# Chapter 4
# Multilayer Microcapsules with Tailored Structures and Properties as Delivery Carriers for Drugs and Growth Factors


**Weijun Tong and Changyou Gao**

**Abstract** Multilayer microcapsules fabricated via the layer-by-layer (LbL) assembly method with tailored structures and functionalities are promising candidates as carriers of drugs, growth factors, and other bioactive agents in biomedicine. The capsules loaded with growth factors also can be integrated with scaffolds to form bioactive scaffolds for regenerative medicine. This chapter discusses first the recent progress of the fabrication of LbL microcapsules including manipulation of their properties by chemical cross-linking, microcapsules based on new driving forces, microcapsules with subcompartments, and microcapsules which can transform their shape. Their potential applications as drug delivery carriers, emphasizing on the new encapsulation methods, the fabrication of nanoparticles and nanocapsules, the triggered release of encapsulated substances, and the deformation and recovery behavior of microcapsules, are demonstrated. Finally, the loading of growth factors into multilayer capsules and their incorporation into scaffolds are introduced.

**Keywords** Layer-by-layer · Polyelectrolytes · Microcapsules · Drug carriers · Growth factors · Loading and release · Scaffolds

## 4.1 Introductions

Hollow capsules are of great interest due to their potential applications and fundamental importance as new colloidal structures in areas such as medicine, catalysis, cosmetics, as well as biotechnology [1]. One of the promising methods which can fabricate hollow capsules with tailored structures and functions is the layer-by-layer

W. Tong (✉) · C. Gao
MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou, China
e-mail: tongwj@zju.edu.cn; cygao@zju.edu.cn

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2023

C. Gao (ed.), *Polymeric Biomaterials for Tissue Regeneration*,
https://doi.org/10.1007/978-981-99-6948-7_4

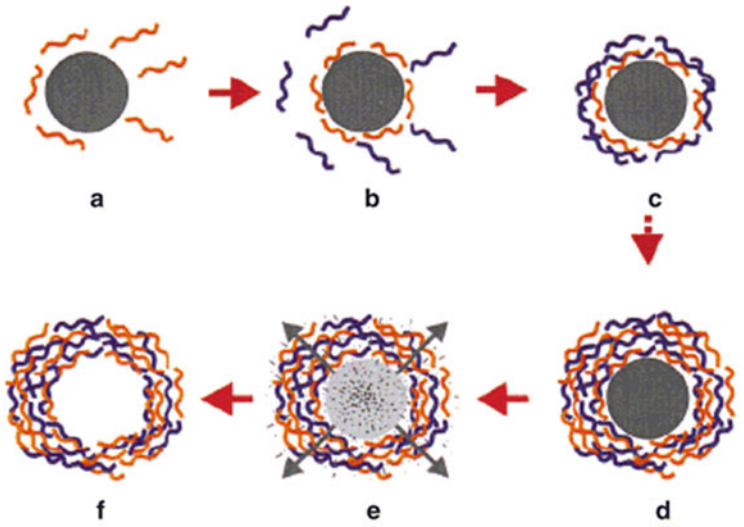


**Fig. 4.1** The initial steps (**a-d**) involve step-wise film formation by repeated exposure of the colloids to polyelectrolytes of alternating charge. After the desired number of polyelectrolyte layers are deposited, the core is removed (**e**) to obtain a suspension of polyelectrolyte hollow shells (**f**).

(LbL) assembly [2, 3] of multilayer films onto colloidal particles, followed by core removal (Fig. 4.1) [4, 5]. Through this method, the capsules with well-controlled size and shape, finely tuned wall thickness, and variable wall compositions have been obtained [6]. The LbL microcapsules with integrated multifunctionality have high capacity for loading of a wide range of substances and sensitive response to diverse stimuli and thus are highly attractive for the biorelated applications [7–9]. At the beginning, the studies of LbL multilayer capsules are mainly focused on the fabrication and basic physicochemical properties [6]. However, the past decade has witnessed a rapid increase of researches concerning their functionalization and applications, particularly in the biomedical fields such as drug delivery [10]. In this chapter, we first focus on the recent progress of the LbL microcapsules in our lab with respect to manipulation of their properties by chemical cross-linking and fabrication of the microcapsules based on new driving forces, the capsules with subcompartments, and the capsules which can transform their shape. Then, we will discuss the potential applications of LbL capsules as drug delivery carriers, emphasizing on the new encapsulation methods developed in our lab, the surface modification of smaller particles, as well as the deformation and recovery behavior of microcapsules passing through a model capillary vessel. Finally, the loading of growth factors into multilayer capsules and their incorporation into scaffolds are discussed.

## 4.2 Multilayer Microcapsules with Tailored Structures, Properties, and Functions

### *4.2.1 Cross-Linking to Tailor the Properties of Microcapsules*

The electrostatic interaction is first used for the fabrication of LbL microcapsules. Although it is generally strong enough to hold the integrity of the microcapsules, in some cases cross-linking is still necessary for the capsules to survive through harsh conditions such as high ionic strength, extreme pH, and strong polar organic solvent [11, 12]. Moreover, cross-linking also can effectively manipulate the permeability and mechanical strength of the capsules [11, 13]. For the multilayer films and capsules based on hydrogen bonding, further stabilization is also required for biomedical applications since most of them will be disassembled at physiological conditions [14, 15]. Many methods have been developed to cross-link the multilayer films and capsules, such as carbodiimide chemistry [16–18], UV irradiation [13], as well as thermal cross-linking [19]. More recently, disulfide [20, 21] and click chemistry [22–28] have also been proved effective for cross-linking the hydrogen-bonded multilayers and microcapsules.

The above mentioned techniques are exclusively based on a reaction between the functional groups of the two components in the multilayers. We demonstrated that the multilayer microcapsules assembled from poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) could be considerably stabilized by cross-linking of only the PAH component with glutaraldehyde (GA) [29]. After cross-linking, the capsule wall was apparently thicker and with higher folds. The capsules were quite stable in 0.1 M NaOH even after 24 h. The elasticity modulus (680 MPa) of the capsule walls was doubled compared with that of the control. Furthermore, the permeability of the capsule walls was also greatly reduced after cross-linking. We further applied this method to the poly(ethylenimine) (PEI) and poly(acrylic acid) (PAA) weak polyelectrolyte microcapsules [30]. The cross-linked microcapsules can maintain their macroscopic topology at extreme low or high pH while reorganizing their localized microstructure to enable selective permeation or rejection of macromolecules at lower (<pH 4) and higher pH (>pH 6), respectively. Thus, it is possible to produce capsules that are dual-pH responsive and stable over a broad pH range.

### *4.2.2 Capsules Directly Assembled Based on Nonelectrostatic Interactions*

Different driving forces can endow the microcapsules with different physicochemical structures, stimuli response, and thereby their functionality and applicability. At the beginning, the electrostatic interaction was the first driving forces for the LbL assembly [4, 5, 31]; thus, the building blocks were mainly charged species. Then hydrogen bonding has been employed for the assembly of microcapsules. Recently,

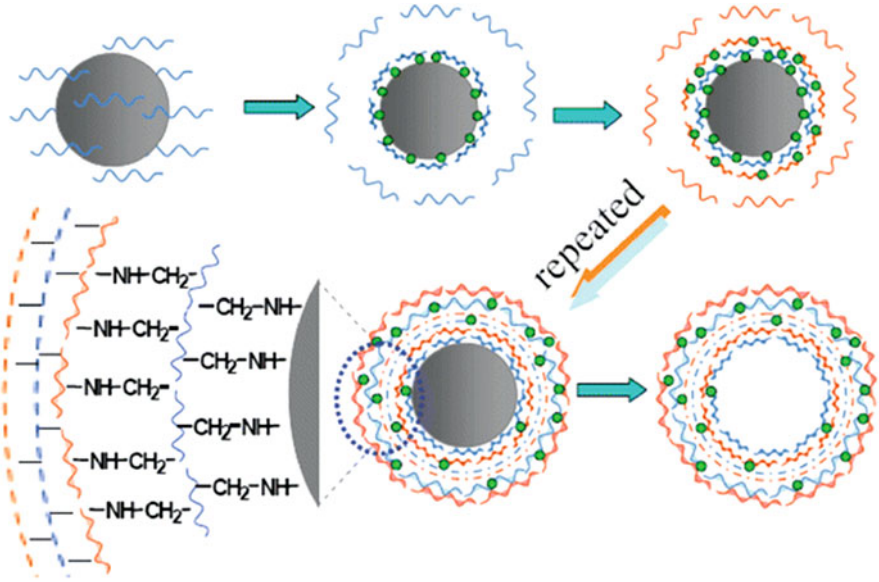


**Fig. 4.2** Schematic illustration of the process of direct covalent LbL assembly on a silica particle and fabrication of a hollow capsule by etching out the template core. The *blue lines* represent PGMA, the *red lines* represent PAH, and the *green dots* represent the covalent linkage between layers. (Reprinted with permission from Ref. [33]. Copyright 2007 by Wiley-VCH)

the multilayer hollow microcapsules based on other nonelectrostatic interactions such as covalent bonding, host–guest interaction, and bio-specific interactions have been fabricated, which show unique properties.

Covalent LbL-assembled microcapsule is stable enough to withstand the long-time etching of strong polar organic solvent [32]. We recently fabricated a new structure of microcapsules with high modulus and high stability through the covalent LbL assembly (Fig. 4.2) [33]. Aminosilanized $SiO_2$ microparticles were used as templates. Poly(glycidyl methacrylate) (PGMA) and PAH were alternately immobilized onto the particle surfaces through a coupling reaction between the epoxides and the amines. Thus, a highly cross-linked structure was produced in this process. The templates were removed by HF etching, resulting in hollow microcapsules. The microcapsules are stable in extreme pHs and elevated temperature. Using the method of osmotic-induced invagination [34, 35], the elastic modulus of the microcapsule walls without any treatments was found to be as high as 910 MPa, which is quite stable even under acid and base treatment.

The reaction between amine and aldehyde is fast and efficient in aqueous solution at room temperature; based on this reaction, single polyelectrolyte component multilayers and microcapsules can be fabricated through direct covalent assembly of PAH with GA [36]. The structure and the cutoff molecular weight of the capsule walls are dependent on the molecular weight of used polymers [37]. This method can be applied not only on biomacromolecules with amine groups such as polypeptides

and proteins but also on polysaccharides, because GA also can readily react with hydroxyl groups at very mild conditions.

However, the main drawback of the abovementioned methods is that the obtained capsules may largely lose their stimuli-responsive properties due to the uncontrollable covalent reactions [36, 37]. One solution to this problem is to carefully control the content of reactive groups in polymer chains, leading to a controllable reaction degree and a number of functional groups [38]. Bovine serum albumin (BSA) is a kind of biocompatible and biodegradable natural protein. It has a high content of aspartic and glutamic acids, lysine, and arginine [39, 40]. Only the amine groups of lysine can be cross-linked by GA [41], while the other amino acids with free carboxylic groups still exist, which can induce the pH response of the resultant capsules. We recently demonstrated that [42] BSA hollow capsules could be obtained by covalent assembly of BSA and GA on a template followed by core removal. The capsules possessed reversible pH-responsive permeability, which can be used to encapsulate macromolecules.

Host–guest interaction is another type of driving forces frequently employed in supramolecular chemistry. It is known that the host–guest interaction is readily mediated by the host and guest molecules with respect to their matching degree and concentration. If charge interaction is further introduced, multiresponsive microcapsules can thus be expected. According to this design, multilayer microcapsules were fabricated by using the interaction between β-cyclodextrin (β-CD) and ferrocene grafted to a weak polyelectrolyte PAH, which can further introduce charge interaction into the capsule walls (Fig. 4.3) [43]. The microcapsules that consist of PAH-g-β-CD and PAH-g-ferrocene indeed show multiresponsiveness to environmental stimuli. For example, they swell and shrink at low and high pH, respectively. Incubation in a salt or β-CD solution can also mediate their swelling and shrinking behaviors. With these smart features, the microcapsules can serve as reservoirs for drugs, DNAs, enzymes, and so on.

