Chapter 4
Polysaccharides-Based Microcapsules

Yi Jia, Xiyun Feng and Junbai Li

Abstract LbL-assembled microcapsules have gained great interests in diverse fields due to their versatile and engineering properties. In this chapter, we focus on the microcapsules fabricated from biocompatible and biodegradable polysaccharides. Various interactions, including electrostatic interaction, hydrogen bonding, covalent crosslinking, ionic crosslinking, and host–guest interaction are introduced and employed as driving forces to construct polysaccharide microcapsules with specific stimuli-responsivity. The functionalization of polysaccharide microcapsules with bioactive moieties (such as cell surface receptor ligands) is presented and their applications in cancer therapy and blood substitutes are highlighted.

Keywords Polysaccharides · Layer-by-layer assembly · Schiff base · Microcapsule · Cancer therapy · Blood substitute

4.1 Introduction

Multifunctional microcapsule systems have attracted great attention because of their wide potential applications in fields of catalysis, energy storage, cosmetics, especially in drug delivery, and other biomedical applications. In the past few decades, numerous approaches have been developed for the construction of multifunctional microcapsule systems and significant progress has been made. As a powerful molecular assembly technique, layer-by-layer (LbL) assembly has been extensively used for the fabrication of multifunctional microcapsules because it possesses engineered features including size, shape, thickness, composition, permeation, and

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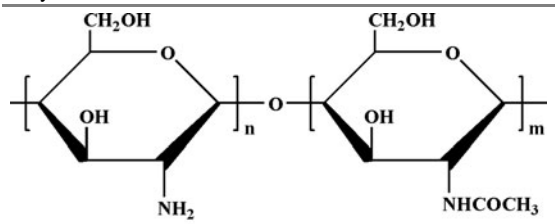
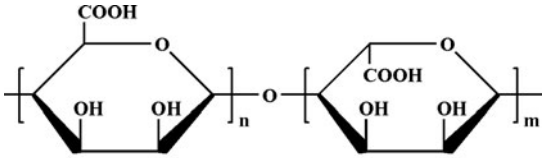
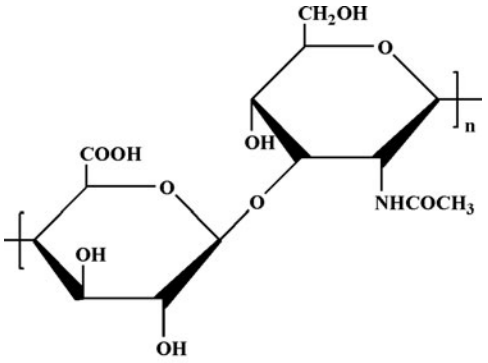
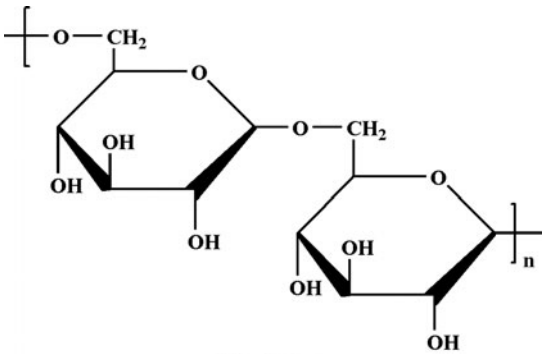
the capability of incorporating different functional molecules [1–4]. The microcapsules are fabricated by the sequential deposition of polymers onto a sacrificial template followed by the removal of template. Almost all the interactions (such as electrostatic interaction, hydrogen bonding, covalent crosslinking, charge transfer, metal coordination, specific recognition, etc.) could be used as the driving force for the LbL assembly of microcapsules. Therefore, an unprecedented variety of molecules or materials were selected as wall components and a wide variety of LbL-assembled microcapsules were obtained [1, 2, 5–7]. Microcapsules fabricated from biomolecules are of special interest owing to their biocompatibility, biodegradability, specific molecular recognition ability, simple modifiability, and easy availability for bottom-up fabrication. Many biomolecules, such as phospholipids, polysaccharides, peptides, and proteins are employed as building blocks to construct multifunctional microcapsule systems and have successfully applied in drug carriers, bioreactors, biosensors, and other biomedical fields [2, 7–11].

In this chapter, we specifically focus on polysaccharides-based microcapsules and show some of their most significant applications in biomedical field. In details, we first describe the main features of polysaccharides and outline the basic principles for the preparation of polysaccharides-based microcapsules via LbL assembly techniques. We then present the biomedical applications of these polysaccharides-based microcapsules, including cancer therapy and blood substitutes.

4.2 The Structure and Properties of Polysaccharides

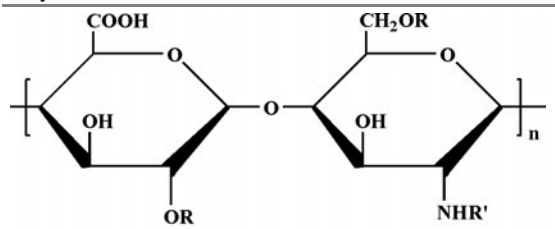
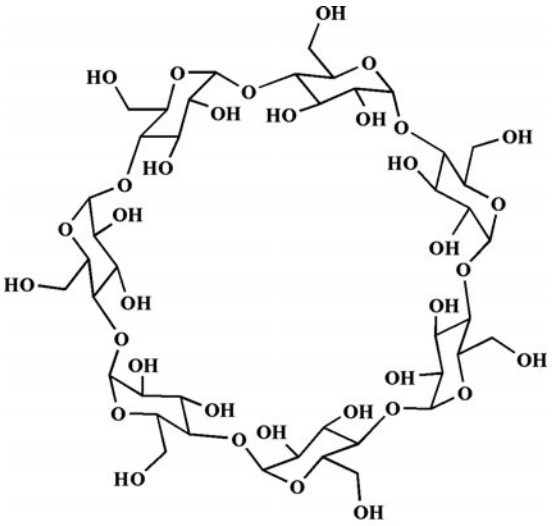
Polysaccharides are polymers of monosaccharides linked by glycosidic bonds. These natural polymers are the most abundant renewable resource on the Earth, with an annual formation rate exceeding the world production rate of synthetic polymers by several orders of magnitude. They have various origins including algal origin (e.g., alginate, agar, and carrageenan), microbial origin (e.g., dextran and xanthan gum), plant origin (e.g., cellulose, starch, pectin, and guar gum), and animal origin (e.g., chitosan, chondroitin, heparin, and hyaluronic acid) [8, 12, 13]. The chemical structures and main properties of some conventional polysaccharides are listed in Table 4.1. These polysaccharides are diverse in chemical structures, molecular weight, physicochemical properties as well as biological activities. However, all of the polysaccharides have some favorable characteristics in common such as excellent biocompatibility, biodegradability, stability, hydrophilicity, low toxicity, low immunogenicity, and ease of chemical modification. These outstanding behaviors of polysaccharides are mainly attributed to, (i) originating from extracellular matrices of plant and animal tissues, (ii) bearing numerous terminal hydroxyl groups of glucose units, (iii) existence of a large number of active functional groups (free carboxyl, primary amino, acetamido, etc.). Due to the presence of these active groups on the polysaccharides backbone, chemical functionalization can be easily realized through oxidation, sulfation, amidation,

