation of ethanol within the head groups of the lipid molecules. Above T_m the lipid sheets become flexible, close on themselves and spontaneously encapsulate whatever is present in the surrounding liquid, including other lipid vesicles. The process is irreversible, *i.e.*, the vesicles stay intact after the temperature is lowered below T_m, and proceeds without disruption of the membranes. Furthermore, the size of the structures formed from the sheets can be controlled by the addition of cholesterol and by varying the ethanol concentrations in the interdigitated lipid sheets.

A noteworthy feature of the vesosomes is their content release behaviour as compared to the single-compartment liposomes prepared from the same lipid. In serum, the extra layer can be expected to protect the subcompartments from attack by phospholipases and protein insertion leading to lysis. Indeed the simple vesosome architecture led to a greatly reduced susceptibility (albeit *in vitro*) to attack by phospholipases.[38] After 20 h of incubation, the release of carboxyfluorescein from the vesosomes was negligible compared to a negative control (no added lipase), while liposomes formed from the same lipids showed complete release in 4–8 h. In calf serum, the release of dyes from the vesosomes showed a half-life of 50 hours, whereas significant release only occurred after a 10 hour delay, possibly reflecting the degradation of the outer lipid layer. For the weakly basic drug ciprofloxacin a half-life of 6 h in 50% calf-serum was observed for its release from the vesosome subcompartments (Fig. 1b), while for liposomes the content was released in half an hour. This enhanced retention time can be of use for drugs where premature leakage from the carrier is a problem. It should be noted however that the circulation half-time in mouse was only 2 hours, which was comparable to liposomes. This possibly reflects the larger size, and thus reduced circulation time of the vesosomes.[29,38,39] Evidently, permeability is not the only hurdle to overcome to design a successful drug delivery system. For lipid vesicles, the size and composition are equally important determinants of the performance of the drug delivery vehicles *in vivo*.[40] Considering

the diameter of vesosomes and that of multi-compartmentalized structures discussed later it is evident that their potential as drug delivery vehicles will only start to materialize if their diameters can be tuned to the narrow window (200–500 nm) that is generally considered to be prerequisite for circulation *in vivo*.

A two-step hydration of lipid films was used by Bolinger *et al.* to obtain a multi-compartmentalized lipid vesicle integrated reactor system (Fig. 2a).[41] First, small unilamellar vesicles (SUVs) were formed and extruded through 400 nm cut-off filters. Then, large unilamellar vesicles (LUVs) were formed in the presence of the SUVs in the hydrating solution. Dyes and biomolecules could be successfully incorporated into the SUVs by adding them to the hydration solution. Attolitre to zeptolitre volumes of reagents were thus selectively encapsulated into one of the compartments. The resulting LUVs displayed a large size distribution (+/−50%) making the evaluation of encapsulation efficiency difficult. On average, however, based on a dilution calibration curve, it was estimated that there were ~50 SUVs of $D = 250$ nm diameter incorporated into a single 2 μm diameter LUV. Including negatively charged lipids into the LUVs and SUVs suppressed the interaction between the vesicles. Sudden increase in permeability (to polar solutes) of lipid bilayers can be achieved upon reaching the melting temperature and this was used for mixing the contents of the compartmentalized vesicles. By tuning the temperature of the system, controlled mixing of attolitre amounts of reactants inside the femtolitre container was demonstrated by compartmentalizing small unilamellar vesicles of relatively high T_m (41 °C; using high T_m lipids) inside a large unilamellar vesicle of much lower T_m (−18 °C). The release from SUVs nested inside the LUV was monitored by using a dye and increasing the temperature from 25 to 41 °C. The LUV membrane efficiently confined the dyes released from the SUVs so that they did not leak outside the big vesicles. When an enzyme (alkaline phosphatase) was encapsulated in the LUVs and its substrate in the sub-compartments, the turnover of the substrate could be controlled by temperature (Fig. 2b). This set-up was extended to consecutive enzymatic reactions by encapsulation of different sorts of SUVs having different T_m and different enclosed substrates. In principle, such reactions inside the LUV can be monitored with single molecule precision, using, *e.g.*, fluorescence correlation spectroscopy.[42]