The specific interactions between complementary DNA bases are stable enough under physiological conditions in nature, which can be used for the assembly of multilayer films and capsules [44]. Moreover, carbohydrate–protein interaction, which is a combination of multiple hydrogen bonding and hydrophobic interactions and participates in a wide variety of biological events [45–47], is also quite stable at physiological conditions. Therefore, this interaction can be used to assemble microcapsules which simultaneously possess good stability and responsiveness to external stimulus due to its noncovalent nature. Concanavalin A (Con A) can specifically bind to polysaccharides such as dextran and glycogen [48–50]; thus, it can be utilized to fabricate thin films with them through lectin–carbohydrate interactions [51–53]. These films can respond to glucose [50, 53]. Recently, the hollow capsules assembled by Con A and glycogen through LbL method were also obtained. They are stable at physiological pH range due to the relatively strong multiple hydrogen bonding but still can respond to glucose [54]. The sequential multilayer film growth proceeds successfully on both planar and curved substrates when the Con A molecules adopt confirmation of tetramers or more complicated aggregates. The obtained capsules show layer-number-dependent shell shrinkage, distortion, and

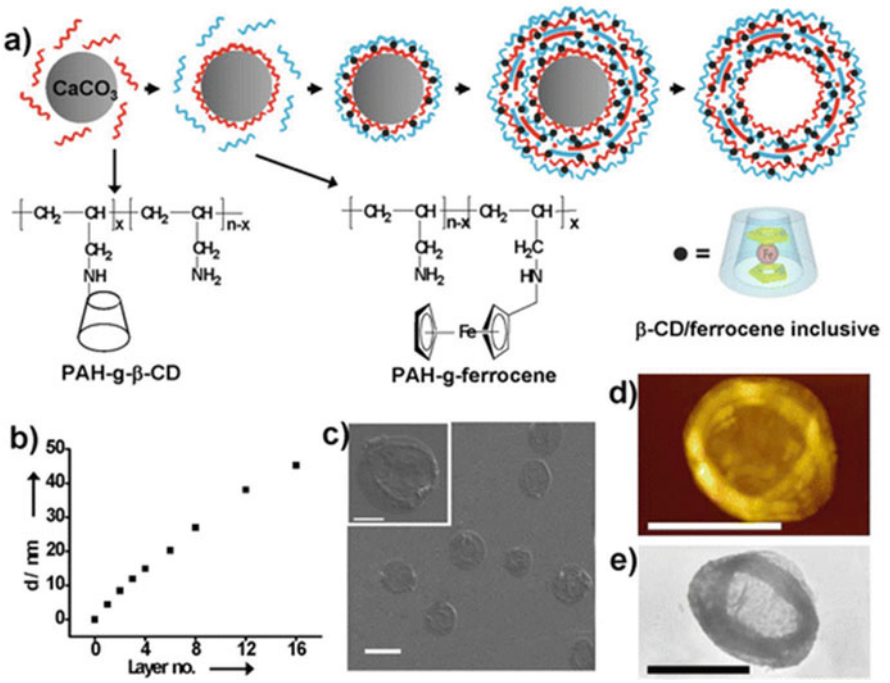


**Fig. 4.3** Fabrication process and structure characterization of host–guest microcapsules. (**a**) LbL assembly of same polyelectrolyte on carbonate particles to obtain hollow microcapsules using host–guest interaction. The chemical structure of PAH-g-β-CD, PAH-g-ferrocene, and β-CD/ferrocene inclusive are shown in the second row. (**b**) The thickness of the PAH-g-β-CD/PAH-g-ferrocene multilayers assembled on silicon wafer as a function of layer no. (**c**) SEM, (**d**) SFM, and (**e**) TEM images of the prepared (PAH-g-β-CD/PAH-g-ferrocene)3 microcapsules, respectively; bar is 5 μm. Inset in (**c**) is a higher magnification image of one capsule; bar is 2 μm. (Reprinted with permission from Ref. [43]. Copyright 2008, American Chemical Society)

densification. The capsules are stable in a pH range of 6–9 and show specific responses to glucose, mannose, fructose, and dextran. Triggered by these stimuli, the preloaded cargoes in the capsules can be released.

### *4.2.3 Capsules with Subcompartments*

The multicompartmental micro- and nanostructures can be loaded with multiple cargoes and mimic the structure of cells; thus, they have received tremendous attention recently [55–58]. Hollow capsules with subcompartments are ideal models which resemble the structure of cells. By combining of other techniques, diverse LbL capsules with different subcompartments have been obtained [59]. The

subcompartments can be incorporated through two different ways: wall decoration and interior loading.

Kreft et al. first reported the LbL microcapsules with a shell-in-shell structure for integrated and spatially confined enzymatic reactions [60]. De Geest et al. reported the assembly of multilayers on big hydrogel particles (hundreds of microns) in which tens of hollow LbL microcapsules or microparticles are loaded [57, 61]. Recently, Caruso group incorporated intact liposomes into LbL capsule walls or inside capsules to prepare "capsosomes," which can be then employed as enzymatic reactors and delivery vehicles for hydrophobic cargoes [62–66].

Alternatively, polymeric micelles also can be incorporated into the walls or interiors of LbL capsules as the subcompartments. The micelles possess advantages of sustained release of hydrophobic substances. In particular, polymeric micelles possess a unique core/shell structure and relatively good stability [67]. Thus, the micelle-incorporated microcapsules combine the advantages of both micro- and nanostructures. Polymeric micelles can be loaded into the shell through alternating assembly of poly(styrene-*b*-acrylic acid) (PS-*b*-PAA) micelles and oppositely charged polyelectrolyte on templates [68]. After core removal, the as-prepared microcapsules show extraordinary stability in concentrated HCl (37%) and 0.1 M NaOH. This extraordinary stability against highly acidic or alkaline conditions is possibly due to the hydrophobic interaction between PS cores of the micelles and hydrogen bonding of the PAA chains in adjacent layers and PAH chains. The incorporation of polymeric micelles in LbL capsule interiors has been presented by Li et al. [69] as well as Tong et al. [70]. In the latter method [70], LbL assembly was conducted on $CaCO_3$ microparticles predoped with PS-*b*-PAA micelles, resulting in encapsulation of micelles after core removal. The micelles inside the capsules can form a chain and network-like structure with more micelles near the capsule walls. Hydrophobic drugs as such can be loaded into the hydrophobic cores of micelles, while the negatively charged PAA corona of the micelles can result in spontaneous deposition [71–75] of water-soluble and positively charged drugs. The apparent concentrations of hydrophobic and water soluble are much higher than that of the feeding values. Therefore, capsules with this synergetic feature show their great promise in loading of drugs with different physicochemical properties.

### *4.2.4 Shape Transformation of Capsules*

The smart capsule systems are of high attraction due to their ability to respond to the alteration of environment conditions. LbL-assembled capsules can change their structure and properties intelligently in response to various stimuli. But most of the intelligence of the hollow structures results from controllable swelling and shrinking, accompanying with permeability change [76]. Less concern is paid to shape transformation of the hollow structures, which is only observed in vesicles and hollow silica spheres previously [77–83]. Recently, single-component microcapsules were fabricated in our lab by an in situ reaction of reactive

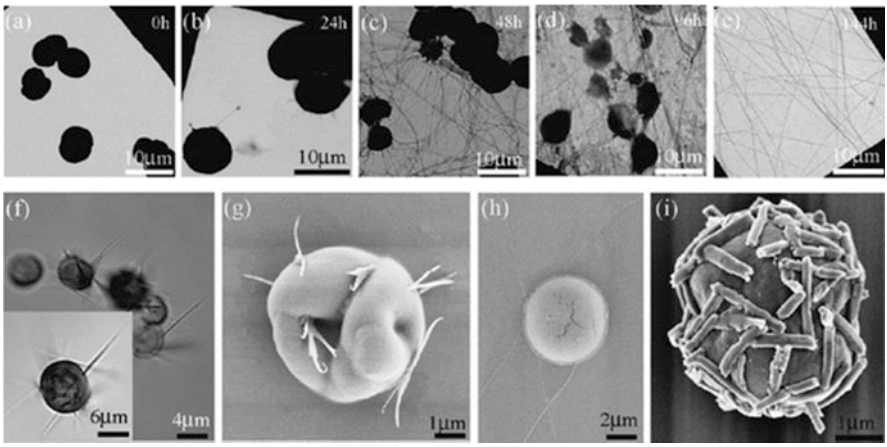


**Fig. 4.4** TEM images showing the process of nanotube protruding from the PAH-Py microcapsules incubated in pH 0 HCl for 0(**a**), 24(**b**), 48(**c**), 96(**d**), and 144 h(**e**), respectively. (**f**) Optical images (inset, a higher magnification) showing the protruded nanotubes from the PAH-Py microcapsules incubated in pH 0 HCl for 30 h. SEM images of a microcapsule with nanotubes after treatment in pH 0 HCl for (**g**) 30 h and (**h**) 72 h, respectively. (**i**) SEM image of a microcapsule with nanorods after treatment in pH 2 HCl for 1 h. (Reprinted with permission from Ref. [85]. Copyright 2011, American Chemical Society)

hydrophobic low-molecular-weight molecules with corresponding PE-doped $CaCO_3$ microparticles, followed by core removal [84, 85].

The first example is the capsules made of ferrocenecarboxaldehyde (Fc-CHO) and PAH-doped $CaCO_3$ microparticles [84]. This single-component microcapsule is stabilized by hydrophobic aggregation of Fc moieties. Due to the redox properties of Fc, the PAH-Fc microcapsules can reversibly swell and shrink in response to oxidation and reduction. At the same time, the permeability also can be changed reversibly. PAH-pyrene (Py) microcapsules also can be fabricated through the reaction of pyrenecarboxaldehyde with the doped PAH [85]. When this kind of capsules is incubated in acidic solution, one-dimensional nanotubes (1D-NTs) or nanorods (1D-NRs) are protruded from the microcapsules. The 1D-NTs keep growing with incubation time and eventually form a network. Meanwhile, the microcapsules are degraded gradually and disappear completely after 144 h (Fig. 4.4). The micelles assembled from PAH-Py polymers treated at similar conditions also can be transformed into one-dimensional structures. The one-dimensional nanotubes are formed by 1-pyrenecarboxaldehyde with ordered π–π stacking and exhibit a helical structure and anisotropic property. The final nanostructures are determined by the different hydrolysis rate of Schiff base at different pH values. The linear PAH also can guide the building-up process especially for the nanotubes. Through this mechanism, hollow capsules budded with nanotubes or nanorods mimicking the cellular protrusion of filopodia are successfully fabricated by controlling the incubation time in solutions with different pH (Fig. 4.4).

By chemical cross-linking and surface modification, 1D-NR growth state from the PAH-Py microcapsules can be well controlled [86]. The 1D-NRs also can grow in the LbL-assembled capsules in a controllable manner [87]. For this purpose, PSS/PAH multilayers were assembled on the surface of $CaCO_3$ (PAH-Py) microparticles, yielding PAH-Py and (PSS/PAH)n double-shell capsules. By incubation of the obtained capsules in pH 2 solution, the 1D-NRs grow within the PSS/PAH multilayer capsules in three dimensions. The fluorescence emission intensity of Py NRs inside the capsules can be tuned by a charge-transfer pair. This novel composite structure with PAH-Py NRs inside PE multilayer microcapsules provides a creative strategy for in situ nanomaterials fabrication, illuminating the trend for controllable properties and functions of smart nanodevices. The modulation of the protrusion of NRs also can be achieved by addition of small molecules such as 1-pyrenesulfonic acid sodium salt ($PySO_3Na$) [88], demonstrating the tunable properties of such kind of nanostructures.