Table 4.1 The chemical structures and main properties of conventional polysaccharides

Polysaccharides	Main properties
 Chitosan (CHI)	The only natural polycationic polysaccharide; soluble in acidic solution; facile adherence to mucosal surfaces; wide availability, superior wound-healing, and antibacterial properties
 Alginate (ALG)	Water soluble at physiological pH and highly mucoadhesive
 Hyaluronic Acid (HA)	Water soluble; non-immunogenic; the only non-sulfated glycosaminoglycans; plays key roles in cell motility, proliferation, and differentiation; can function as a lubricant and shock absorber; active targeting to specific membrane receptors present on most mammalian cells; water retention ability
 Dextran	Highly water soluble, lacks nonspecific cell binding, and resistant to protein adsorption

(continued)

Table 4.1 (continued)

Polysaccharides	Main properties
 R=H or SO_3H and R'=H, SO_3H or $COCH_3$ **Heparin (HP)**	High negative charge density; anticoagulant activity and antiviral activity; regulate angiogenesis and inhibit complement activation
 **Cyclodextrin (CD)**	Unique cup-like morphology with a hydrophilic exterior and a hydrophobic cavity that enables them to encapsulate small hydrophobic molecules

esterification, or grafting methods, resulting in many kinds of polysaccharide derivatives [8, 14]. Moreover, these hydrophilic groups could form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes), forming specific bioadhesion on mucosal surfaces (such as colon) and prolonging the drug residence time. All these merits make polysaccharides excellent building blocks for biomedical applications.

4.3 Preparation of Multifunctional Polysaccharide-Based Microcapsules

With the development of biotechnology and nanotechnology, more and more polysaccharide-based microcapsules emerge, which greatly enrich the versatility of polysaccharide-based carriers in terms of category and function. According to structural characteristics, these polysaccharide-based microcapsules are prepared mainly via five mechanisms, namely electrostatic interaction, hydrogen bonding, covalent crosslinking, ionic crosslinking, and host–guest interaction.

4.3.1 Polysaccharide Microcapsules Fabricated via Electrostatic Interaction

Electrostatic interaction is considered as one of the main driving forces exploited for LbL assembly. It is attractive for its simplicity and mild preparation conditions. In a general strategy, oppositely charged components are alternately deposited on templates and hollow capsules are obtained followed by removal of templates. Therefore, the assembly of polysaccharide-based microcapsules can be easily obtained through electrostatic interaction of ionic polysaccharides with oppositely charged polysaccharides or polymers. From the standpoint of polyelectrolyte, polysaccharides can be divided into polycationic polysaccharides (chitosan (CHI)) and polyanionic polysaccharides [alginate (ALG), heparin (HP), chondroitin sulfate (CS), hyaluronic acid (HA), carboxymethyl cellulose (CMC), dextran sulfate (DEXS), etc.]. Among these polysaccharides, CHI is the only natural polycationic polysaccharide derived from partial deacetylation of chitin, which consists of glucosamine and N-acetyl glucosamine units. Owing to its wide availability, superior wound-healing and antibacterial properties, CHI is widely used as polycations in the preparation of polysaccharide-based microcapsules. Any polyanionic polysaccharides or their derivatives could be assembled with CHI through electrostatic interaction, such as CHI/ALG, CHI/HP, CHI/CMC, CHI/DEXS multilayer microcapsules, and so on [15–20]. As alternatives to polysaccharides or their derivatives, other charged polymers like polyelectrolyte, proteins, and polypeptides were also exploited as oppositely charged polymers for the fabrication of polysaccharides-based microcapsules [21–23], which could endow the microcapsules with specific properties or functions. Moreover, these polysaccharides-based microcapsules fabricated through electrostatic interaction are often responsive to pH and ionic strength, making it well suitable for stimulus-responsive drug release.

4.3.2 Hydrogen-Bonded Polysaccharide Microcapsules

Since most polysaccharides have numerous active functional groups (typically, carboxyl, carbonyl, hydroxyl and amine groups, etc.) that can serve as hydrogen bond donors and acceptors, hydrogen bonding was also employed in the LbL assembly of polysaccharides-based microcapsules. Compared to electrostatic interaction, hydrogen bonding allows the fabrication of multilayer microcapsules both in aqueous solution and organic solvents, which increase the diversity of components used in the construction of microcapsules. What is more, hydrogen-bond interaction is sensitive to pH and humidity change, endowing the microcapsules with stimulus-responsive drug release behaviors as well as those fabricated via electrostatic interaction [24]. As an example, poly(N-isopropylacrylamide) (PNIPAAm)/ALG multilayer microcapsules were fabricated through hydrogen bonding between amide carboxyl groups of the PNIPAAm and hydroxyl groups of the ALG [25]. These hydrogen-bonded (PNIPAAm)/ALG microcapsules were demonstrated to be pH-responsive for fluorescent probe molecules FITC-dextran (Mw $\sim$ 2000 kDa). It is permeable for FITC-dextran below pH 5.8, while impermeable above pH 6.8. The distinct permeability of the microcapsules is mainly ascribed to the strength of hydrogen bond and ALG-Mn^{2+} complexation formed at different pH values. In addition, the introduction of PNIPAAm also enabled the polysaccharide microcapsules thermosensitivity. This dual sensitivity may provide new opportunities for these capsules as efficient drug delivery carriers.

In general, hydrogen bonding was not used alone in LbL assembly of polysaccharide-based microcapsules, which is always used in combination with other interactions. In the case of the insulin/ALG microcapsules, both hydrogen bonding and electrostatic interaction contributed to the successful assembly of microcapsules [26].