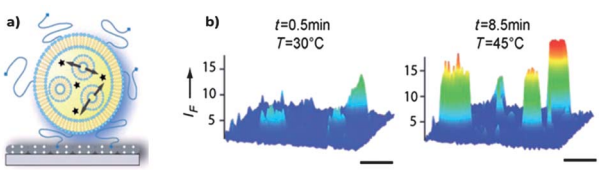

Fig. 2 (a) Schematic representation of the surface-immobilized multi-compartment system for consecutive enzymatic reactions in a single architecture. Alkaline phosphatase (ALP) is incorporated in the outer compartment together with two subcompartments of which the bilayers have different T_m ($T_m \approx 23$ °C and 41 °C) and are loaded with different substrates for ALP. (b) Fluorescence intensity images obtained at $T = 30$ °C (the substrate is restricted to the subcompartments) and at $T = 45$ °C. At $T \approx T_m$ the substrate, fluorescein diphosphate, diffuses through the bilayer and is converted by the enzyme to a fluorescent product.[42] Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Phase separating systems

A conceptually different way of compartmentalization was presented by Keating and co-workers.[43] Their approach was based on the phase separation behaviour of certain concentrated aqueous polymer solutions. When used as encapsulants, these were capable of phase separation reminiscent of intracellular dynamic compartmentalization mechanisms. This was demonstrated by encapsulating concentrated aqueous solutions of PEG and dextran (both ~10 wt%) into lipid giant unilamellar vesicles (GUVs; $D = 5$–30 μm (Fig. 3)). At high temperature a single homogeneous phase was present in the vesicles. Upon cooling, however, phase separation occurred and two separate compartments could be clearly identified.

The process was completely reversible and the phase separation process was accelerated compared to bulk solutions; it occurred in minutes compared to hours or days for bulk solutions of the same composition and polymer concentration. Dynamic control over the formation of the compartments and protein partitioning between the liquid phases was achieved by varying the temperature, pH, and osmotic pressure.[44] Changes in pH allowed for reversible shuttling of proteins between the compartments without affecting the phase-separated compartments. For example, at pH 6.5 human serum albumin (HSA) partitioned to the dextran-rich phase. When changing the pH to 4 or 12 the protein denatured and partitioned to the PEG rich phase.[45] Adding sucrose to the exterior solution resulted in an osmotic gradient leading to transport of water out of the vesicle and thus to an increase of the (local) concentration of the encapsulated polymers. When PEG-ylated lipids were used this led to budding of the vesicles, with the dextran-rich phase enriched in the budding compartment.[44] In an extension of this work, by using raft-forming lipids, this budding process could be controlled such that the daughter vesicles produced by the fission of the mother cells differed in membrane composition as well as in the 'cytosolic' composition.[46]

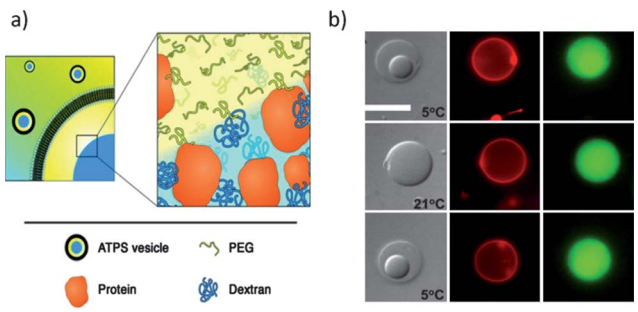

Fig. 3 (a) Illustration of the model cell aqueous two-phase system (ATPS), where giant lipid vesicles are loaded with concentrated aqueous solutions of dextran and PEG, which phase separate at low temperatures. (b) Reversibility of the segregation of proteins in the ATPS. Soybean agglutinin lectin partitions to the dextran-rich phase at $T = 5$ °C (top row: dextran phase separates), spreads over the whole volume at $T = 21$ °C (middle row: PEG and dextran phase mix), and re-compartmentalizes again at 5 °C (bottom row). The middle panel and right panel represent fluorescence from rhodamine tagged lipids and lectin (scale bar is 10 μm).[43] Copyright (2005) National Academy of Sciences, U.S.A.