Inspired by the above results, PAH-Py NRs consisting of a Py-CHO core and PAH shell can be prepared by surface grafting of PAH onto Py-CHO NRs [89]. After coated with PAH, The NRs become more curved and flexible as a result of partial loss of Py-CHO from the NRs. The PAH-Py NRs with a hydrophilic and charged PAH layer can be suspended stably in water for at least 3 months. Because of the charge attraction and coordination effect of amino groups, Au NPs can be either adsorbed or in situ synthesized on the PAH-Py NR surface. The initial fluorescence emission of Py is largely remained due to the excellent isolation effect of PAH, which avoids direct contact between Py and the Au NPs. Using the similar process, other hybrid organic–inorganic functional nanomaterials with controlled physicochemical structures can be synthesized, such as tetraphenylethylene (TPE) nanoparticles [90]. TPE-substituted poly(allylamine hydrochloride) (PAH-g-TPE) was synthesized by a Schiff base reaction between PAH and TPE-CHO. The PAH-g-TPE forms micelles in water at pH 6, which are further transformed into pure TPE-CHO nanoparticles (NPs) with a diameter of ~300 nm after incubation in a solution of low pH value. In contrast, only amorphous precipitates are obtained when TPE-CHO methanol solution is incubated in water. The aggregation-induced emission feature of the TPE molecule is completely retained in the TPE NPs, which can be internalized into cells and show blue fluorescence. Formation mechanism of the TPE NPs is proposed by taking into account the guidance effect of linear and charged PAH molecules and the propeller-stacking manner between the TPE-CHO molecules.

## 4.3 Microcapsules as Drug Delivery Carriers

The LbL-assembled capsules with tailored structures and functions are versatile platforms for encapsulation, storage, and delivery of diverse substances. Thus, the LbL-assembled capsules are ideal advanced drug carriers for the delivery of diverse drugs and growth factors. There are already several excellent reviews which

summarized the very recent progress in this field [21, 91–99]. The following sections will mainly focus on the recent advances in this field.

### 4.3.1 Controlled Loading of Low-Molecular-Weight Drugs

Many applications of the multilayer capsules must face a challenge of efficient loading of the desired substances. This is particularly difficult for loading of low-molecular-weight and water-soluble substances because small molecules can freely diffuse through the capsule walls [100]. We developed the "spontaneous deposition" method, which is based on a mechanism of high affinity of the preloaded substances in the capsules with the cargoes that will be loaded. Gao et al. first found that positively charged molecules such as dextran labeled with tetramethylrhodamine isothiocyanate (TRITC-dextran) could deposit into the aged "hollow" microcapsules templated on melamine formaldehyde (MF) particles with a large amount [71]. The strong fluorescence emitted from the interior of capsules could prove the existence of considerable high concentration of dextran in the capsule interiors (the so-called spontaneous deposition) (Fig. 4.5a). Many other water-soluble substances with positive charges such as polyelectrolytes [71], proteins [71], enzymes [72], and low-molecular-weight dyes and anticancer drugs (Fig. 4.5b, c) [73] can be spontaneously deposited with a large quantity. Moreover, the deposition still occurs even if the molecules have very few positive charges such as the TRITC-dextran (Fig. 4.5a), which gets positive charges from a few pendent TRITC groups. The driving force for this phenomenon is the electrostatic interaction between the negatively charged complex (PSS/MF) within the capsule interior and the loaded molecules. The PSS/MF complex is formed by the dissociated PSS from the very initial layer and the positively charged MF degradation product.

The spontaneously deposited low-molecular-weight drugs in the LbL microcapsules templated on MF colloidal particles can be released in a sustained manner [73, 74]. The amount of the loaded drugs can be controlled through changing the feeding concentration of the drugs, temperature, as well as salt concentration. This tailorable deposition behavior is crucial for control release applications. The loaded drugs can be released in a sustained manner. The release profile can be tuned by changing the interaction between the drugs and the PSS/MF complex. The presence of anticancer drug-loaded capsules can effectively kill HL-60 cells, a kind of human leukemia cell [73].

For better control of the spontaneous deposition property, the capsules can be preloaded with charged polyelectrolyte using a polyelectrolyte-doped template [75, 101–104]. At higher drug feeding concentration and higher salt concentration, large amount of daunorubicin (DNR) and DOX can be loaded [75, 103]. The drug concentration within the microcapsules is hundreds of times higher than the feeding concentration. The drug can be released from the capsules through a diffusion-controlled release mechanism at the initial stage (4 h). The in vitro experiments demonstrate that the encapsulated drug can effectively induce the apoptosis of

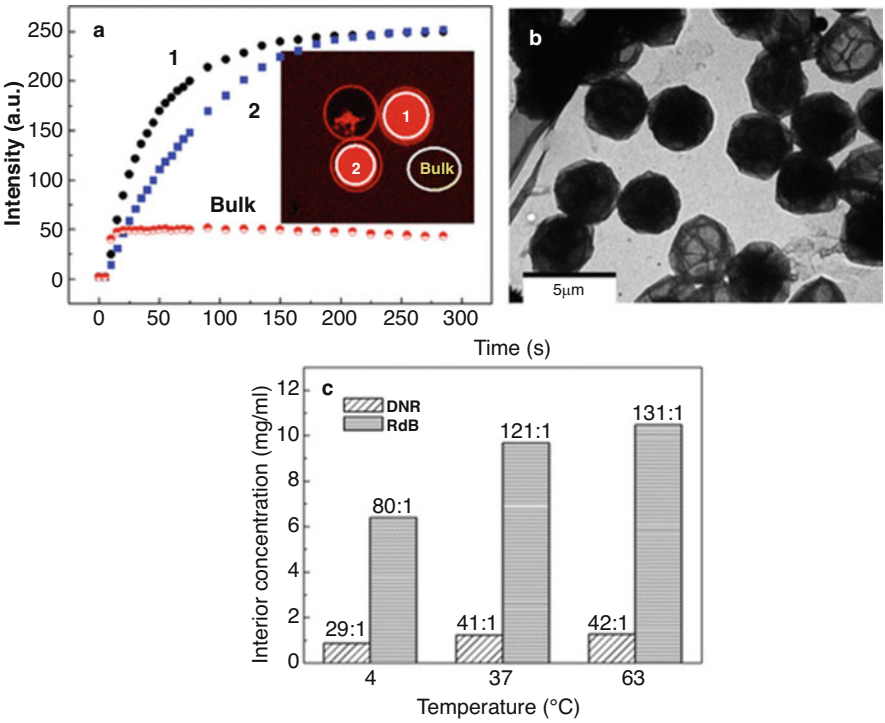


**Fig. 4.5** (**a**) Fluorescence intensity averaged from inside the *circles* as a function of incubation time. TRITC-dextran ($M_w$ ~65 kDa) and preformed MF-(PSS/PAH)5 capsules were used. (**b**) TEM images of daunorubicin (DNR) deposited MF-(PSS/PAH)5 capsules. (**c**) DNR and rhodamine B (*RdB*) concentrations in the capsule interior as a function of temperature. MF-(PSS/PAH)4 (PSS/PDADMAC)5 capsules were used for DNR with a feeding concentration of 30 mg/ml and MF-(PSS/PAH)5 capsules for RdB, 80 mg/ml. The *numbers* in the figure represent the concentration ratios of capsule interior and bulk. *PDADMAC* poly(diallyldimethylammonium chloride). (Reprinted with permission from Ref. [73]. Copyright 2005 by Wiley-VCH)

HepG2 tumor cells. The encapsulated DOX also has better efficacy than that of the free drug in terms of tumor inhibition in a 4-week in vivo culture period [104].

Nonetheless, challenge is still remained to better maintain the drugs inside the capsules and then control their release profile. Previous studies demonstrate that poly (diallyldimethylammonium chloride) (PDADMAC)/PSS capsules with PSS as the outmost layer can shrink dramatically at elevated temperature [105, 106], resulting in a thicker and denser capsule wall. So dextran (Mw from 10 to 70 kDa) can be effectively encapsulated with a slightly higher concentration than the feeding value [107]. The loading of water-soluble small molecular drugs also can be achieved using this method [108]. In our recent work [109], spontaneous deposition and heat-induced shrinkage were combined to fabricate a drug carrier system, showing a high drug loading efficiency and more controllable release profile. Through this strategy,

photosensitizers also can be encapsulated, and most of them are stably retained for a long time and protected by capsule wall against reductive enzyme [110].

Encapsulation of low-molecular-weight drugs through the attractions between the drugs and the preloaded substances in the multilayer capsules also can be applied to different systems via specific interactions besides the electrostatic interactions. For example, Sukhorukov and coworkers reported that low-molecular-weight doxycycline could be encapsulated in LbL microcapsules via attraction to dextran sulfate (DS) in the microcapsule core because doxycycline molecules can penetrate the shells and react with DS to form a complex within the microcapsule [111]. The specific and sustained activity of doxycycline is well maintained. If the capsules are further coated with a lipid layer, the release and sustained activity of encapsulated drug can be enhanced because its leakage is greatly prohibited. This method could provide a long-term delivery system of low-molecular-weight drugs from multilayer capsules. Kharlampieva and coworkers reported a facile method for the efficient encapsulation of a wide range of hydrophilic substances with molecular weight less than 1000 and different charges [112]. The capsules are fabricated via LbL assembly of poly(methacrylic acid) (PMAA) and poly(*N*-vinylpyrrolidone) (PVPON) on silica templates. After cross-linking of the PMAA multilayers, PVOPN is entrapped in the shell to form an interpenetrated network. The capsules show reversible variation in diameter upon pH changes, and thus encapsulation of low-molecular-weight substances could be achieved at pH = 7.5 followed by sealing the capsule wall with high-molecular-weight DS at pH = 5.5. It is interesting that the negatively charged molecules can be entrapped within the capsule cavity, while the positively charged molecules are encapsulated within the negatively charged capsule shell. This approach allows the simultaneous loading of different low-molecular-weight substances at different positions in the capsules; thus, the capsules can deliver multiple drugs. Furthermore, the pH-responsiveness of the capsule also can achieve the controlled release of the drugs.

Normal LbL assembled microcapsules fail to encapsulate low-molecular-weight drugs because of the semipermeable nature of the shell. Sukhorukov and coworkers reported a new method to fabricate poly-L-arginine hydrochloride (PARG)/DS/silica ($SiO_2$) composite capsules [113]. The inorganic $SiO_2$ layer is in situ formed to seal the capsules; thus, low-molecular-weight drug can be effectively encapsulated inside. The cell experiments demonstrate that the PARG/DS/silica capsules can be degraded into fragments and the release of encapsulated molecules is achieved in a relatively short time (2 h), while the capsules with a similar structure using nonbiodegradable polyelectrolytes remain intact even after 3 days.

### *4.3.2 LbL Assembly on Nanoparticles*

Most of the LbL capsules have a diameter of a few micrometers, which are too large for intravenous injection. One possible solution is to assemble multilayers on particles with a smaller size. De Koker et al. have reviewed the progress of LbL

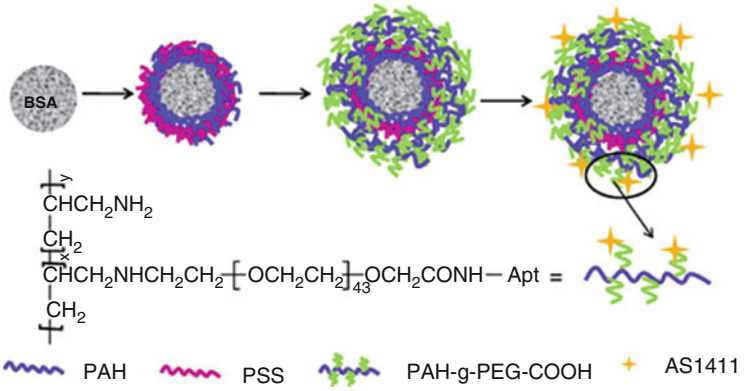


**Fig. 4.6** Schematic illustration to show the preparation process of BSA nanoparticles coated with PAH/PSS multilayers and coupled with aptamer AS1411. (Reprinted with permission from Ref. [119]. Copyright 2012 by RSC)

assembly on ultrasmall (sub-100 nm) particles, which are mainly gold NPs [98]. In our lab, surface modification of biodegradable and nontoxic polyester, poly(lactide-*co*-glycolide) (PLGA) NPs with a size around 200–300 nm using LbL assembly have been extensively investigated. PLGA is one of the commonly used polymers for drug delivery [114, 115]. These particles with such a size can be injected into the blood vessel and may accumulate in cancerous tissues through the well-known enhanced permeability and retention (EPR) effect. Modification of NP surface with targeting molecules can enhance the drug concentration in the targeted organs or tissues and reduce the dosage and toxic side effects. In order to effectively immobilize the ligands, the NPs should possess enough number of active groups and are stable enough for following reactions. The LbL assembly can endow the NPs with uniform surface charge density, numerous active groups, and excellent stability in various mediums. For example, PAA/PEI and chitosan (CS)/alginate (CS/ALG) can be used to build multilayers on the PLGA NPs for further immobilization of PEG and folic acid aiming at long-time circulation and targeting [116, 117]. The surface charge and the thickness of the assembled multilayers can greatly influence the release profile of loaded dyes [118]. The surface with negative charges or PEG also can reduce protein adsorption, whereas surface modified with folic acid can enhance the NP uptake by human hepatoma cells.