4.3.3 Covalent-Bonded Polysaccharide Microcapsules

As polysaccharides are often weak polyelectrolytes, the microcapsules fabricated through electrostatic interactions were not strong enough and thus restricted their practical applications. To obtain robust polysaccharide microcapsules, covalent crosslinking was introduced in the assembly process. Most of covalent crosslinked polysaccharide microcapsule systems were prepared using the available $–NH_2$ and –OH groups and crosslinkers that can form a number of linkage chemistries, including amine–carboxylic acid bonding and amine–aldehyde bonding (commonly known as Schiff base bonding) [2, 21]. Here, we specifically focus on polysaccharide microcapsule systems fabricated through Schiff base interaction. First, it is worth to mention the outstanding superiority of Schiff base interaction [2, 27]. (i) Schiff base reaction proceeds under ambient conditions without activation and involves only water as a by-product, avoiding the contamination of the

microcapsules by impurities. (ii) Compared to noncovalent bonding, the robustness and stability of covalent Schiff base-bonded polysaccharide microcapsules were significantly improved. (iii) The formation of Schiff base bond enables the microcapsules with autofluorescence, attributing to the $n–\pi^*$ transition of the C=N bonds. (iv) Schiff base bond is a dynamic covalent bond that the stability of these bonds decreases as the pH decreases. All these merits make Schiff base-bonded polysaccharide microcapsules attractive in biomedical application, especially for biological tracing and controlled drug delivery.

Typically, Schiff base-bonded polysaccharide microcapsules can be formed by using small molecule aldehyde or aldehyde polymer as crosslinkers.

4.3.3.1 Glutaraldehyde Crosslinked Polysaccharide Microcapsules

As a familiar crosslinking agent, glutaraldehyde (GA) was frequently used to prepare stable microcapsules. The aldehyde groups of GA can easily react with amino groups of polysaccharides to form Schiff base bonds. After alternate assembly of polysaccharide and GA on decomposable template, hollow single-component or multi-component polysaccharide microcapsules can be obtained [28]. Compared to the microcapsules fabricated through electrostatic interaction, the stability of GA-crosslinked polysaccharide microcapsules against extreme pH treatments was substantially improved. Moreover, other amino-containing compounds such as proteins and peptides could also react with GA to endow the polysaccharide microcapsules with specific recognitions or functions. However, it is noteworthy that GA is relatively toxic to living organisms and it is associated with calcification in certain bioapplications [29]. To reduce the toxicity of GA-crosslinked polysaccharide microcapsules without compromising its stability, lower concentration of GA, or posttreatment with GA after LbL assembly was recommended [15, 30].

4.3.3.2 Aldehyde Polysaccharide-Crosslinked Polysaccharide Microcapsules

In order to avoid the use of toxic GA, researchers tend to pre-functionalized polymer chains of polysaccharides with reactive aldehyde groups. Since most of the polysaccharides have cis-diol groups in their structure, mild periodate oxidation is used to confer the polysaccharide chain with aldehyde functionalities (namely aldehyde polysaccharides) [31], which have highly reactive nature toward amino-containing compounds. Aldehyde polysaccharides are excellent biological crosslinkers due to their low toxicity, biocompatibility, and biodegradability, and they are usually employed both as a crosslinker and as a wall component in covalent assembly of polysaccharide microcapsules [31–33]. Recently, we oxidized ALG to generate alginate dialdehyde (ADA) and prepared CHI/ADA multilayers microcapsules through electrostatic interaction and Schiff base interaction [31], Fig. 4.1. The formation of Schiff base bond enabled the CHI/ADA microcapsules

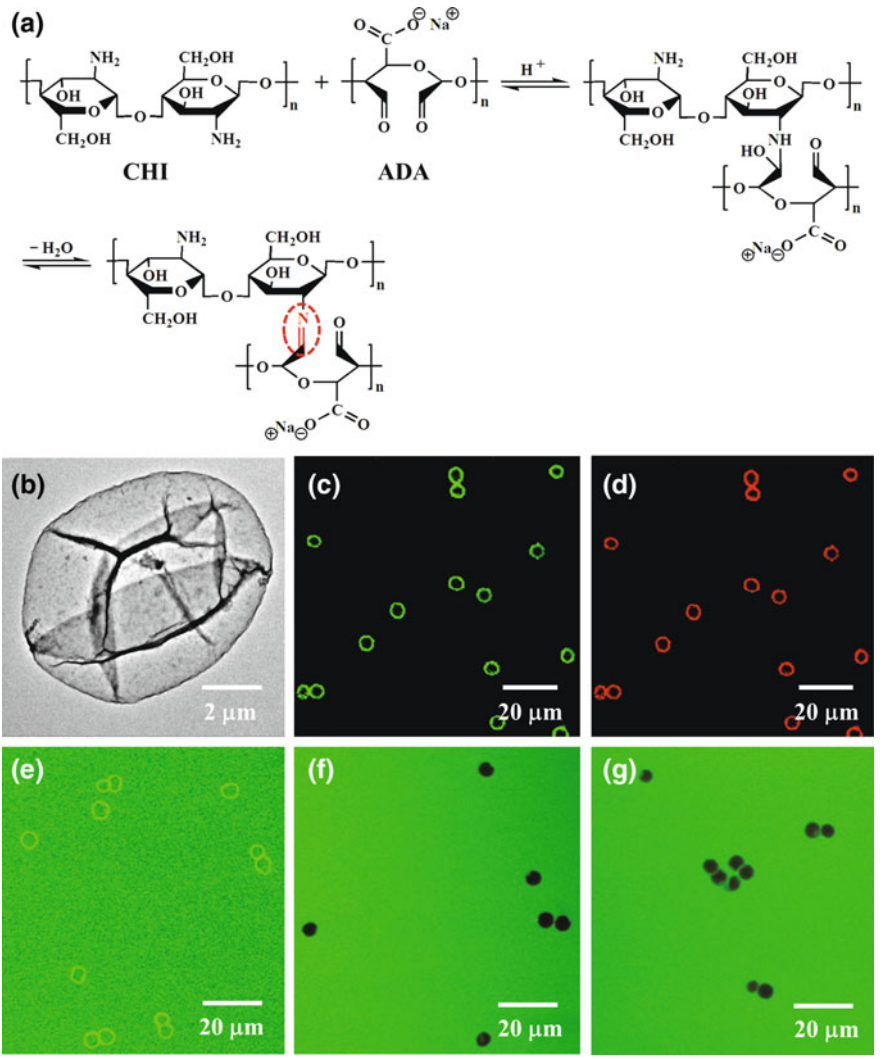


Fig. 4.1 **a** The Schiff base reaction between CHI and ADA, **b** TEM image and **c**–**d** CLSM images of CHI/ADA multilayer microcapsules, **e**–**g** CLSM images of CHI/ADA multilayer microcapsules in different pH media with FITC-dextran (20 kDa) as a probe molecule: pH 5, pH 7, and pH 9 (from *left* to *right*). Reproduced from Ref. [31] by permission of The Royal Society of Chemistry

with enhanced stability and pH-dependent permeability. In acid condition, the crosslinked shells become incompact and permit the diffusion of FITC-dextran (20 KDa), while impermeable in neutral and basic solution. This feature is highly preferable for specific pH-triggered drug release. Moreover, the Schiff base-bonded polysaccharide microcapsules were found to be autofluorescent, which ascribe to

the n–π^* transition of the C=N bonds. The autofluorescence would be beneficial in tracing and monitoring safety and efficacy of microcapsules in organisms, avoiding the use of extra fluorochromes. Therefore, this study provides a simple and promising strategy for making autofluorescent and pH-responsive materials. Based on this, we further used ADA to crosslink with amino-containing small molecule, drugs, and proteins to obtain autofluorescent and pH-responsive polysaccharide microcapsules and confer them with specific or extra functions [33–36]. In addition to ADA, we also synthesized other aldehyde polysaccharides, such as dialdehyde heparin (DHP) and dialdehyde starch (DAS), and then crosslinked them with CHI to prepare polysaccharide microcapsules [31]. The successful preparation of CHI/DHP and CHI/DAS multilayers microcapsules demonstrated that this approach is also applied to other polysaccharides and their derivatives, further confirming the versatility and broad applicability of the method.