In a separate example, phase separated compartments inside GUVs were prepared by microinjection of a temperature responsive polymer poly(*N*-isopropylacrylamide) (PNIPAM). Below the lower critical solution temperature (LCST) PNIPAM is soluble in water, while above the LCST it becomes hydrophobic and phase separates in aqueous medium. Reversible formation of a dense, phase-separated hydrogel compartment inside the GUV accompanied by shrinking/swelling was observed above the LCST of PNIPAM.[47] The size of the separated PNIPAM phase could be tuned to some extent by temperature.

Multiple emulsions

Multiple emulsions are structured fluids consisting of emulsion droplets within larger droplets that are on their turn dispersed in a continuous medium. Emulsions have since long been used to compartmentalize one fluid in another fluid, mostly *via* multiple sequential emulsification processes. However, size monodispersity and control over the internal structure have always been problematic. A major advance to tackle this problem was the introduction of microcapillary microfluidic devices, which could be used to control the flow profile of liquids, to obtain multiple emulsions with control over the droplet size and morphology.[48] Encapsulation of molecules in a particular phase of the double emulsion prepared using such devices is made easy by simply introducing the molecules into the particular fluid stream. As the external and internal fluids never come into contact one can selectively encapsulate molecules in each of the phases, giving researchers the ability to construct multi-compartmentalized systems. For example, Weitz and co-workers described a device, where by controlling the flow rates of three fluids, double emulsions could be generated in a single step, with control over the inner and outer droplet sizes as well as over the number of droplets inside the larger droplets.[48] Further improvements of the microfluidic devices, in particular cascading many components, led to the fabrication of more complex compartmentalized structures with multiple nested droplets with a controlled number of compartments in each droplet. Chu *et al.* prepared hydrogel capsules from a triple emulsion and demonstrated the possibility of entrapment and release of cargo from the compartments formed from the emulsion's oil and aqueous phases.[49] In an extension of this work multi-compartmentalized capsules were fabricated where each internalized compartment could carry a different encapsulated cargo.[50]

In a separate example, by controlling the laminar flow of three non-mixing fluids in a planar microfluidic flow-focusing device, Nie *et al.* reported the production of highly monodisperse, albeit relatively large (20–150 μm), core–shell droplets with a predetermined diameter of the cores and thickness of the shell.[51] Multiple cores in the droplets were obtained by changing the relative flow rates of the three liquids flowing in the device. By carefully controlling the flow conditions, varying the interfacial capillary wavelengths, and shifting their lengths and phases with respect to each other, control over the number of cores inside a single droplet and over the location of the cores in the droplets was achieved (Fig. 4). Monomers could be encapsulated in one of the emulsion phases and photo-polymerized in a wavy channel of the microreactor. Selective polymerization of the monomers inside the droplets resulted in multicore polymeric capsules, or in capsules with hollow capsules with polymeric shells, depending on the emulsion structure.

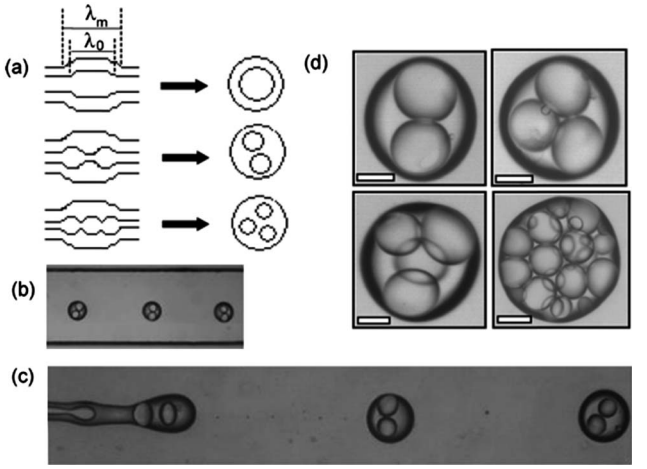

Fig. 4 (a) Schematic representation of the formation of emulsion droplets with multiple cores. Inner phase: silicone oil, outer phase: monomer liquid. (b) Optical microscopy image of core–shell droplets with two cores flowing through the microfluidic device. (c) Break-up of the coaxial jet into two-core droplets. (d) Isolated core–shell droplets comprising a different number of cores engulfed with a polymeric shell. Scale bar is 40 μm. Reprinted with permission from ref. 51. Copyright (2005) American Chemical Society.