The multilayers also can be assembled on the NPs before drug loading, and then different drugs can be loaded into the preformed multilayer-coated particles for different applications. In our recent work [119], BSA NPs with a size about 200 nm were coated with PAH/PSS multilayers, onto which a layer of PAH-*g*-PEG-COOH was further adsorbed. By carbodiimide chemistry, aptamer-AS1411 molecules were immobilized (Fig. 4.6). Aptamer-AS1411 can target to overexpressed nucleolin on cancer cell membrane [120, 121]. The PEGylated multilayer-coated BSA NPs have enhanced colloidal stability even in serum-containing medium [122]. DOX can be effectively loaded into the preformed BSA

NPs through electrostatic interaction between negative charges in BSA and positive charges in DOX. The encapsulation efficiency (98.6%) and loading percentage (9%) are both very high. The loaded drugs can be released faster at pH 5.5 than at pH 7.4. In vitro cell culture demonstrates that the as-prepared BSA NPs can specifically bind to liver cancer cells, leading to higher cellular uptake and cytotoxicity.

Except of the solid nanosize templates, the multilayers also can be assembled on emulsion droplets. For example, Szczepanowicz and coworkers reported the preparation of nanoparticles via direct coating on the emulsion droplets with polyelectrolyte multilayer shells [123]. The oil cores containing paclitaxel can be first stabilized by docusate sodium salt/poly-L-lysine surface complex (AOT/PLL) and are further coated in multilayers formed by the LbL assembly of poly-L-glutamic acid (PGA) and PLL up to five or six layers. Their surfaces can be further modified through the assembly of the pegylated polyelectrolyte, resulting in prolonged persistence of the nanocarriers in the circulation. The obtained nanoparticles can be stabilized in cell culture medium and the encapsulated hydrophobic anticancer drug can be released to kill cancer cells. Due to the dynamic nature of the emulsion droplets and the surfactants, the emulsion-based templates generally have relatively limited colloidal stability and broad size distribution. Cheng and coworkers used crystallized miniemulsion droplets as templates for the fabrication of multilayer nanocapsules [124]. Compared with normal emulsions, the miniemulsions are kinetically more stable and the crystallization of the inside oil phase can result in their higher colloidal stability due to the surface-anchored surfactant molecules. Polyelectrolytes with opposite charges can be alternatively assembled on this kind of templates and the crystallized oil phase can be dissolved by using proper organic solvent, resulting in hollow nanocapsules with well-defined structures and controlled size.

In order to obtain hollow nanocapsules, the nanosize templates should be removed under mild conditions. For example, Cui and coworkers reported the LbL assembly of multilayers on $Cu_2O$ nanoparticles [125]. $Cu_2O$ particles are cheap and can be easily fabricated. After the assembly of multilayers, they can be removed in $Na_2S_2O_3$ solution at neutral pH. During this process, no toxic reagents are needed. Furthermore, $Cu_2O$ nanoparticles with tunable morphologies and sizes can be synthesized via tuning the preparation conditions. Thus, the nanocapsules with different shapes could be obtained by using $Cu_2O$ nanoparticles with different shapes as templates. In addition, the capsule shape significantly influences their interaction with cells. The association of cubic capsules to HeLa cells are significantly increased compared with their tetradecahedral and spherical counterparts. This kind of nanocapsules with tunable morphologies can provide an ideal model system for the investigation of bio–nano interactions.

Although the LbL method have been proved to be a versatile approach for the preparation of multilayer nanocapsules with engineered structure and properties by using diverse building blocks and templates, the fabrication of nanocapsules through this method at large scale is still challenging and time-consuming. The traditional LbL assembly process needs several washing and centrifugation steps before each assembly of polyelectrolyte layer, resulting in a very long preparation time and accumulated particle loss after multiple centrifugation steps. In order to solve this

problem, Elizarova and coworkers reported a continuous method to the preparation of nanosize multilayer capsules using calcium phosphate nanoparticles as templates [126]. This method uses a tubular flow type reactor which can fabricate tens of milligrams of nanocapsules in 1 h. In the fabrication process, the template nanoparticles and polyelectrolyte solution are first mixed in the tubing to form the first layer on the templates. Then the modified nanoparticles pass into the next segment of tubing, where they meet the second polyelectrolyte with opposite charges. After mixing, the second layer is assembled on the particles. These steps can be continuously repeated until the required number of layers is assembled. The key point for the successful fabrication of nanocapsules via this method is to avoid the presence of any excess polyelectrolyte in the tubing, otherwise severe coagulation may happen. Thus, the careful control of the added amount of polyelectrolyte in the tubing is critical. The results demonstrate that slightly under dosing the amount of added polyelectrolyte can ensure the negligible free polyelectrolyte in solution. During the alternative assembly steps, the typical charge reversal can be observed and the relatively strong surface charges can make the particles stable during the fabrication process. Finally, after the required number of layers are assembled, the calcium phosphate templates can be facilely removed by incubation in mild acidic solution to obtain the hollow nanocapsules.

### *4.3.3 Capsules Squeeze Through a Confined Capillary*

Compared with their nanometer-sized counterparts, the LbL microcapsules can be fabricated in an easier way [127]. However, microcapsules are difficult for intravenous injection. But in human blood, the circulation cells have a size of several microns. One example is red blood cell (RBC), which has extreme reversible deformability under physiological flow, so that it can easily pass through the smallest blood capillary vessel (~3 μm). One can imagine that if the capsules have proper shape and flexibility, they may easily squeeze through narrow capillary as natural RBC. This kind of capsules may have great potential applications as drug carriers. The deformability of polymeric microparticles (mainly hydrogel microparticles) with different shapes and sizes through a narrow constriction has been studied under flow conditions. For example, RBC-mimicking particles, which are flexible enough to flow through narrow glass capillaries and able to recover to discoidal shape, have been successfully fabricated [128]. Hayashi et al. demonstrated that 3.5 μm biconcave disk-shaped particles fabricated by electrospraying of cellulose derivative polymers can maintain RBC-like shape after filtrated through a membrane with a pore size of 1 μm [129]. Haghgooie et al. synthesized PEG hydrogel particles with different shapes including disks, rings, crosses, and S-shapes and demonstrated the modes of particles' passage through poly(dimethyl siloxane) (PDMS) channels [130]. However, little is known about the deformation behaviors of multilayer microcapsules with a size similar to RBC under flow in a smaller microchannel

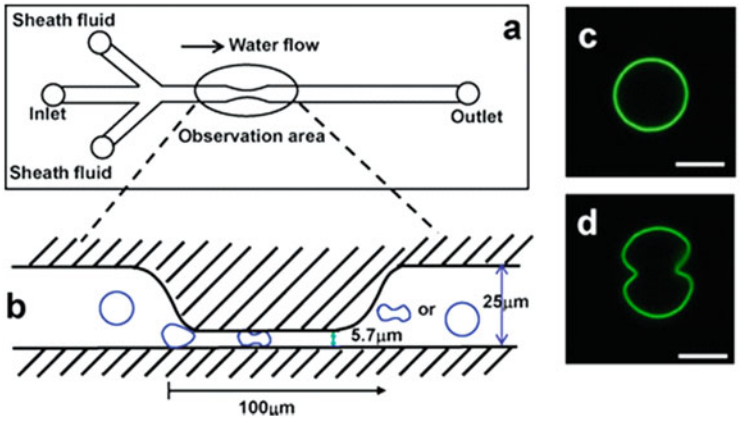


**Fig. 4.7** Scheme drawing to show the structure of the microchannel device by (**a**) *top view* and (**b**) *side view*. CLSM images of the 6.8 μm (**c**) and 8.6 μm (**d**) (PAH/PSS)5 microcapsules after being squeezed through the microchannel with a height of 5.7 μm. The former can recover its original spherical shape (**c**), while the latter keeps its deformed shape (**d**). (Adapted with permission from Ref. [133]. Copyright 2012, American Chemical Society)

[131], although the static deformation behaviors have been systematically studied under the press of a colloidal probe [132] or osmotic pressure [34, 35].

Recently, the deformability of multilayer microcapsules under flow in a confined microchannel was studied in our lab (Fig. 4.7) [133]. The influences of capsule size, wall thickness, cross-linking, and the filling of PSS inside on the deformation and recovery behaviors of the capsules were systematically investigated. The recovery ability of capsules is dependent on the deformation extent but not mechanical strength. The squeezed hollow microcapsules can recover their original spherical shape when the deformation extent is smaller than 16%, whereas permanent physical deformation takes place at a larger deformation extent such as 34%. In a sharp contrast, all the intact capsules prefilled with PSS can recover their original shape even when the deformation extent is as large as 47%. The spontaneously loaded dyes can be well maintained after the deformation and recovery process. It is the first time to disclose the alteration of drug amount in multilayer microcapsules after flowing through a constriction.

Furthermore, the RBC-like multilayer microcapsules also have been successfully fabricated by templating on $Ca(OH)_2$ particles with an RBC-like shape through covalent LbL method. The capsules can preserve their RBC-like morphology well in water after template removal. When the RBC-like capsules (6.7 μm) are trapped in a microcapillary with a smaller size (5 μm), they deform only in the areas in contact with the capillary wall. After they are forced to pass through, 90% of the RBC-like capsules recover their original discoidal shape. By assembling additional hemoglobin layers on the RBC-like capsules, they can be endowed with oxygen-binding and release capacity [134].

Yet, this is only the first step toward the fabrication of RBC-mimicking multilayer capsules. Nonetheless, the current results are important not only for understanding of capsule properties but also for their practical applications as drug delivery carriers. For example, the capsules for injection application should have a smaller size, soft wall structure, and RBC-like shape, while those for embolization should have a stiff wall which can clog the blood vessels with higher efficiency.

### *4.3.4 Anisotropic Capsules Interact with Cells*

As drug carriers, multilayer capsules should interact with different cells and may be internalized, which is of practical important for delivery of cargoes into cells. Thus, their interactions with cells draw much attention recently. It is well known that the physicochemical properties of colloidal particles can strongly influence their interactions with biological systems [135, 136]. Especially, the small differences on their physicochemical characteristics may strongly influence the interactions between particles and cells and further affect their cellular uptake, intracellular distribution, and ultimate cellular fate [137]. Recently, the shape of particles has been found to play a crucial role in the interactions between cells and particles and is regarded as a new important parameter for designing materials to realize specific biological functions [138, 139]. Smith and coworkers [140] demonstrated that the polystyrene particles with three shapes (spheres, prolate ellipsoids, and oblate ellipsoids) could manipulate different attachment and internalization of macrophages. Mitragotri and coworkers [141] found that compared with rods, the spherical polystyrene particles with identical total volumes exhibited significant perinuclear accumulation. When ovalbumin is used as a model antigen conjugated to particle surfaces, the regulation of immune response could be achieved by changing sizes and shapes of nanoparticles [142]. Moreover, in a model microvascular network, elongated particles exhibited higher adhesion and binding probability than spheres [143, 144]. In an in vivo experiment, the hydrogel microparticles mimicking the shape of red blood cells could possess the increased blood circulation and enhanced adhesion ability [145].