4.3.4 Ionic-Crosslinked Polysaccharide Microcapsules

Ionic crosslinking is a specific electrostatic interaction. In ionic crosslinking, polyions or small ionic molecules react with the polyelectrolytes and the formed ionic bond act like bridges along the polymer chains. Compared with other interactions, ionic crosslinking is more simple and mild. Once opposite charged polymers and ions are mixed together, ionic networks are formed immediately. For polycationic CHI and their derivatives, polyanion tripolyphosphate (TPP) is the most widely used ionic crosslinkers [37, 38]. It can form a gel by ionic interaction between positively charged amino groups of CHI and negatively charged counterions of TPP. Compared to other polyphosphates that only bind on the surface of CHI droplets, TPP can diffuse into CHI droplets or films freely to form ionically crosslinked chitosan beads or films [39]. For polyanionic polysaccharides bearing carboxylic groups on the molecular chains, di- and tri-valent ions (Ca^{2+}, Ba^{2+}, Sr^{2+}, Al^{3+}) are suitable ionic crosslinkers. Typically, ALG forms hydrogels by means of Ca^{2+}, which positions in the interstices between G blocks, leading to an ordered conformational structure called “egg-box” array [38]. In our earlier study, we used Ca^{2+} as crosslinkers to alternatively assemble with ALG, nano-hydroxyapatite (nHA) on tube-like template, and 3D hydrogel scaffolds were finally obtained [40]. By simply regulating the Ca^{2+} crosslinking intensity, the elastic modulus, swelling behavior, permeability, and diffusivity of the hydrogel scaffold can be easily tuned. In another study, porous spherical $CaCO_3$ are used as effective ‘casting’ template to prepare Ca^{2+}-crosslinked ALG microcapsules [41]. Here, $CaCO_3$ microspheres were used not only as a template but also as an ionic crosslinking agent provider. The crosslinking of Ca^{2+} and ALG happened when $CaCO_3$ template was dissolved. Such Ca^{2+}-crosslinked ALG microcapsules or beads are pH-responsive and have

been intensively explored for site-specific oral delivery. Generally, Ca^{2+} crosslinked ALG microcapsules showed slow drug release in acidic pH due to the poor swelling. Oppositely, in phosphate buffer and simulated intestinal fluid, the swelling and the release are promoted as phosphate ions extract the Ca^{2+} from ALG hydrogels microcapsules.

4.3.5 Polysaccharide Microcapsules Fabricated via Host–Guest Interaction

Cyclodextrins (CDs) are cyclic oligomers of glucose that have a hydrophilic exterior and a hydrophobic cavity. They can act as hosts to hydrophobic molecules and form water-soluble inclusion complexes with small molecules and portions of large compounds. This unique ability enables CDs-based assemblies widely utilized in the biomedical and pharmaceutical fields to improve bioavailability of poorly soluble or biodegradable drugs and to enhance permeability of biological membranes [42, 43]. Supramolecular microcapsules (SMCs) based on CDs and their derivatives exploited host–guest interactions as the main driving force. Compared to the well-known electrostatic interactions, supramolecular host–guest interactions may offer additional benefits, such as tunable binding affinity, reversibility, incorporation of neutral molecules and/or biomolecules, straightforward control over the growth process and layer thickness by structural and steric design of the building blocks, and so on. Zhang and co-workers reported the preparation of SMCs by alternately depositing carboxymethyl dextran-graft-β-CD (CMD-g-β-CD) and polyaldehyde dextran-graft-adamantane (PAD-g-AD) on $CaCO_3$ particles via host–guest interaction [44]. Simultaneously, adamantine-modified doxorubicin (AD-Dox) was also loaded on the LbL wall via host–guest interaction. Because the AD groups were linked to dextran or Dox via pH-cleavable hydrazone bonds, AD moieties can be removed under the weak acidic condition, leading to destruction of SMCs and release of Dox. Meanwhile, they designed photo-switchable polysaccharide microcapsules based on host–guest interactions between α-cyclodextrin (α-CD) and azobenzene (Azo) [45]. The obtained capsules could be dissociated upon UV irradiation due to the transformation of trans–Azo to cis–Azo, followed by the release of the drug. Analogously, some functional molecules or responsive polymers could be assembled or grafted with CDs to provide the polysaccharide microcapsules with multi-responsiveness or specific recognitions and serve as smart drug reservoir [42, 46].

4.4 Biomedical Applications of the Polysaccharide-Based Microcapsules

4.4.1 Cancer Therapy

LbL-assembled polysaccharide microcapsules usually have good biocompatibility, biodegradability, low immunogenicity with tunable size, morphology, surface properties, and permeability. Thus, they have great advantages and important applications in biomedicine. One of the most important applications of polysaccharide microcapsules is used as drug carriers for cancer therapy. Their high loading capacity and tunable properties are favorable to deliver and unload anticancer drugs. Effective cancer therapy is achieved by specific targeting to tumor and controlled drug release. Typical examples are summarized in Table 4.2 and introduced.