Layer-by-layer assembly

One of the very successful routes for capsule formation is based on sequential, layer-by-layer (LbL) absorption of polymers on a template *via* electrostatic, covalent, or non-covalent interactions.[52] Subsequent template dissolution leads to free-standing capsules (*e.g.*, calcium carbonate template can be easily removed by using EDTA).[53–55] Entrapment of molecules inside the capsules can be achieved by switching the permeability of the capsules in response to an external stimulus, by incorporating the molecular cargo into the sacrificial template, or by absorbing the molecules on the template during the assembly process. Because the capsule walls are made of hydrophilic polymers they are permeable to water and small organic solutes, while, in general, large macromolecules, such as proteins, are retained.[56] This also allows the removal of the template dissolution products.

Kreft *et al.* demonstrated the feasibility of conducting enzymatic reactions in multi-compartment shell-in-shell capsules.[57,58] The assembly process proceeded *via* deposition of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) multilayers on a $CaCO_3$ template followed by a second $CaCO_3$ precipitation and a second round of PSS/PAH multilayer formation. Co-precipitation of biomacromolecules with subsequent nanoparticle adsorption and template dissolution led to a two compartment, shell-in-shell structure, which allowed for the selective compartmentalization of different molecules in each of the compartments. The semi-permeability of the capsule walls was tested in a model coupled enzymatic reaction (cascade reaction) involving glucose oxidase (GOx) and horseradish peroxidase (HRP). The experiments showed that, indeed, species

such as glucose, H_2O_2 or the fluorogenic substrate Amplex Red were able to diffuse freely between the compartments, while the enzyme remained in the individual compartments. In a subsequent investigation the cascade was extended to three enzymes by introducing β-glucosidase, which generated glucose for the GOx/HRP cascade from fluorescein-diglucose. Importantly, it was also shown that the kinetics of the cascade reaction could be slowed down by introducing layers of a protein, bovine serum albumin (BSA), in-between the separate compartments.[59]

Triggered release from such compartments was demonstrated by incorporating light-absorbing 20 nm gold nanoparticles into the shell of the inner compartment. Absorption of the 830 nm laser light by the gold nanoparticles resulted in locally induced heating, disruption of the shell and triggered mixing of minute volumes of liquids inside the capsules (Fig. 5).[60] The absorption of the gold nanoparticles at near infrared wavelengths (a biologically 'transparent' window where the absorption by living tissue or cells is low), which was outside of their plasmon resonance band at ~530 nm, was explained by the interactions between the particles as well as by their particular shape and size, which may have shifted the absorption band to the near infrared part of the electromagnetic spectrum. A similar phenomenon was observed for silver nanoparticles embedded in LbL-capsules, making such capsules attractive for biomedical applications.[61] Another enzymatic cascade reaction system was prepared by LbL two-compartment assembly on inorganic templates co-precipitated with enzyme.[62] An inner template of $CaCO_3$ containing formaldehyde dehydrogenase and an outer shell-template of silica-containing formate dehydrogenase served for the deposition of bilayers of protamine/*o*-alginate and a layer of titania/protamine, respectively. The templates could be easily removed with EDTA and HF/NH_4F. The formed capsules showed good mechanical stability and could be recycled up to 8 times. A cascade reaction where carbon dioxide was converted to formaldehyde by two consecutive reductions was compared to a system with freely diffusing, not compartmentalized enzymes. It was found that the yield was the highest for the multi-compartmentalized capsules, followed by single-compartment capsules, and that the yield of formaldehyde for the freely diffusing enzymes in the bulk solution was the lowest. This phenomenon was ascribed to the close proximity and the relative location of the enzymes. Although this is a plausible explanation, the enzyme concentration in the capsules was not accurately measured. The observed effect might equally well have resulted from a more optimal ratio of the enzymes used. Related studies, where the enzyme concentration was adequately assessed, showed a more modest, albeit still a significant increase of the yield.[63,64]