Polyelectrolyte multilayer microcapsules with tailored structures and properties have gained much interest in biomedical field especially for drug loading and release [10, 91–99]. However, researches about the interactions between anisotropic polyelectrolyte microcapsules and cells are rare [146, 147], and the mechanism of shape-induced difference in interactions between capsules and cells needs further investigation. For example, Caruso and coworkers [146] reported the fabrication of rod-shaped hydrogel capsules with tunable aspect ratios by a templating method. With increasing of the aspect ratios, slower and less cellular internalization of capsules was observed. Kharlampieva and coworkers [147] obtained polymer capsules with hemispherical geometry by drying poly(*N*-vinyl pyrrolidone)/tannic acid (PVPON/TA)n multilayer capsules and found that compared with their spherical and

cubic counterparts, the hemispherical capsules are taken up in a greater extent. However, the mechanism behind is not very clear.

More recently, bowl-like microcapsules were fabricated by osmotic-induced invagination of microcapsule in concentrated PSS solution [148]. Both the bowl-like and spherical capsules maintained their colloidal stability and shape in cell culture medium up to 7 days. The bowl-like microcapsules could be internalized with a faster rate and higher number by SMCs and macrophages than their spherical counterparts. Preferential attachment onto the cell membrane from the bend side and easier enwrapping by cell membranes are likely the major reasons enabling the uptake of the bowl-like capsules in priority than their spherical counterparts. Such results may help people to understand the role of capsule shape in the interaction with cells and provide useful guidance for further design of more efficient carriers.

### *4.3.5 Triggered Release of Encapsulated Substances from the Capsules*

Although encapsulation of active substances into multilayer capsules can protect them from the influence of the environment, the encapsulated substances should be released in a triggered way at desired positions. Among various triggers, the remote stimuli-light and ultrasound (US) have the advantages of high temporal and spatial resolution and controllable power without direct contact. Thus in the following part, we will focus on these two methods.

The LbL method can integrate light-responsive building blocks into the shells of multilayer capsules. For example, Zapotoczny and coworkers incorporated a relatively small amount of multiwalled carbon nanotubes (MWCNTs) into the shells of multilayer capsules, leading to an almost 20-fold increase of the apparent elastic modulus of the obtained capsules [149]. Due to their absorption in the near-infrared region and specific arrangement of MWCNTs in the shells, the capsules can show a light-triggered enhancement of permeability in a reversible, nondestructive manner. Using this feature, durability and facile encapsulation/release of desired substances into/from microcapsules can be achieved, which is crucial for their practical applications.

US imaging has the advantages of low cost, fast real-time visualization, deep penetration in tissues, and noninvasiveness. More important, the US can trigger drug release via inertial cavitation-caused mechanical damage to the capsules, and also can achieve spatiotemporal controllable drug release. Compared with light, the penetration depth in tissues is much higher. By choosing proper functional building blocks during the assembly of multilayer capsules, the capsules can be endowed with US-sensitive properties. For example, integration of metal and metal oxide nanoparticles in multilayer capsules can improve their US sensitivity because of the increased shell density [150]. $Fe_3O_4$ nanoparticles-modified capsules can be broken into pieces after 60 s sonication at an energy density of 377 W/cm$^2$

[151]. ZnO nanoparticles-modified capsules also can be totally ruptured by US after 9 s at an energy density of 30 W/cm$^2$ [152]. However, the higher-power US used in these studies may result in tissue damage and other side effects. Kharlampieva and coworkers reported high US imaging contrast and low-power diagnostic or high-power therapeutic US-triggered drug release from hydrogen-bonded TA and PVPON multilayer capsules [153]. The capsules possess good and long-term US imaging contrast. Upon low-power diagnostic US irradiation, the encapsulated drug can be gradually released, while its fast release can be achieved via high-power therapeutic US irradiation. Furthermore, the US imaging contrast of capsules can be regulated by changing the number of layers, and the type and molecular weight of used polymers.

The integration of functional building blocks into the shells of multilayer capsules not only can increase the US sensitivity but also can endow the capsules with other specific functionalities. For example, Sukhorukov and coworkers developed an method to the in situ fabrication and assembly of fluorescent carbon dots (CDs) into the shells of multilayer capsules [154]. CDs are synthesized in situ in capsule shells by carbonization of dextran molecules via hydrothermal treatment. The obtained nanocomposite capsules have luminescence which can be used for imaging. The heat treatment also can encapsulate low-molecular-weight drug into the capsules. The in situ formation of CDs in capsule shells endows with US responsiveness; thus, the loaded drug can be released upon US treatment. The shells of multilayer capsules also can be functionalized with radionuclide for imaging via positron emission tomography (PET) [155]. The capsules are prepared via LbL assembly of TA and deferoxamine (DFO)-functionalized PVPON. DFO can chelate the 89Zr radionuclide. The in vivo PET imaging can track the capsules in vivo and reveal their biodistribution. The encapsulated hydrophilic anticancer drug can be released upon the irradiation of therapeutic US to the Zr-functionalized capsules. Similarly, multilayer capsules with iron oxide nanoparticles-incorporated shells also possess magnetic resonance imaging (MRI) and US-triggered drug release abilities and thus can achieve real-time tracking and targeted delivery in vivo [156]. Such kinds of capsules with imaging ability as well as US-triggered drug release should have broad applications in the biomedical field.

In order to better control the release of encapsulated substances, the multilayer capsules which can respond to multistimuli have been developed. For example, iron oxide and graphene oxide (GO) are assembled with polysaccharides through the LbL method to form the shells of multilayer capsules. The capsules can be loaded with drugs through pH control, while the iron oxide and GO empower the capsules with magnetic and light responsiveness. Thus, the alternative magnetic field and near-infrared laser can trigger the release of drugs on demand [157]. Sukhorukov and coworkers also designed triple-responsive inorganic–organic hybrid microcapsules for the controlled release of encapsulated drugs [158]. The UV light and US responses are endowed by the in situ deposited $TiO_2$ and $SiO_2$ nanostructures in the capsule shells through a sol–gel process. This process also can reduce the permeability of the capsules, leading to the encapsulation of low-molecular-weight drugs. The enzymatic response is achieved by using biodegradable polypeptides and

polysaccharides for the fabrication of capsules. Upon employing different stimuli, the encapsulated drug can be released according to different mechanisms at desired times. This work demonstrates that the multilayer capsules are ideal platforms for the design of multimodal-responsive drug carriers.

## 4.4 Microcapsules as Growth Factor Carriers and Their Incorporation into Scaffold

Spatial- and temporal-controlled delivery of growth factors is crucial for the efficient repair upon tissue injury or failure in tissue engineering. But delivery of growth factors to the site of tissue regeneration is challenging since these proteins have short half-lives, high molecular weight, and slow tissue penetration. Thus, the generally used strategy to enhance in vivo efficacy of growth factors is the use of growth factor-loaded delivery systems, which release growth factors in a controlled way. Due to the tailored structures, multiple functionalities, as well as controlled permeability, the multilayer capsules are promising candidates as growth factor carriers.

Several methods have been successfully developed to load different growth factors into multilayer capsules. Akashi and coworkers first developed biodegradable multilayer capsules to encapsulate basic fibroblast growth factor (bFGF) as a cytokine release carrier [159]. The multilayer capsules were fabricated via the layer-by-layer (LbL) assembly of chitosan and dextran sulfate. The bFGF was encapsulated into the capsules by reversibly controlling the capsule permeability. At pH $<$ 8.0, the capsule shell was nonpermeable for macromolecules. However, FITC-dextran with a molecular weight as high as 250 kDa could easily penetrate the capsules at pH $>$ 8.0. Using the pH-controlled reversible shell permeability, bFGF was successfully encapsulated into the capsules. Release of the encapsulated bFGF was sustained over 70 h. Due to the local and sustained release of bFGF, mouse L929 fibroblast cells proliferated well for 2 weeks. Antipina and coworker used a coprecipitation-based layer-by-layer encapsulation method to load bFGF into the microcapsules [160]. In this method, bFGF was first protected by heparin and bovine serum albumin and then coprecipitated into $CaCO_3$ microparticles. Low cytotoxic and biodegradable polyelectrolytes dextran sulfate and poly-L-arginine were used for capsule shell assembly on the $CaCO_3$ microparticles. The encapsulation efficiency was greatly influenced by the shell thickness. Under optimized conditions, a maximum encapsulation efficiency of 42% could be achieved. The controlled release of FGF2 from the microcapsules was helpful to enhance the proliferation of L929 cells. De Geest and coworkers introduced a postloading approach by engineering the capsules in such a way that they acted as growth factor-binding "microsponges" [161]. In this method, $CaCO_3$ microparticles doped with heparin were first fabricated by a coprecipitation method. Subsequently, these microparticles were coated with heparin/poly-L-arginine multilayers, followed by decomposition of the $CaCO_3$ core. In this way, hollow capsules were obtained with

heparin both as membrane component and being suspended in their hollow void. Heparin is well known to have a high affinity for several growth factors. Therefore, the engineered microcapsules with high heparin content will enhance their growth factor-binding capacity. Transforming growth factor-beta 1 (TGF-beta 1) could be loaded and released from such kind of heparin-engineered microcapsules without affecting its biological activity. The growth factor-loaded multilayer capsules could be easily incorporated within a gelatin tissue engineering scaffold without affecting the properties of this scaffold.

Benkirane-Jessel and coworkers first demonstrated that a hydrogel scaffold incorporated with the growth factor-loaded multilayer capsules could induce bone formation in vivo [162]. In this research, bone morphogenetic proteins ($BMP_2$) and TGF-beta 1 were assembled into the shell of biodegradable multilayer microcapsules. The stem cells were differentiated into bone cells when cocultured with growth factor-loaded multilayer capsules. More importantly, when such kind of capsules were integrated with alginate gel and implanted into mice, inducing bone formation in vivo was observed. The in vivo results demonstrate the promising application potential of multilayer microcapsules as growth factor carriers in the field of tissue engineering and regenerative medicine.

## 4.5 Conclusions and Outlooks

The LbL assembly technique is a highly versatile and powerful platform for the fabrication of capsules with tailored structures and functions. They have already shown their great promise of applications in many areas, especially in the field of controlled release. Recently, much attention has been paid on the multilayer capsules assembled by new driving forces and those with highly sophisticated structures for biomedical applications, such as drug and growth factor carriers, as highlighted in this chapter.

Although the significant advances have been made in this area, there are still some key obstacles which should be overcome. First, for the real practical applications of multilayer capsules, rapid, scalable, and efficient new preparation methods should be developed. One recent example is the microcapsule preparation technique utilizing a fluidized bed for the LbL assembly of polymers [163]. The properties of obtained microcapsules are in close agreement with conventionally prepared LbL capsules. The technique provides a new way to rapidly generate microcapsules, while being also amenable to scale-up and mass production. Furthermore, a fully flow-based technique using tangential flow filtration (TFF) for LbL assembly on particles was developed [164]. Multilayered particles and capsules with size ranging from micrometers to submicrometers can be assembled on different templates using diverse polymers. The well-controlled, integrated, and automatable nature of the TFF LbL system provides significant progress of the practical applications of LbL systems. Second, the in vivo behaviors of LbL capsules such as degradation and toxicity are largely unexplored. For intravenous injection, the LbL capsules are

required to circulate in the bloodstream and have good hemocompatibility. Several recent researches have shown that coating of blood-compatible multilayers on the ultrasmall (~20 nm) [165, 166] and submicron (~500 nm) [167] particles is beneficial to obtain injectable capsule drug delivery systems. Therefore, particles with a submicron size are attractive for preparation of LbL capsules, which may accumulate in cancerous tissues through EPR effect.

The LbL capsules with tailed structures and functions can be loaded with both drugs and imaging agents within a single system to form theranostic carriers, which can selectively accumulate in diseased tissues and simultaneously report their biochemical and morphological characteristics. At the same time, the synergistic carriers which carry chemo-, radio-, and gene therapeutics can enhance the treatment efficacy [168, 169].

For successful tissue regeneration, it is extremely important to provide cells with a local environment using biomaterials which enable them to proliferate and differentiate efficiently and correctly, resulting in cell-induced tissue regeneration. For this purpose, capsules or spheres can be integrated into different scaffolds to provide for prolonged, site-specific delivery of loaded growth factors, drugs, as well as other bioactive species. The capsules should be well designed with properly controlled release profile as well as surface properties, so that they can act as an integral part of the porous three-dimensional scaffolds, and their incorporation does not significantly affect the scaffold properties but can release their cargoes to meet the needs of cells.