Table 4.2 LbL-assembled polysaccharide microcapsules and their applications

Assembled pairs	Driving force	Application	References
CHI/ALG	Electrostatic interaction	Folate receptor mediated targeting	[16]
TRAIL/ALG	Electrostatic interaction	Death receptor mediated targeting	[47]
CHI/HP	Electrostatic interaction	Enzyme-responsive drug release	[18]
CHI/DEXS	Electrostatic interaction	Enzyme-responsive drug release	[20]
PNIPAAm/ALG	Hydrogen bond	pH- and temperature-responsive drug release	[25]
Insulin/ALG	Electrostatic interaction and hydrogen bond	Sustained drug release	[26]
CHI/ADA	Electrostatic interaction and covalent Schiff base bond	pH-responsive drug release	[31]
Dox/ADA	Electrostatic interaction and covalent Schiff base bond	pH-responsive drug release	[35]
ADA/CM	Electrostatic interaction and covalent Schiff base bond	pH- and redox-responsive drug release	[34]
PLGA/CHI	Electrostatic interaction and covalent amide bond	pH-responsive drug release	[23]

(continued)

Table 4.2 (continued)

Assembled pairs	Driving force	Application	References
ADA/Ca^{2+}	Ca^{2+} crosslinking	Folate receptor mediated targeting, chemotherapy and PDT	[41]
HA-CD/PLL	Host–guest interaction	CD44 receptor mediated targeting,	[46]
CMD-g-β-CD/PAD-g-AD	Host–guest interaction	pH-responsive drug release	[44]
PAA-C_{12}-Azo/CMD-g-α-CD	Host–guest interaction	Photo-responsive drug release	[45]
ADA/CAT	Covalent Schiff base bond	PDT	[36]
ADA/fLuc	Covalent Schiff base bond	PDT	[33]
CHI/ALG	Electrostatic interaction	PTT	[48]
Hb/DHP	Electrostatic interaction and covalent Schiff base bond	Blood substitutes	[32]

Abbreviations
TRAIL Tumor necrosis factor-related apoptosis-inducing ligands; *ACHI* Adenine-modified chitosan; *HA-CD* HA-modified CD; *CMD-g-α-CD* Carboxymethyl dextran-graft-α-CD; *PAA-C12-Azo* poly(acrylic acid) Naminododecane p-azobenzeneaminosuccinic acid; *PLGA* poly (l-glutamic acid); *fLuc* firefly luciferase

4.4.1.1 Specific Targeting to Tumor

Specific targeting to tumor cells could increase bioavailability of anticancer drugs as well as reduce toxic side effects to normal cells resulting from nonspecific uptake [49]. The strategies for specific targeting polysaccharide microcapsules to tumor cells mainly employ surface functionalization with bioactive moieties such as cell surface receptor ligands (receptor mediated targeting).

Hyaluronic acid (HA) is a natural anionic polysaccharide that represents one of the main constituents of the extracellular matrix (ECM) in mammalian organisms. Moreover, HA can bind to specific receptor cluster determinant 44 (CD44) that overexpress in various cancer cells [50]. When HA is bound to CD44, it is cleaved into lower molecular weight segments as low as 20 kDa by the membrane anchored enzyme Hyal-2 and readily internalized to the cells. Therefore, polysaccharide HA itself is a tumor cell surface receptor ligand and HA-containing microcapsules are suitable for tumor-targeted drug release. Making use of the specific tumor recognition of HA and the unique inclusion complexation of CD, Auzély-Velty and co-workers fabricated polysaccharide microcapsules through LbL deposition of HA-modified CD molecules and poly(L-lysine) (PLL) on calcium carbonate particles [46]. The poorly water-soluble anticancer drug paclitaxel (PTX) molecules

were loaded in the LbL assembled wall via selectively complexed by CD. PTX-loaded polysaccharide microcapsules showed greater cytotoxicity than PTX in solution for breast cancer cells that overexpressed HA receptor, suggesting that they were specifically bound to cancer cells and taken up by the cancer cells via HA receptor-mediated endocytosis.

The folate receptor (FR) is a confirmed tumor-associated antigen that binds folate and folate-modified assemblies with very high affinity and shuttles these assemblies inside cells via an endocytic mechanism. A wide variety of drug carriers employed folate as the ligand for FR-positive tumor cells targeting and showed enhanced cytotoxicity to tumors. Our group fabricated CHI/ALG and ALG/Ca^{2+} microcapsules and coated these polysaccharide microcapsules with folate-linked liposomes [16, 41]. In vitro tests show that the introduction of folate on the surfaces of polysaccharide microcapsules leads to selective recognition to cancer cells with increased cell uptakes and enhanced anticancer efficiency. Besides folate, we also exploited TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) as tumor cell surface receptor ligands to modify polysaccharide microcapsules. TRAIL is a cognate ligand of tumor necrosis factor (TNF) receptors. It binds or interacts with cell surface proapoptosis receptors to assemble a cell-death-inducing signaling complex that can induce cell apoptosis. Specially, TRAIL can induce apoptosis selectively in many cancer cell lines, whereas normal cells are relatively resistant [49]. Based on this, we fabricated TRAIL/ALG multilayers on the surfaces of Dox-loaded $CaCO_3$ particles via LbL assembly technique (Fig. 4.2) [47]. Flow-cytometry analysis demonstrated that TRAIL as the outer layer of the system retained its activity and remarkably enhanced the selectivity of polysaccharide nanoparticles to recognize cancer cells. The ratio of TRAIL-functionalized polysaccharide particles uptake by cancer cells reached up to 91.3 ± 4.1%, while nude $CaCO_3$ particles were only 43.5 ± 3.3%. After cell endocytosis, DOX loaded in the polysaccharide particles slowly released in tumor cell. Notable anticancer activity of the nanocomposite was observed due to the synergistic effect of TRAIL targeted extrinsic apoptosis pathway and DOX triggered apoptosis pathway.

4.4.1.2 Controlled Drug Release

Controlled drug delivery systems (DDSs) provide an alternative approach to regulating the bioavailability of therapeutic agents. In DDSs, an active therapeutic is incorporated into a polymeric network structure and the drug is released from the system in a predefined manner. LbL-assembled polysaccharide microcapsules are excellent DDSs that have good biocompatibility, biodegradability, and tunable properties. Controlled drug release from polysaccharide microcapsules can be fine-tuned via endogenous and exogenous stimuli (such as pH, redox, enzyme, glucose, temperature, light illumination, etc.).

Since the tissues around cancer cells have a lower pH than those of normal cells, pH-responsive polysaccharide microcapsules were designed for the controlled release of anticancer drugs. Doxorubicin (Dox) is one of the most common