Self-rupturing or exploding capsules made of a (charged) degradable hydrogel encapsulated into a polyelectrolyte shell were introduced by De Geest *et al.*[65,66] The degradable microgel network made, for instance, of dextran cross-linked by a hydrolysable linker, dissolved upon introduction of such capsules in water, leading to an osmotic pressure build-up between the dissolving dextran (which cannot permeate the shell layers) and the bulk solvent. This, in turn, eventually caused shell rupture, and the release time could in principle be tuned by the crosslinking density of the gel network. The authors extended this concept to multi-compartmentalized systems by loading 3 μm diameter layer-by-layer assembled microcapsules into gel beads of $D = 150$ μm.[55] The small LbL capsules were formed by alternating absorption of dextran sulfate/poly-L-arginine on a sacrificial $CaCO_3$ template and then the capsules were dispersed in an emulsion of concentrated PEG and dextran-precursor, which was subsequently polymerized. The resulting gel beads were then coated with the same dextran sulfate/poly-L-arginine LbL pair resulting in a multi-compartmentalized architecture (Fig. 6). By addition of sodium hydroxide, the internal LbL capsules loaded with FITC-dextran as a model drug were rapidly ejected from the capsule through the cracks that formed in the outer shell membrane.

Smaller size, sub-compartmentalized hydrogel capsules were prepared by the sequential deposition of poly(*N*-vinyl pyrrolidone) (PVP) and thiolated poly(methacrylic acid) (PMA) onto silica substrates.[67] These polymers interact *via* hydrogen-bond formation and their capsules are stable at pH = 6.5 (or lower)

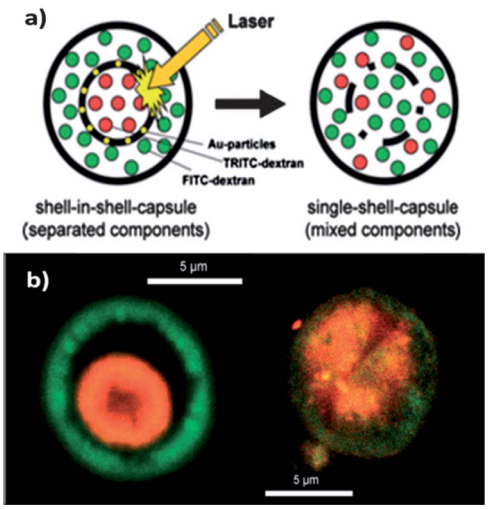

Fig. 5 (a) Spatial separation of biomacromolecules (*e.g.*, enzymes) within distinct compartments of a single capsule produced by a double process of LbL assembly, and their laser induced mixing *via* absorption of light by gold nanoparticles embedded in the inner shell. (b) Fluorescence images of the capsules before and after laser irradiation.[60] Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

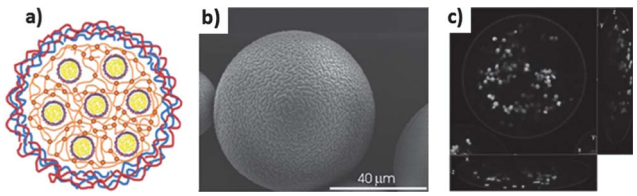

Fig. 6 (a) Schematic structure of a dextran sulfate/poly-L-arginine polyelectrolyte-coated degradable gel bead loaded with dextran sulfate/poly-L-arginine LbL microcapsules. When the polyelectrolyte coated gel beads explode, the smaller LbL microcapsules are suddenly released. (b) SEM image of the LbL coated gel beads. (c) Confocal microscope images of a gel bead loaded with LbL microcapsules containing FITC-dextran.[55] Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

and disintegrate above this pH value. Cross-linking the thiolated PMA *via* the thiol groups led to capsules that were stable for pH values between 5 and 9, a range suitable for biomedical applications. Using this methodology, 300 nm capsules were incorporated into larger 3 μm capsules. Interestingly, by employing chemically distinct cross-linkers for the PMA, the embedded capsules could be selectively degraded, while preserving the integrity of the outer shell wall.