The researches and applications of LbL multilayer capsules are highly multidisciplinary. With the efforts afforded by the experts from fields of chemistry, materials science, mechanical engineering, biology, and so on, the abovementioned obstacles will be overcome sooner or later, and more achievements in this field can be expected in the future.

**Acknowledgments** The research is financially supported by the Natural Science Foundation of China (51120135001 and 21374101, 21174130, 20804036), Ph.D. Programs Foundation of the Ministry of Education of China (20110101130005), and Zhejiang Provincial Natural Science Foundation of China (Y4110064).

## References

1. Caruso F. Hollow capsule processing through colloidal templating and self-assembly. Chem Eur J. 2000;6:413–9.
2. Decher G, Hong JD. Buildup of ultrathin multilayer films by a self-assembly process.1. Consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces. Makromol Chem Macromol Symp. 1991;46:321–7.
3. Decher G. Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science. 1997;277:1232–7.
4. Caruso F, Caruso RA, Mohwald H. Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. Science. 1998;282:1111–4.
5. Donath E, Sukhorukov GB, Caruso F, Davis SA, Mohwald H. Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. Angew Chem Int Ed. 1998;37:2202–5.

6. Peyratout CS, Dahne L. Tailor-made polyelectrolyte microcapsules: from multilayers to smart containers. Angew Chem Int Ed. 2004;43:3762–83.
7. Tong WJ, Gao CY. Multilayer microcapsules with tailored structures for bio-related applications. J Mater Chem. 2008;18:3799–812.
8. Sukhorukov GB, Rogach AL, Garstka M, Springer S, Parak WJ, Munoz-Javier A, Kreft O, Skirtach AG, Susha AS, Ramaye Y, Palankar R, Winterhalter M. Multifunctionalized polymer microcapsules: novel tools for biological and pharmacological applications. Small. 2007;3: 944–55.
9. Sukhorukov GB, Mohwald H. Multifunctional cargo systems for biotechnology. Trends Biotechnol. 2007;25:93–8.
10. Tong WJ, Song XX, Gao CY. Layer-by-layer assembly of microcapsules and their biomedical applications. Chem Soc Rev. 2012;41(18):6103–24.
11. Pastoriza-Santos I, Scholer B, Caruso F. Core-shell colloids and hollow polyelectrolyte capsules based on diazoresins. Adv Funct Mater. 2001;11:122–8.
12. Mauser T, Dejugnat C, Sukhorukov GB. Reversible ph-dependent properties of multilayer microcapsules made of weak polyelectrolytes. Macromol Rapid Commun. 2004;25:1781–5.
13. Zhu HG, McShane MJ. Macromolecule encapsulation in diazoresin-based hollow polyelectrolyte microcapsules. Langmuir. 2005;21:424–30.
14. Sukhishvili SA, Granick S. Layered, erasable, ultrathin polymer films. J Am Chem Soc. 2000;122:9550–1.
15. Sukhishvili SA, Granick S. Layered, erasable polymer multilayers formed by hydrogen-bonded sequential self-assembly. Macromolecules. 2002;35:301–10.
16. Yang SY, Rubner MF. Micropatterning of polymer thin films with ph-sensitive and cross-linkable hydrogen-bonded polyelectrolyte multilayers. J Am Chem Soc. 2002;124:2100–1.
17. Yang SY, Lee D, Cohen RE, Rubner MF. Bioinert solution-cross-linked hydrogen-bonded multilayers on colloidal particles. Langmuir. 2004;20:5978–81.
18. Kozlovskaya V, Ok S, Sousa A, Libera M, Sukhishvili SA. Hydrogen-bonded polymer capsules formed by layer-by-layer self-assembly. Macromolecules. 2003;36:8590–2.
19. Tong WJ, Gao CY. Stable microcapsules assembled stepwise from weak polyelectrolytes followed by thermal crosslinking. Polym Adv Technol. 2005;16:827–33.
20. Zelikin AN, Quinn JF, Caruso F. Disulfide cross-linked polymer capsules: En route to biodeconstructible systems. Biomacromolecules. 2006;7:27–30.
21. Such GK, Johnston APR, Caruso F. Engineered hydrogen-bonded polymer multilayers: from assembly to biomedical applications. Chem Soc Rev. 2011;40:19–29.
22. Connal LA, Kinnane CR, Zelikin AN, Caruso F. Stabilization and functionalization of polymer multilayers and capsules via thiol-ene click chemistry. Chem Mater. 2009;21:576–8.
23. Kinnane CR, Such GK, Antequera-Garcia G, Yan Y, Dodds SJ, Liz-Marzan LM, Caruso F. Low-fouling poly(n-vinyl pyrrolidone) capsules with engineered degradable properties. Biomacromolecules. 2009;10:2839–46.
24. Ochs CJ, Such GK, Yan Y, van Koeverden MP, Caruso F. Biodegradable click capsules with engineered drug-loaded multilayers. ACS Nano. 2010;4:1653–63.
25. Leung MKM, Such GK, Johnston APR, Biswas DP, Zhu ZY, Yan Y, Lutz JF, Caruso F. Assembly and degradation of low-fouling click-functionalized poly(ethylene glycol)-based multilayer films and capsules. Small. 2011;7:1075–85.
26. Ng SL, Such GK, Johnston APR, Antequera-Garcia G, Caruso F. Controlled release of DNA from poly(vinylpyrrolidone) capsules using cleavable linkers. Biomaterials. 2011;32:6277–84.
27. Ochs CJ, Such GK, Caruso F. Modular assembly of layer-by-layer capsules with tailored degradation profiles. Langmuir. 2011;27:1275–80.
28. Liang K, Such GK, Zhu ZY, Yan Y, Lomas H, Caruso F. Charge-shifting click capsules with dual-responsive cargo release mechanisms. Adv Mater. 2011;23:H273–7.
29. Tong WJ, Gao CY, Mohwald H. Manipulating the properties of polyelectrolyte microcapsules by glutaraldehyde cross-linking. Chem Mater. 2005;17:4610–6.

30. Tong WJ, Gao CY, Mohwald H. Stable weak polyelectrolyte microcapsules with ph-responsive permeability. Macromolecules. 2006;39:335–40.
31. Sukhorukov GB, Donath E, Lichtenfeld H, Knippel E, Knippel M, Budde A, Mohwald H. Layer-by-layer self assembly of polyelectrolytes on colloidal particles. Colloids Surf A Physicochem Eng Asp. 1998;137:253–66.
32. Zhang YJ, Yang SG, Guan Y, Cao WX, Xu J. Fabrication of stable hollow capsules by covalent layer-by-layer self-assembly. Macromolecules. 2003;36:4238–40.
33. Feng ZQ, Wang ZP, Gao CY, Shen JC. Direct covalent assembly to fabricate microcapsules with ultrathin walls and high mechanical strength. Adv Mater. 2007;19:3687–91.
34. Gao C, Donath E, Moya S, Dudnik V, Mohwald H. Elasticity of hollow polyelectrolyte capsules prepared by the layer-by-layer technique. Eur Phys J E Soft Matter Biol Phys. 2001;5:21–7.
35. Gao CY, Leporatti S, Moya S, Donath E, Mohwald H. Stability and mechanical properties of polyelectrolyte capsules obtained by stepwise assembly of poly(styrenesulfonate sodium salt) and poly(diallyldimethyl ammonium) chloride onto melamine resin particles. Langmuir. 2001;17:3491–5.
36. Tong WJ, Gao CY, Mohwald H. Single polyelectrolyte microcapsules fabricated by glutaraldehyde-mediated covalent layer-by-layer assembly. Macromol Rapid Commun. 2006;27:2078–83.
37. Tong WJ, Gao CY, Mohwald H. Poly(ethyleneimine) microcapsules: glutaraldehyde-mediated assembly and the influence of molecular weight on their properties. Polym Adv Technol. 2008;19:817–23.
38. Such GK, Tjipto E, Postma A, Johnston APR, Caruso F. Ultrathin, responsive polymer click capsules. Nano Lett. 2007;7:1706–10.
39. Peters T. Serum-albumin. Adv Protein Chem. 1985;37:161–245.
40. Carter DC, Ho JX. Structure of serum-albumin. Adv Protein Chem. 1994;45:153–203.
41. Rubino OP, Kowalsky R, Swarbrick J. Albumin microspheres as a drug-delivery system – relation among turbidity ratio, degree of cross-linking, and drug-release. Pharm Res. 1993;10: 1059–65.
42. Tong WJ, Gao CY, Moehwald H. Ph-responsive protein microcapsules fabricated via glutaraldehyde mediated covalent layer-by-layer assembly. Colloid Polym Sci. 2008;286:1103–9.
43. Wang ZP, Feng ZQ, Gao CY. Stepwise assembly of the same polyelectrolytes using host-guest interaction to obtain microcapsules with multiresponsive properties. Chem Mater. 2008;20: 4194–9.
44. Johnston APR, Read ES, Caruso F. DNA multilayer films on planar and colloidal supports: sequential assembly of like-charged polyelectrolytes. Nano Lett. 2005;5:953–6.
45. Quiocho FA. Carbohydrate-binding proteins – tertiary structures and protein-sugar interactions. Annu Rev Biochem. 1986;55:287–315.
46. Lee YC, Lee RT. Carbohydrate-protein interactions – basis of glycobiology. Acc Chem Res. 1995;28:321–7.
47. Nelson RM, Venot A, Bevilacqua MP, Linhardt RJ, Stamenkovic I. Carbohydrate-protein interactions in vascular biology. Annu Rev Cell Dev Biol. 1995;11:601–31.
48. Lis H, Sharon N. Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem Rev. 1998;98:637–74.
49. Becker JW, Reeke GN, Cunningham BA, Edelman GM. New evidence on location of saccharide-binding site of concanavalin-a. Nature. 1976;259:406–9.
50. Goldstein IJ, Hollerman CE, Smith EE. Protein-carbohydrate interaction. 2. Inhibition studies on interaction of concanavalin a with polysaccharides. Biochemistry. 1965;4:876–83.
51. Lvov Y, Ariga K, Ichinose I, Kunitake T. Layer-by-layer architectures of concanavalin a by means of electrostatic and biospecific interactions. J Chem Soc Chem Commun. 1995;22: 2313–4.