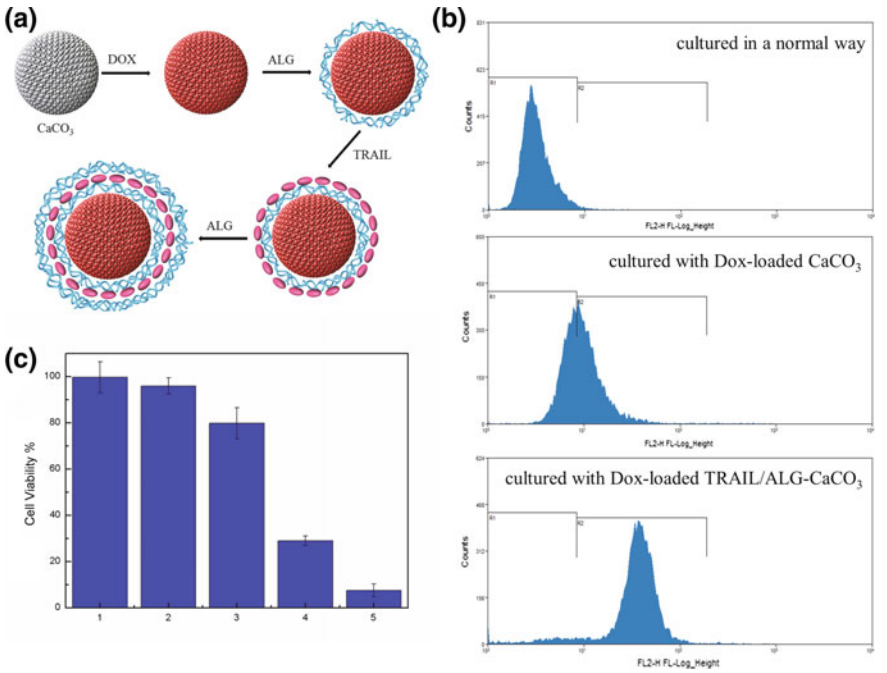


Fig. 4.2 **a** Schematic illustration of the fabrication of Dox-loaded TRAIL/ALG–$CaCO_3$ nanocomposites, **b** Flow-cytometry diagrams of the uptake of hollow shells by HeLa cells cultured in three different way, **c** HeLa cells viability in vitro measured by MTT assay, and the cells were cultured: [1] in a normal way; [2] with nude $CaCO_3$ nanocomposites; [3] with TRAIL/ALG-$CaCO_3$ nanocomposites; [4] with Dox-loaded $CaCO_3$ nanocomposites; [5] with Dox-loaded TRAIL/ALG-$CaCO_3$ nanocomposites. Reproduced from Ref. [47] by permission of The Royal Society of Chemistry

low-molecular chemotherapeutic agents used in the cancer treatment. It could be encapsulated in capsules through preloading in porous template or postloading via physical adsorption. In view of the existence of abundant amino groups on Dox, our group used ADA to crosslink with Dox through covalent Schiff base bond (Fig. 4.3) [35]. As discussed before, the stability of Schiff base bonds decreases as the pH decreases [2]. Thus, controlled release of Dox could be achieved through pH stimulus. As shown in Fig. 4.3b, the release rate of Dox from Dox/ADA capsules was much faster at pH 5.5 compared to that at pH 7.4. Moreover, the Dox/ADA microcapsules induced sustained drug release and exhibited high efficiency against tumor cell proliferation.

Considering the differences between the environment in the bloodstream (neutral pH and low concentration of GSH) and subcellular structures in tumor cells (low pH and high concentration of GSH), we further prepared pH and redox-responsive $(ADA/CM)_5$ microcapsules via covalent Schiff base bond and disulfide bond [34]. The microcapsules were shown to be permeable at low pH due to the hydrolysis of

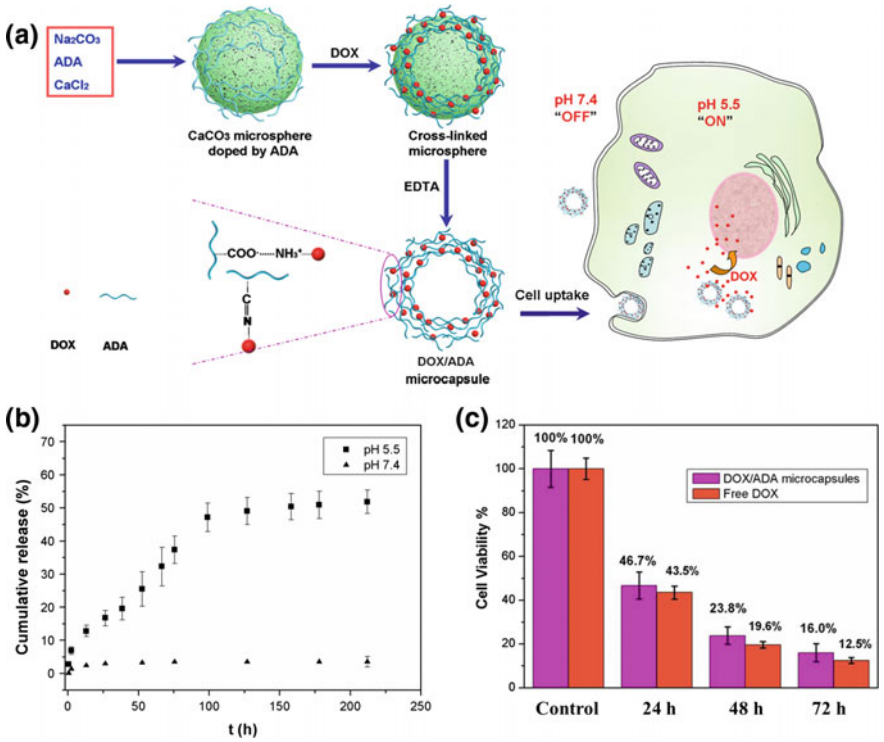


Fig. 4.3 **a** Schematic illustration of the fabrication of DOX/ADA microcapsules against tumor cell proliferation, **b** quantitative analyses of in vitro release of DOX from DOX/ADA microcapsules at pH 5.5 and 7.4 at 37 °C, **c** cytotoxicity results for MCF-7 cells with a drug concentration (14 μg mL^{-1}) incubated with different time stages. Reproduced from Ref. [35] by permission of John Wiley & Sons Ltd

Schiff base bond, or after adding a reducing agent to cleave the disulfide bonds within the formed multilayers. Thus docetaxel-loaded $(ADA/CM)_5$ capsules were readily triggered drug release in tumor cells and prevented unwanted release in bloodstream.

In comparison with endogenous stimuli (pH, redox, enzyme, glucose), exogenous stimuli (light, ultrasound, magnet) are more simple and easy to manipulate. Light is a superior external stimulus for drug release since it can be remotely manipulated to attain both spatial and temporal control with high precision [51]. Photosensitive polysaccharide microcapsules were fabricated by assembling polysaccharides with photosensitive polymers or particles [33, 36, 45]. Drugs can be released from these polysaccharide microcapsules by photocleavage, photoisomerization, or photoactivation of the assembled polymers upon light irradiation.

Photodynamic therapy (PDT) and photothermal therapy (PTT) are two typical and much more promising cancer therapies that have been widely reported [52, 53]. Both therapies possess obviously outstanding advantages such as specific

spatiotemporal selectivity, minimal side effects, low toxicity and remote controllability, especially avoiding chemo-resistance. Photodynamic therapy (PDT) is a treatment that employs light illumination produced reactive oxygen species (ROS) to kill cancer cells. It usually consists of three components: photosensitizers (PSs), light and oxygen-containing substrates (e.g., molecular oxygen, water). These three components are indispensable and play crucial roles in the efficacy of PDT. In the PDT treatment, PSs were first injected into patients and then irradiated with light of appropriate wavelength at the diseased tissues. Nevertheless, most of PSs are insoluble and easily aggregate in aqueous media, which reduced the quantum yield of ROS and limited their anticancer efficacy. Therefore, many kinds of PSs' carriers have been developed to overcome this problem. For example, our group employed LbL-assembled ALG/CHI microcapsules as PSs' carriers and loaded hypocrellin B (HB) on shells of microcapsules [54]. These HB-loaded polysaccharide microcapsules showed high cytotoxicity after exposure to visible light. It indicates that polysaccharide microcapsules are suitable as PSs' carriers and can efficiently avoid unwanted aggregation.