Capsosomes

LbL capsules have been successfully combined with liposomes to form novel compartmentalized systems termed capsosomes. These are LbL capsules, generally based on PMA/PVP multilayers, where liposomes have been sandwiched in between the polymer multilayers and the template colloid (Fig. 7).[68–71] For stabilization purposes a layer of polylysine is first deposited onto the template onto which liposomes are absorbed. Cholesterol or oleic acid modified PMA is used to cover the liposomes; cholesteryl and oleyl groups are able to interact non-covalently with the lipid layers, thus anchoring the vesicles to the capsule walls and overall stabilizing the resulting capsosomes. Dissolution of the template results in structurally stable polymer hydrogel capsules with liposomes incorporated in the capsule shell, or dispersed in the interior.[70,72–74] In these compartmentalized systems the stability and permeability control are provided by the polymeric capsule, while the liposomes serve as protective carriers of specific cargo, such as drugs or enzymes.[75]

A large quantity of liposomes (800–8000 for capsosomes of D = 1–5 μm respectively) can be incorporated into a single hydrogel capsule. Release of substances from the liposome compartments can be induced by a stimulus that either lyses the liposomes (*e.g.*, Triton X) or by the more subtle variation of temperature to control the permeability of the lipid bilayer. The latter strategy was used to trigger enzymatic reactions, making capsosomes interesting platforms for the study of enzymatic cascade processes.[76]

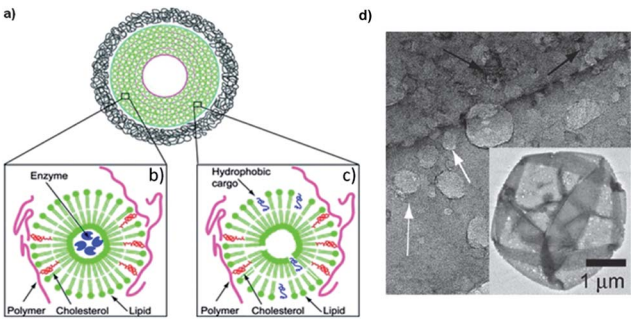

Fig. 7 (a–c) Schematic representation of capsosomes. Multiple liposome layers are adsorbed on a sacrificial template (not shown) and stabilized by cholesteryl-functionalized poly(methacrylic acid). The liposomes can function as carriers of hydrophilic or hydrophobic cargo.[68] Copyright (2009) American Chemical Society. (d) High magnification TEM image of the multilayers with incorporated liposomes. Black arrows identify intact liposomes, while the white arrows point to holes in the capsosome shell where the liposomes were displaced. The inset shows a single capsosome.[71] Copyright WILEY-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Amphiphilic block copolymers, polymersomes

Spontaneous self-assembly of amphiphilic polymers into higher-order, discrete supramolecular assemblies and the use of amphiphilic block copolymers to obtain nanomaterials with distinct compartments are well-established.[77] The morphology of the assemblies can be tailored by the primary chemical structure and length of the individual polymer blocks. Depending on the block copolymer structure, a wealth of different morphologies, across the length scales can be obtained.[78] Simple A–B copolymers generally form one-compartment micelles, while multi-compartment structures form from more complex polymeric architectures, *e.g.*, multi-block, graft, star-block, or miktoarm star copolymers.[79–82] However, these will not be discussed in detail given the scope of this review. Instead, we focus our attention on one type of self-assembled polymeric structures obtained using amphiphilic block copolymers, the so-called polymersomes. These structures are reminiscent of liposomes, in that they form similar mono- or multi-layered vesicular structures with an aqueous core, but they have proven to be more mechanically resilient.[83–85] Mechanical robustness makes them amenable for functionalization without affecting their stability, while at the same time they can serve as carriers for high loadings of drugs, macromolecules or nanoparticles.[86–88] In the past decade, polymersomes have been studied alongside with liposomes, LbL-capsules, and other biocontainers, such as viruses, as promising vehicles for drug delivery and enzyme immobilization.[10,89–92] Of recent date is their use for the construction of multi-compartmentalized architectures.