52. Lvov Y, Ariga K, Ichinose I, Kunitake T. Molecular film assembly via layer-by-layer adsorption of oppositely charged macromolecules (linear polymer, protein and clay) and concanavalin a and glycogen. Thin Solid Films. 1996;284:797–801.
53. Sato K, Imoto Y, Sugama J, Seki S, Inoue H, Odagiri T, Hoshi T, Anzai J. Sugar-induced disintegration of layer-by-layer assemblies composed of concanavalin a and glycogen. Langmuir. 2005;21:797–9.
54. Zhu Y, Tong WJ, Gao CY. Molecular-engineered polymeric microcapsules assembled from concanavalin a and glycogen with specific responses to carbohydrates. Soft Matter. 2011;7: 5805–15.
55. Walker SA, Kennedy MT, Zasadzinski JA. Encapsulation of bilayer vesicles by self-assembly. Nature. 1997;387:61–4.
56. Lutz JF, Laschewsky A. Multicompartment micelles: has the long-standing dream become a reality? Macromol Chem Phys. 2005;206:813–7.
57. De Geest BG, De Koker S, Immesoete K, Demeester J, De Smedt SC, Hennink WE. Self-exploding beads releasing microcarriers. Adv Mater. 2008;20:3687–91.
58. Zhang Y, Ruder WC, Leduc PR. Artificial cells: building bioinspired systems using small-scale biology. Trends Biotechnol. 2008;26:14–20.
59. Delcea M, Yashchenok A, Videnova K, Kreft O, Mohwald H, Skirtach AG. Multicompartmental micro- and nanocapsules: hierarchy and applications in biosciences. Macromol Biosci. 2010;10:465–74.
60. Kreft O, Prevot M, Mohwald H, Sukhorukov GB. Shell-in-shell microcapsules: a novel tool for integrated, spatially confined enzymatic reactions. Angew Chem Int Ed. 2007;46:5605–8.
61. De Geest BG, McShane MJ, Demeester J, De Smedt SC, Hennink WE. Microcapsules ejecting nanosized species into the environment. J Am Chem Soc. 2008;130:14480–2.
62. Stadler B, Chandrawati R, Price AD, Chong SF, Breheney K, Postma A, Connal LA, Zelikin AN, Caruso F. A microreactor with thousands of subcompartments: enzyme-loaded liposomes within polymer capsules. Angew Chem Int Ed. 2009;48:4359–62.
63. Hosta-Rigau L, Stadler B, Yan Y, Nice EC, Heath JK, Albericio F, Caruso F. Capsosomes with multilayered subcompartments: assembly and loading with hydrophobic cargo. Adv Funct Mater. 2010;20:59–66.
64. Chandrawati R, Stadler B, Postma A, Connal LA, Chong SF, Zelikin AN, Caruso F. Cholesterol-mediated anchoring of enzyme-loaded liposomes within disulfide-stabilized polymer carrier capsules. Biomaterials. 2009;30:5988–98.
65. Hosta-Rigau L, Chung SF, Postma A, Chandrawati R, Stadler B, Caruso F. Capsosomes with "free-floating" liposomal subcompartments. Adv Mater. 2011;23:4082–7.
66. Stadler B, Chandrawati R, Goldie K, Caruso F. Capsosomes: subcompartmentalizing polyelectrolyte capsules using liposomes. Langmuir. 2009;25:6725–32.
67. Gohy JF. Block copolymer micelles. In: Block copolymers ii. Berlin: Springer; 2005. p. 65–136.
68. Zhu Y, Tong WJ, Gao CY, Mohwald H. Assembly of polymeric micelles into hollow microcapsules with extraordinary stability against extreme ph conditions. Langmuir. 2008;24:7810–6.
69. Li XD, Lu T, Zhang JX, Xu JJ, Hu QL, Zhao SF, Shen JC. A study of properties of "micelle-enhanced" polyelectrolyte capsules: structure, encapsulation and in vitro release. Acta Biomater. 2009;5:2122–31.
70. Tong WJ, Zhu Y, Wang ZP, Gao CY, Mohwald H. Micelles-encapsulated microcapsules for sequential loading of hydrophobic and water-soluble drugs. Macromol Rapid Commun. 2010;31:1015–9.
71. Gao CY, Donath E, Mohwald H, Shen JC. Spontaneous deposition of water-soluble substances into microcapsules: phenomenon, mechanism, and application. Angew Chem Int Ed. 2002;41:3789–93.

72. Gao CY, Liu XY, Shen JC, Mohwald H. Spontaneous deposition of horseradish peroxidase into polyelectrolyte multilayer capsules to improve its activity and stability. Chem Commun. 2002;17:1928–9.
73. Liu XY, Gao CY, Shen JC, Mohwald H. Multilayer microcapsules as anti-cancer drug delivery vehicle: deposition, sustained release, and in vitro bioactivity. Macromol Biosci. 2005;5: 1209–19.
74. Mao ZW, Ma L, Gao CY, Shen JC. Preformed microcapsules for loading and sustained release of ciprofloxacin hydrochloride. J Control Release. 2005;104:193–202.
75. Zhao QH, Zhang SA, Tong WJ, Gao CY, Shen JC. Polyelectrolyte microcapsules templated on poly(styrene sulfonate)-doped $CaCO_3$ particles for loading and sustained release of daunorubicin and doxorubicin. Eur Polym J. 2006;42:3341–51.
76. He Q, Song WX, Moehwald H, Li JB. Hydrothermal-induced structure transformation of polyelectrolyte multilayers: from nanotubes to capsules. Langmuir. 2008;24:5508–13.
77. Wang JG, Xiao Q, Zhou HJ, Sun PC, Yuan ZY, Li BH, Ding DT, Shi AC, Chen TH. Budded, mesoporous silica hollow spheres: hierarchical structure controlled by kinetic self-assembly. Adv Mater. 2006;18:3284–8.
78. Yu K, Zhang L, Eisenberg A. Novel morphologies of "crew-cut" aggregates of amphiphilic diblock copolymers in dilute solution. Langmuir. 1996;12:5980–4.
79. Tung PH, Kuo SW, Chan SC, Hsu CH, Wang CF, Chang FC. Micellization and the surface hydrophobicity of amphiphilic poly(vinylphenol)-*block*-polystyrene block copolymers. Macromol Chem Phys. 2007;208:1823–31.
80. Sha K, Li D, Li Y, Zhang B, Wang J. The chemoenzymatic synthesis of a novel cbabc-type pentablock copolymer and its self-assembled "crew-cut" aggregation. Macromolecules. 2007;41:361–71.
81. Menger FM, Seredyuk VA. Internally catalyzed separation of adhered lipid membranes. J Am Chem Soc. 2003;125:11800–1.
82. Zhou YF, Yan DY. Real-time membrane fission of giant polymer vesicles. Angew Chem Int Ed. 2005;44:3223–6.
83. Zhou YF, Yan DY. Real-time membrane fusion of giant polymer vesicles. J Am Chem Soc. 2005;127:10468–9.
84. Wang ZP, Mohwald H, Gao CY. Preparation and redox-controlled reversible response of ferrocene-modified poly(allylamine hydrochloride) microcapsules. Langmuir. 2011;27:1286–91.
85. Wang ZP, Mohwald H, Gao CY. Nanotubes protruding from poly(allylamine hydrochloride)-graft-pyrene microcapsules. ACS Nano. 2011;5:3930–6.
86. Wang ZP, Xie Y, Gao CY. Repeated protrusion of fluorescent pyrene nanorods on the surface of crosslinked poly(allylamine hydrochloride) microcapsules. RSC Adv. 2012;2:11354–8.
87. Wang ZP, Liu MY, Xie Y, Gao CY. In situ fabrication of pyrene derivative nanorods inside polyelectrolytes microcapsules with tunable fluorescent properties. J Mater Chem. 2012;22: 2855–8.
88. Guan EJ, Wang TX, Wang ZP, Gao CY. Modulating the nanorods protrusion from poly (allylamine hydrochloride)-*g*-pyrene microcapsules by 1-pyrenesulfonic acid sodium salt. J Colloid Interface Sci. 2013;405:10–6.
89. Wang ZP, Skirtach AG, Xie Y, Liu MY, Mohwald H, Gao CY. Core-shell poly(allyamine hydrochloride)-pyrene nanorods decorated with gold nanoparticles. Chem Mater. 2011;23: 4741–7.
90. Wang TX, Cai YB, Wang ZP, Guan EJ, Yu DH, Qin AJ, Sun JZ, Tang BZ, Gao CY. Decomposition-assembly of tetraphenylethylene nanoparticles with uniform size and aggregation-induced emission property. Macromol Rapid Commun. 2012;33:1584–9.
91. De Geest BG, De Koker S, Sukhorukov GB, Kreft O, Parak WJ, Skirtach AG, Demeester J, De Smedt SC, Hennink WE. Polyelectrolyte microcapsules for biomedical applications. Soft Matter. 2009;5:282–91.

92. De Geest BG, Sukhorukov GB, Mohwald H. The pros and cons of polyelectrolyte capsules in drug delivery. Expert Opin Drug Deliv. 2009;6:613–24.
93. Johnston APR, Such GK, Ng SL, Caruso F. Challenges facing colloidal delivery systems: from synthesis to the clinic. Curr Opin Colloid Interface Sci. 2011;16:171–81.
94. Johnston APR, Such GK, Caruso F. Triggering release of encapsulated cargo. Angew Chem Int Ed. 2010;49:2664–6.
95. De Cock LJ, De Koker S, De Geest BG, Grooten J, Vervaet C, Remon JP, Sukhorukov GB, Antipina MN. Polymeric multilayer capsules in drug delivery. Angew Chem Int Ed. 2010;49: 6954–73.
96. Yan Y, Such GK, Johnston APR, Lomas H, Caruso F. Toward therapeutic delivery with layer-by-layer engineered particles. ACS Nano. 2011;5:4252–7.
97. De Koker S, Lambrecht BN, Willart MA, van Kooyk Y, Grooten J, Vervaet C, Remon JP, De Geest BG. Designing polymeric particles for antigen delivery. Chem Soc Rev. 2011;40:320–39.
98. De Koker S, Hoogenboom R, De Geest BG. Polymeric multilayer capsules for drug delivery. Chem Soc Rev. 2012;41:2867–84.
99. Ariga K, McShane M, Lvov YM, Ji QM, Hill JP. Layer-by-layer assembly for drug delivery and related applications. Expert Opin Drug Deliv. 2011;8:633–44.
100. Sukhorukov GB, Brumen M, Donath E, Mohwald H. Hollow polyelectrolyte shells: exclusion of polymers and donnan equilibrium. J Phys Chem B. 1999;103:6434–40.
101. Tong WJ, Dong WF, Gao CY, Mohwald H. Charge-controlled permeability of polyelectrolyte microcapsules. J Phys Chem B. 2005;109:13159–65.
102. Tong WJ, Song HQ, Gao CY, Mohwald H. Equilibrium distribution of permeants in polyelectrolyte microcapsules filled with negatively charged polyelectrolyte: the influence of ionic strength and solvent polarity. J Phys Chem B. 2006;110:12905–9.
103. Zhao QH, Mao ZW, Gao CY, Shen JC. Assembly of multilayer microcapsules on $CaCO_3$ particles from biocompatible polysaccharides. J Biomater Sci Polym Ed. 2006;17:997–1014.
104. Zhao QH, Han BS, Wang ZH, Gao CY, Peng CH, Shen JC. Hollow chitosan-alginate multilayer microcapsules as drug delivery vehicle: doxorubicin loading and in vitro and in vivo studies. Nanomed Nanotechnol Biol Med. 2007;3:63–74.
105. Kohler K, Shchukin DG, Mohwald H, Sukhorukov GB. Thermal behavior of polyelectrolyte multilayer microcapsules. 1. The effect of odd and even layer number. J Phys Chem B. 2005;109:18250–9.
106. Kohler K, Mohwald H, Sukhorukov GB. Thermal behavior of polyelectrolyte multilayer microcapsules: 2. Insight into molecular mechanisms for the pdadmac/pss system. J Phys Chem B. 2006;110:24002–10.
107. Sadasivan S, Kohler K, Sukhorukov GB. Fabrication of organized porphyrin-nanotube-attached heat-sensitive polyelectrolyte capsules. Adv Funct Mater. 2006;16:2083–8.
108. Song WX, He Q, Mohwald H, Yang Y, Li JB. Smart polyelectrolyte microcapsules as carriers for water-soluble small molecular drug. J Control Release. 2009;139:160–6.
109. Tong WJ, She SP, Xie LL, Gao CY. High efficient loading and controlled release of low-molecular-weight drugs by combination of spontaneous deposition and heat-induced shrinkage of multilayer capsules. Soft Matter. 2011;7:8258–65.
110. Han YY, Bu J, Zhang YY, Tong WJ, Gao CY. Encapsulation of photosensitizer into multilayer microcapsules by combination of spontaneous deposition and heat-induced shrinkage for photodynamic therapy. Macromol Biosci. 2012;12:1436–42.
111. Luo D, Gould DJ, Sukhorukov GB. Local and sustained activity of doxycycline delivered with layer-by-layer microcapsules. Biomacromolecules. 2016;17:1466–76.
112. Kozlovskaya V, Chen J, Zavgorodnya O, Hasan MB, Kharlampieva E. Multilayer hydrogel capsules of interpenetrated network for encapsulation of small molecules. Langmuir. 2018;34: 11832–42.