PSs are usually excited by short-wavelength UV–Vis light, so its poor tissue penetration has become the obstacle in treating deep-seated tumors below the skin, which hinders the widespread clinical use of PDT [52]. Fortunately, the external near-infrared (NIR) light/X-ray-excited PDT, as well as internal self-illuminating PDT have brought about a novel technique of deep PDT with great promise for the efficient treatment of deep-seated tumors. Recently, we designed and fabricated a kind of bioluminescent microcapsules by covalent LbL assembly of ADA and luciferase (fLuc) on luciferin-coprecipitated $CaCO_3$ microparticles [33]. In the presence of O_2, Mg^{2+}, and ATP, fLuc could catalyze the oxidation of its substrate luciferin to produce light. This light was then used to activate PSs for the production of active oxygen ($^1O_2$), as shown in Fig. 4.4. Cytotoxicity tests confirmed that PSs in the capsules could be activated by the excitation of bioluminescent microcapsules in the dark without external light and effectively prevent the proliferation of tumor cells.

In addition to PSs' aggregation and poor penetration depth of light, PDT is also restricted by the low concentration of molecular oxygen (O_2) in tumor's hypoxic microenvironment [53]. To increase the availability of O_2, an oxygen-generating system was constructed by LbL assembly of CAT/ADA [36]. In this system, catalase (CAT) catalyzes the decomposition of intracellular hydrogen peroxide (H_2O_2) into H_2O and O_2. The produced O_2 can be utilized by PSs to produced $^1O_2$ under light excitation and thus effectively enhanced the anticancer efficiency of PDT. Moreover, the reduced cellular levels of H_2O_2 may also help to inhibit tumor proliferation.

In combination with desirable components, LbL-assembled polysaccharide microcapsules hold great promise for efficient cancer therapy and well tackle the challenges of both chemotherapy and PDT. This also applies for PTT. LbL-assembled CHI/ALG multilayer microcapsules loaded with gold nanorods (GNRs) were constructed and can be used for enhanced PTT (Fig. 4.5) [48]. These polysaccharide microcapsules were served as support matrices and can avoid

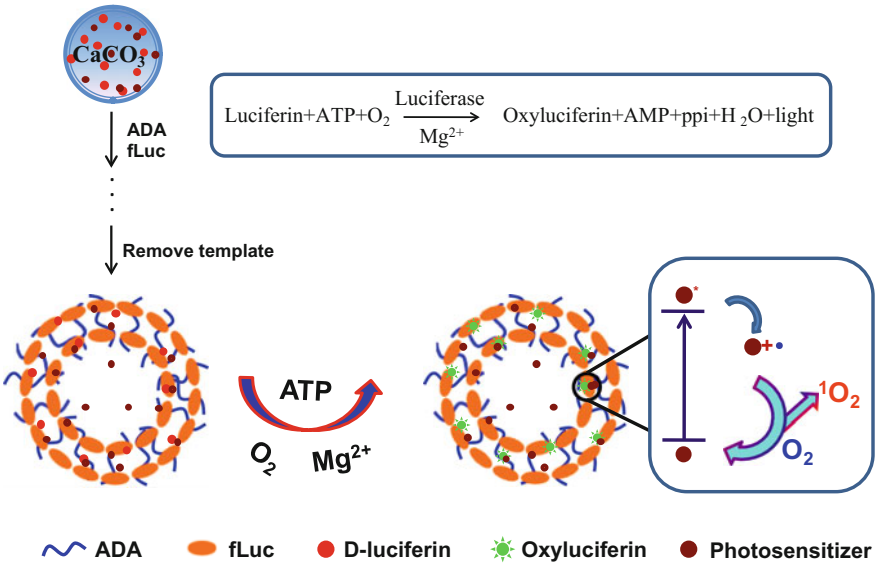


Fig. 4.4 The fabrication and bioluminescent process of ADA/fLuc microcapsules. Reproduced from Ref. [33] by permission of John Wiley & Sons, Ltd

unwanted aggregation of GNRs. GNRs were reported to have a strong absorption in the NIR region and induce faster and higher heating effects compared to other gold materials. Upon irradiation with NIR light, the GNRs absorb the energy and quickly transform it into heat, which may selectively ablate cancerous cells within the irradiated zone and achieve high efficiency to suppress tumor growth. More interestingly, the significant increase of local temperature triggered the autonomous movement of these polysaccharide microcapsules, with the speed ranging from 1.3 to 23.27 μm/s upon the increase of incident laser power. This system is promising as a versatile drug carrier and paves a new way to design self-propelled drug delivery system for diverse biomedical application in future.

4.4.2 Blood Substitutes

As essential structural components of both plant and animal cells, polysaccharides play many different roles in vivo. For instance, cellulose and chitin are the major components of cell walls; starch and glycogen act as energy reserves in biological systems; HA and CS are responsible for the unique hydration and mechanical properties of cartilage, synovial fluid, and tendons [55]. Therefore, proper polysaccharides need to be selected for specific bioapplications.

Heparin (HP) is known as an anticoagulant and growth factor-binding agent. It was also found to inhibit cancer cell adhesion, deactivate heparinase, and activate