For the controlled generation of multi-compartment polymersomes, where possibly content and wall properties can be modulated, two different approaches have been reported. Non-spherical multi-compartment polymersomes fabricated *via* double emulsions were demonstrated by Weitz and co-workers.[93] Using a capillary microfluidic device, W/O/W double emulsions containing amphiphilic block copolymers in the oil phase, and a controlled number of dispersed internal aqueous droplets, were generated. By the slow evaporation of the organic phase the compartments were pulled together and the amphiphilic polymer, which initially stabilized the interfaces, now formed the polymersome membrane. The number of compartments could be defined by the number of water droplets in the organic phase, which on its turn could be tuned by controlling the flow rate of fluids in the microfluidic device. In this way, multi-compartment polymersomes were formed from poly(butyl acrylate)-*b*-poly(acrylic acid) and a biodegradable poly(ethylene glycol)-*b*-poly(lactic acid). For the latter, by employing a two-capillary microfluidic device the separate compartments could be loaded with different cargoes (Fig. 8).[94,95]

Chiu *et al.* presented a multi-compartmentalized system made of a copolymer of acrylic acid and distearin acrylate.[96] By dissolving the copolymer in the organic phase of a W/O/W double emulsion and subsequent evaporation of the solvent, polymer vesicles were obtained. The size of the vesicles could be tuned by changing the content of the polymer in the organic phase or by changing the organic phase solvent composition. Multi-compartmentalized vesicles were prepared by a second emulsification process, whereby the small vesicles were introduced into the dispersed water phase. Interestingly, at pH = 5.0 calcein added to the vesicle dispersion did

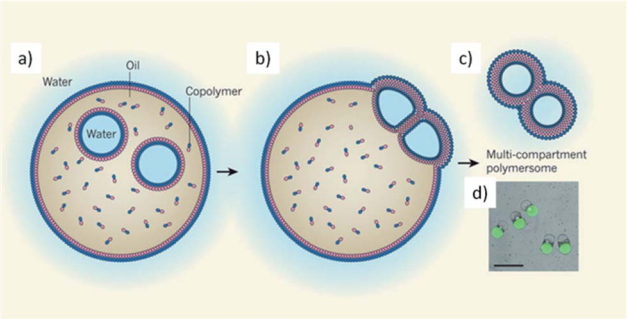

Fig. 8 (a–c) Formation process of multi-compartment polymersomes *via* double-emulsion templating. A water–oil–water emulsion is prepared containing the block copolymer in the oil phase. As the organic solvent is evaporated, the block copolymers come in close contact at the interface and subsequently form a bilayer. Complete evaporation of the organic phase yields the compartmentalized polymersomes. (d) Fluorescence microscopy image of the architectures with a fluorescent dextran selectively encapsulated in one compartment (scale bar is 200 μm).[94] Reprinted with permission from Macmillan Publishers Ltd.: Nature, copyright (2011).

not penetrate the vesicle membrane, however, at pH = 8.0 the calcein readily partitioned into the polymersome interior. By adjusting again the pH to the acidic range, the calcein was entrapped in the polymersome. This phenomenon was explained by the pH-induced ionization of the acrylic acid groups located in phase-separated, acrylic acid-rich patches of the membrane.