113. Gao H, Goryacheva OA, Tarakina NV, Sukhorukov GB. Intracellularly biodegradable polyelectrolyte/silica composite microcapsules as carriers for small molecules. ACS Appl Mater Interfaces. 2016;8:9651–61.
114. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. Curr Opin Solid State Mater Sci. 2002;6:319–27.
115. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev. 1999;99:3181–98.
116. Zhou J, Romero G, Rojas E, Ma L, Moya S, Gao CY. Layer by layer chitosan/alginate coatings on poly(lactide-*co*-glycolide) nanoparticles for antifouling protection and folic acid binding to achieve selective cell targeting. J Colloid Interface Sci. 2010;345:241–7.
117. Zhou J, Romero G, Rojas E, Moya S, Ma L, Gao CY. Folic acid modified poly(lactide-*co*-glycolide) nanoparticles, layer-by-layer surface engineered for targeted delivery. Macromol Chem Phys. 2010;211:404–11.
118. Zhou J, Moya S, Ma L, Gao CY, Shen JC. Polyelectrolyte coated plga nanoparticles: templation and release behavior. Macromol Biosci. 2009;9:326–35.
119. Xie L, Tong W, Yu D, Xu J, Li J, Gao C. Bovine serum albumin nanoparticles modified with multilayers and aptamers for ph-responsive and targeted anti-cancer drug delivery. J Mater Chem. 2012;22:6053–60.
120. Kim JK, Choi KJ, Lee M, Jo MH, Kim S. Molecular imaging of a cancer-targeting theragnostics probe using a nucleolin aptamer- and microrna-221 molecular beacon-conjugated nanoparticle. Biomaterials. 2012;33:207–17.
121. Iwasaki H, Nabeshima K, Nishio J, Jimi S, Aoki M, Koga K, Hamasaki M, Hayashi H, Mogi A. Pathology of soft-tissue tumors: daily diagnosis, molecular cytogenetics and experimental approach. Pathol Int. 2009;59:501–21.
122. Xie LL, Tong WJ, Xu JQ, Gao CY. Multilayers and poly(allylamine hydrochloride)-graft-poly (ethylene glycol) (pah-g-peg) modified bovine serum albumin nanoparticles: improved stability and ph-responsive drug delivery. Chin J Polym Sci. 2012;30:719–26.
123. Szczepanowicz K, Bzowska M, Kruk T, Karabasz A, Bereta J, Warszynski P. Pegylated polyelectrolyte nanoparticles containing paclitaxel as a promising candidate for drug carriers for passive targeting. Colloids Surf B. 2016;143:463–71.
124. Jafari A, Sun HT, Sun BY, Mohamed MA, Cui HG, Cheng C. Layer-by-layer preparation of polyelectrolyte multilayer nanocapsules via crystallized miniemulsions. Chem Commun. 2019;55:1267–70.
125. Pei HY, Bai YY, Guo JM, Gao ZL, Dai Q, Yu Q, Cui JW. Tunable morphologies of polymer capsules templated from cuprous oxide particles for control over cell association. Chin Chem Lett. 2020;31:505–8.
126. Elizarova IS, Luckham PF. Fabrication of polyelectrolyte multilayered nano-capsules using a continuous layer-by-layer approach. J Colloid Interface Sci. 2016;470:92–9.
127. Schneider G, Decher G. Functional core/shell nanoparticles via layer-by-layer assembly. Investigation of the experimental parameters for controlling particle aggregation and for enhancing dispersion stability. Langmuir. 2008;24:1778–89.
128. Doshi N, Zahr AS, Bhaskar S, Lahann J, Mitragotri S. Red blood cell-mimicking synthetic biomaterial particles. Proc Natl Acad Sci U S A. 2009;106:21495–9.
129. Hayashi K, Ono K, Suzuki H, Sawada M, Moriya M, Sakamoto W, Yogo T. Electrosprayed synthesis of red-blood-cell-like particles with dual modality for magnetic resonance and fluorescence imaging. Small. 2010;6:2384–91.
130. Haghgooie R, Toner M, Doyle PS. Squishy non-spherical hydrogel microparticles. Macromol Rapid Commun. 2010;31:128–34.
131. Prevot M, Cordeiro AL, Sukhorukov GB, Lvov Y, Besser RS, Mohwald H. Design of a microfluidic system to investigate the mechanical properties of layer-by-layer fabricated capsules. Macromol Mater Eng. 2003;288:915–9.
132. Dubreuil F, Elsner N, Fery A. Elastic properties of polyelectrolyte capsules studied by atomic-force microscopy and ricm. Eur Phys J E Soft Matter Biol Phys. 2003;12:215–21.

133. She S, Xu C, Yin X, Tong W, Gao C. Shape deformation and recovery of multilayer microcapsules after being squeezed through a microchannel. Langmuir. 2012;28:5010–6.
134. She S, Li Q, Shan B, Tong W, Gao C. Fabrication of red-blood-cell-like polyelectrolyte microcapsules and their deformation and recovery behavior through a microcapillary. Adv Mater. 2013;25:5814–8.
135. Toy R, Peiris PM, Ghaghada KB, Karathanasis E. Shaping cancer nanomedicine: the effect of particle shape on the in vivo journey of nanoparticles. Nanomedicine. 2014;9:121–34.
136. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng. 2012;14:1–16.
137. Nel AE, Maedler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater. 2009;8:543–57.
138. Huang X, Teng X, Chen D, Tang F, He J. The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. Biomaterials. 2010;31:438–48.
139. Alexander JF, Kozlovskaya V, Chen J, Kuncewicz T, Kharlampieva E, Godin B. Cubical shape enhances the interaction of layer-by-layer polymeric particles with breast cancer cells. Adv Healthc Mater. 2015;4:2657–66.
140. Sharma G, Valenta DT, Altman Y, Harvey S, Xie H, Mitragotri S, Smith JW. Polymer particle shape independently influences binding and internalization by macrophages. J Control Release. 2010;147:408–12.
141. Kolhar P, Mitragotri S. Polymer microparticles exhibit size and shape dependent accumulation around the nucleus after endocytosis. Adv Funct Mater. 2012;22:3759–64.
142. Kumar S, Anselmo AC, Banerjee A, Zakrewsky M, Mitragotri S. Shape and size-dependent immune response to antigen-carrying nanoparticles. J Control Release. 2015;220:141–8.
143. Doshi N, Prabhakarpandian B, Rea-Ramsey A, Pant K, Sundaram S, Mitragotri S. Flow and adhesion of drug carriers in blood vessels depend on their shape: a study using model synthetic microvascular networks. J Control Release. 2010;146:196–200.
144. Shah S, Liu Y, Hu W, Gao J. Modeling particle shape-dependent dynamics in nanomedicine. J Nanosci Nanotechnol. 2011;11:919–28.
145. Merkel TJ, Jones SW, Herlihy KP, Kersey FR, Shields AR, Napier M, Luft JC, Wu H, Zamboni WC, Wang AZ, Bear JE, DeSimone JM. Using mechanobiological mimicry of red blood cells to extend circulation times of hydrogel microparticles. Proc Natl Acad Sci U S A. 2011;108:586–91.
146. Shimoni O, Yan Y, Wang Y, Caruso F. Shape-dependent cellular processing of polyelectrolyte capsules. ACS Nano. 2013;7:522–30.
147. Chen J, Kozlovskaya V, Goins A, Campos-Gomez J, Saeed M, Kharlampieva E. Biocompatible shaped particles from dried multilayer polymer capsules. Biomacromolecules. 2013;14:3830–41.
148. Li HY, Zhang WB, Tong WJ, Gao CY. Enhanced cellular uptake of bowl-like microcapsules. ACS Appl Mater Interfaces. 2016;8:11210–4.
149. Chojnacka-Gorka K, Wolski K, Zapotoczny S. Durable polyelectrolyte microcapsules with near-infrared-triggered loading and nondestructive release of cargo. ACS Appl Mater Interfaces. 2021;13:1562–72.
150. Skirtach AG, De Geest BG, Mamedov A, Antipov AA, Kotov NA, Sukhorukov GB. Ultrasound stimulated release and catalysis using polyelectrolyte multilayer capsules. J Mater Chem. 2007;17:1050–4.
151. Shchukin DG, Gorin DA, Möhwald H. Ultrasonically induced opening of polyelectrolyte microcontainers. Langmuir. 2006;22:7400–4.
152. Kolesnikova TA, Gorin DA, Fernandes P, Kessel S, Khomutov GB, Fery A, Shchukin DG, Möhwald H. Nanocomposite microcontainers with high ultrasound sensitivity. Adv Funct Mater. 2010;20:1189–95.

153. Chen J, Ratnayaka S, Alford A, Kozlovskaya V, Liu F, Xue B, Hoyt K, Kharlampieva E. Theranostic multilayer capsules for ultrasound imaging and guided drug delivery. ACS Nano. 2017;11:3135–46.
154. Gao H, Sapelkin AV, Titirici MM, Sukhorukov GB. In situ synthesis of fluorescent carbon dots/polyelectrolyte nanocomposite microcapsules with reduced permeability and ultrasound sensitivity. ACS Nano. 2016;10:9608–15.
155. Kozlovskaya V, Alford A, Dolmat M, Ducharme M, Caviedes R, Radford L, Lapi SE, Kharlampieva E. Multilayer microcapsules with shell-chelated Zr-89 for PET imaging and controlled delivery. ACS Appl Mater Interfaces. 2020;12:56792–804.
156. Alford A, Rich M, Kozlovskaya V, Chen J, Sherwood J, Bolding M, Warram J, Bao YP, Kharlampieva E. Ultrasound-triggered delivery of anticancer therapeutics from MRI-visible multilayer microcapsules. Adv Therap. 2018;1:1800051.
157. Deng L, Li Q, Al-Rehili S, Omar H, Almalik A, Alshamsan A, Zhang JF, Khashab NM. Hybrid iron oxide-graphene oxide-polysaccharides microcapsule: a micro-matryoshka for on-demand drug release and antitumor therapy in vivo. ACS Appl Mater Interfaces. 2016;8:6859–68.
158. Timin AS, Muslimov AR, Lepik KV, Saprykina NN, Sergeev VS, Afanasyev BV, Vilesov AD, Sukhorukov GB. Triple-responsive inorganic-organic hybrid microcapsules as a biocompatible smart platform for the delivery of small molecules. J Mater Chem B. 2016;4:7270–82.
159. Itoh Y, Matsusaki M, Kida T, Akashi M. Locally controlled release of basic fibroblast growth factor from multilayered capsules. Biomacromolecules. 2008;9(8):2202–6.
160. She Z, Wang CX, Li J, Sukhorukov GB, Antipina MN. Encapsulation of basic fibroblast growth factor by polyelectrolyte multilayer microcapsules and its controlled release for enhancing cell proliferation. Biomacromolecules. 2012;13(7):2174–80.
161. De Cock LJ, De Wever O, Van Vlierberghe S, Vanderleyden E, Dubruel P, De Vos F, Vervaet C, Remon JP, De Geest BG. Engineered (hep/pARG)(2) polyelectrolyte capsules for sustained release of bioactive TGF-beta 1. Soft Matter. 2012;8(4):1146–54.
162. Facca S, Cortez C, Mendoza-Palomares C, Messadeq N, Dierich A, Johnston APR, Mainard D, Voegel JC, Caruso F, Benkirane-Jessel N. Active multilayered capsules for in vivo bone formation. Proc Natl Acad Sci U S A. 2010;107(8):3406–11.
163. Richardson JJ, Teng D, Bjornmalm M, Gunawan ST, Guo J, Cui JW, Franks GV, Caruso F. Fluidized bed layer-by-layer microcapsule formation. Langmuir. 2014;30(33):10028–34.
164. Bjornmalm M, Roozmand A, Noi KF, Guo JL, Cui JW, Richardson JJ, Caruso F. Flow-based assembly of layer-by-layer capsules through tangential flow filtration. Langmuir. 2015;31(33):9054–60.
165. Poon Z, Chang D, Zhao XY, Hammond PT. Layer-by-layer nanoparticles with a ph-sheddable layer for in vivo targeting of tumor hypoxia. ACS Nano. 2011;5:4284–92.
166. Poon Z, Lee JB, Morton SW, Hammond PT. Controlling in vivo stability and biodistribution in electrostatically assembled nanoparticles for systemic delivery. Nano Lett. 2011;11:2096–103.
167. Yu L, Gao YG, Yue XL, Liu SQ, Dai ZF. Novel hollow microcapsules based on iron-heparin complex multilayers. Langmuir. 2008;24:13723–9.
168. Chen X, Gambhir SS, Cheon J. Theranostic nanomedicine. Acc Chem Res. 2011;44:841.
169. Lammers T, Aime S, Hennink WE, Storm G, Kiessling F. Theranostic nanomedicine. Acc Chem Res. 2011;44:1029–38.