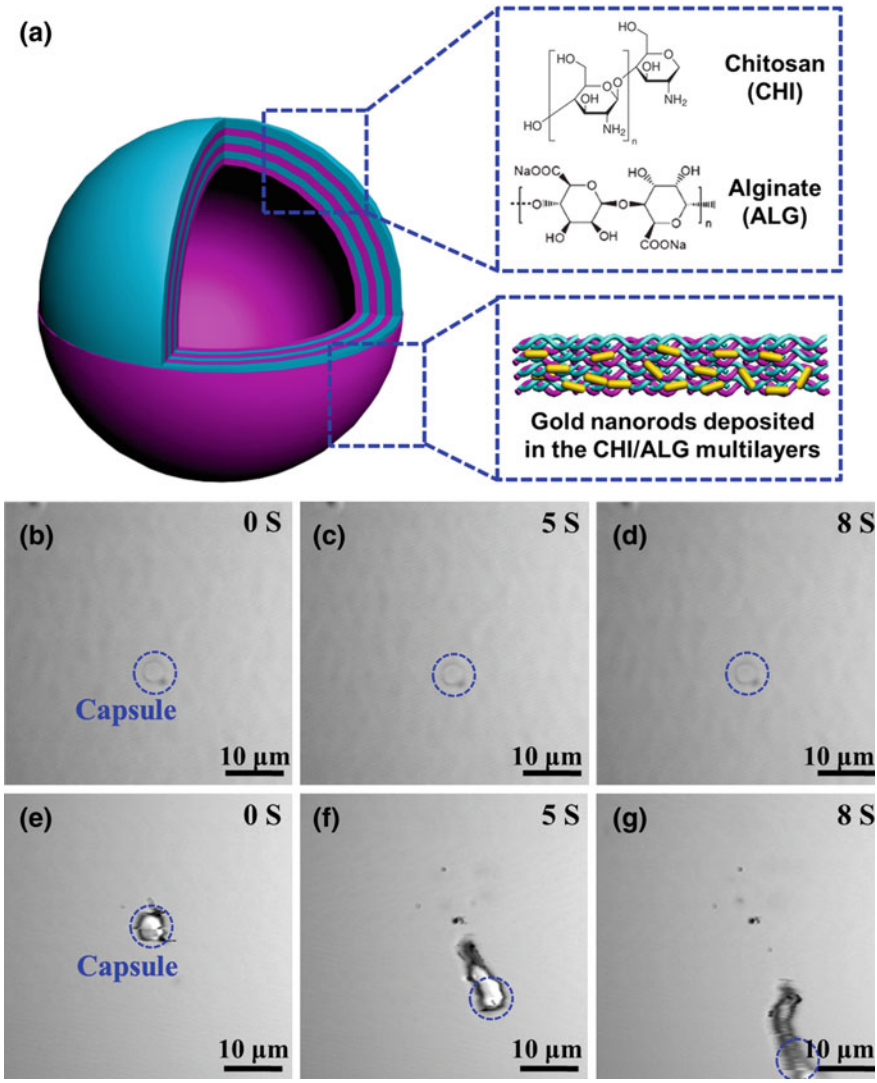


Fig. 4.5 **a** Schematic illustration of CHI/ALG/GNRs multilayer microcapsules, **b–d** video frames of CHI/ALG/GNRs microcapsules without NIR laser illumination; **e–g** video frames of CHI/ALG/GNRs microcapsules under continuous NIR laser illumination (3.23 J/cm^2). Reprinted from Ref. [48], Copyright 2015, with permission from Elsevier

natural killer (NK) cells. Thus, it has been extensively exploited to develop biofunctional multilayer films [17, 56]. However, strong anticoagulant activity limits its use. Dialdehyde heparin (DHP) produced by periodate oxidation of HP, was shown to be a good alternative to HP [32, 56]. It has lower anticoagulant properties than HP, which could prevent severe bleeding complications in biomedicine. Meanwhile, DHP has good biocompatibility, biodegradability, and hemocompatibility as same as HP. In view of its excellent features, DHP was used both as a wall component and as a crosslinker in the fabrication of Hb/DHP multilayer microcapsules to mimic artificial red blood cells (RBCs) (Fig. 4.6) [32]. Hemoglobin (Hb) in RBCs possesses the ability of delivering and releasing oxygen. However, stroma-free Hb could not directly be used as an oxygen carrier since it is liable to dissociate into dimers, leading to severe renal toxicity. The Schiff base bond formed between Hb and DHP in the microcapsules effectively prevent the dissociation of the Hb tetramer into dimers and thus avoiding renal toxicity. Moreover, the Schiff base-bonded Hb/DHP microcapsules were found to be autofluorescent. This is helpful to monitor the microcapsules in humans, avoiding the use of extra fluorochromes. DHP as the outer layer also provides the microcapsules with good "stealth" effect similar to PEGylation, which would be favorable to reduce uptake by macrophages and prolong the blood retention time of microcapsules in vivo. More importantly, DHP-crosslinked Hb maintained its intrinsic bioactivity of reversibly binding and releasing oxygen. All these characteristics make Hb/DHP microcapsules good candidates as blood substitutes.

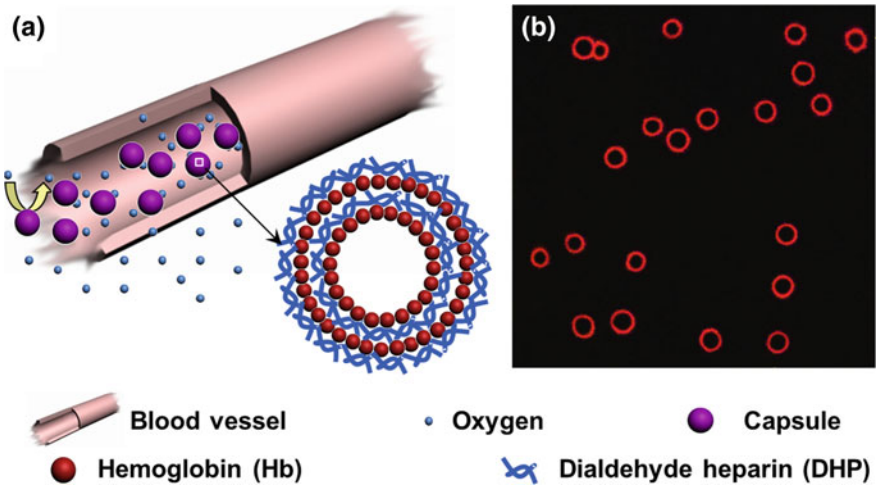


Fig. 4.6 **a** Hb/DHP multilayer microcapsules fabricated through Schiff base interactions for use as blood substitutes, **b** CLSM image of Hb/DHP microcapsules. Reproduced from Ref. [32] by permission of John Wiley & Sons Ltd

4.5 Conclusions

Polysaccharides are the most abundant renewable resources on the Earth that possess excellent biocompatibility, biodegradability, stability, hydrophilicity, low immunogenicity, and ease of chemical modification. They are gaining increasing attention as components to construct well-defined micro/nanometer-sized structures. In this chapter, we highlight recent progress on LbL-assembled polysaccharide microcapsules. Different interactions were used as driving forces to prepare polysaccharide microcapsules and contributed to diverse stimuli-responsivity. In combination with specific bioactive moieties (such as cell surface receptor ligands), the as-prepared functional polysaccharide microcapsules are highly promising as excellent drug carriers for both specific targeting and controlled release in various cancer therapy (chemotherapy, PDT, PTT, combined therapy, etc.). In addition, taking advantages of their unique properties or native functions, polysaccharide microcapsules could also be applied in other biomedical fields, such as blood substitute. With the rapid development of science and nanobiotechnology, LbL-assembled polysaccharide microcapsules are bound to embrace a brighter future and eventually realize important applications in biomedical field.

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