Multi-compartmentalized polymersomes formed by more straightforward self-assembly procedures were first reported for a block copolymer poly(ethylene oxide)$_{113}$-*b*-poly(*n*-butyl acrylate)$_{243}$, which, upon dissolution in dioxane and dialysis *versus* water, spontaneously assembled into multi-compartmentalized polymersomes.[97] In order to control the individual compartment membrane chemical structure and to selectively encapsulate species in each of the compartments we have recently constructed multi-compartmentalized polymersomes *via* a multistep self-assembly procedure.[98] To prepare two chemically distinct compartments we started with polymersomes made of poly[(2-methyloxazoline)-poly-(dimethylsiloxane)–poly-(2-methyloxazoline)] (PMOXA–PDMS–PMOXA, ABA), formed by film rehydration, which we then encapsulated into polymersomes made of polystyrene-*b*-poly[(L-isocyanoalanine(2-thiophen-3-yl-ethyl)amide)] (PS–PIAT) (Fig. 9a). Whereas the ABA block

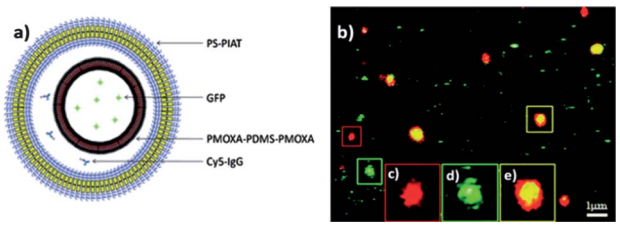

Fig. 9 (a) Scheme of multi-compartmentalized polymersomes; ABA forms the inner compartment and PS-PIAT forms the outer compartment. For abbreviations see text. (b) Scanning confocal fluorescence image of the polymersomes, where GFP (green, d) is encapsulated in the ABA-compartment and Cy-5 Ig-G (red, c) into the PS-PIAT compartment. Yellow colour results from the overlap of the ABA and PS-PIAT signals and indicates multi-compartmentalized polymersomes (e).[98]

copolymer forms a shell which is impermeable, PS–PIAT forms a semi-permeable membrane that allows the diffusion of small molecules. By entrapping GFP in the ABA sub-compartment and Cy-5-labeled IgG in the PS–PIAT compartment, selective encapsulation of biomacromolecules in individual compartments was clearly demonstrated (Fig. 9b–e). Of practical interest was the relatively high yield of multi-compartmentalized polymersomes as compared to the whole polymersome population, *i.e.*, 45% of polymersomes contained both the Ig-G and GFP.

Conclusions

In summary, we have highlighted a range of multi-compartmentalized soft matter systems including vesosomes, capsosomes, polymersomes, double emulsions, and a combination thereof. In this emerging and rapidly moving field of research, the number of approaches and materials that can be used to prepare multi-compartmentalized systems with aqueous compartments is steadily growing.

In comparison to single-compartment capsules, multi-compartment architectures offer fewer restrictions on the type and size of the co-encapsulated cargo. For instance, nanoparticles could be encapsulated in one compartment, while small molecules, drugs, or proteins could be accommodated in a different compartment, both within one self-standing capsule. A unique feature of multi-compartmentalized architectures is furthermore the possibility of differentiating compartments in terms of their response to external stimuli, such as, pH, redox state, osmolarity or temperature, to selectively trigger the release of substances from individual compartments. Connected with this is the possibility to combine the various available capsule systems into one platform, for instance, combining polymersomes and LbL-capsules, as was recently attempted by Caruso and co-workers.[99]

Important fabrication challenges remain however, especially those related to the control over the number and location of the compartments. Here, microfluidic approaches are showing their strength as they allow preparation of highly monodisperse samples in terms of size and number of compartments. For all fabrication methods, accurate determination of compartment occupancy, such as the number of active enzymes, for quantitative studies, is challenging and nontrivial.[100] Once such hurdles are overcome the field can move from mere demonstrations of multicompartment architectures to applications in nanoreactors, cell mimicry, and eventually drug delivery. For the latter it should be noted that 90% of the studies in this review describe particles with diameters well above one micrometre, which is expected to affect the particle's *in vivo* performance. In this context smaller vesosomes and multicompartmentalized polymersomes appear to hold some promise, but still remain to be investigated.[101,102] Importantly, the selected examples in this review have shown that the multicompartmentalized systems are promising architectures to control chemical reactions in time and space, and thus could possibly serve as simple models of the complex spatial and temporal organization of living cells.

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