# Functional multilayered capsules for targeting and local drug delivery

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One of the key challenges in the field of bio-nanotechnology for drug delivery systems (DDS) is the development of nano- or micro-sized delivery carriers possessing both targeting functionalities for specific tissues or cells, and controlled release properties for encapsulated drug molecules, proteins and genes. Hollow capsules developed by layer-by-layer (LbL) assembly have attracted much attention over the past few years owing to their ability to be modified, their capacity to encapsulate a wide range of chemicals, and the variety of functionalities with which they can be enhanced. Current research on LbL capsules focuses on the development of functionalized capsules for specific targeting of cancer or immune cells, and on controlling their release properties by environmental stimuli. This review discusses recent advances in DDS using functional hollow capsules specific for the cellular and tissue-targeted delivery, as well as stimuli-responsive controlled release. DDS based on functional hollow capsules may contribute to the development of new nano-medicines.

**Keywords:** controlled release, drug delivery system, hollow capsule, layer-by-layer, stimuli-responsive, targeting

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## 1. Introduction

The development of safe and functional drug delivery carriers is a challenging task not only in drug delivery system (DDS), but also in the tissue regeneration fields [1]. A colloidal delivery system is one of the most promising, because it may reduce undesired toxic side effects and improve the therapeutic effect. Over the past few decades, self-assembling drug carriers such as polymeric micelles, particles and liposomes have been actively studied for the development of functional DDS carriers for therapeutic agents, such as small molecules, peptides, proteins and genes [2-8]. In the late 1990s, a new approach to fabricate nano- or micrometer-sized hollow capsules was discovered by Caruso and co-workers [9-11]. This method is based on layer-by-layer (LbL) assembly technology, which is an appropriate method to prepare nanometer-sized films on a substrate through alternative immersion into interactive polymer solutions [12,13]. The hollow capsules obtained by LbL assembly have attracted particular interest, largely because of the capability readily to tailor their properties, such as composition, size, surface charge, porosity and surface functionality. Furthermore, the stepwise formation of these capsules allows the introduction of multiple functionalities, and thus a new class of carriers with unprecedented structures and functions could be designed and fabricated. For example, multilayered capsules can be assembled from synthetic and naturally occurring polymers, polypeptides, proteins and DNA, and their surfaces can be easily modified by the immobilization of functional molecules [14-16]. In addition, the release of encapsulated drug molecules from the multilayered capsules is easily

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controllable by changing the environmental pH and ionic strength, and environmental stimuli such as light and magnetic field [17].

This review focuses on the development of multilayered hollow capsules, which are intelligent in the sense that they recognize target tissues or cells and release their contents as a consequence of the environmental stimuli, whereas other reviews have reported on the physicochemical properties [14,18], permeability [17,19], use as nanoscale reactors [20], or biofunctionality [21] of LbL hollow capsules. Both the advantages and disadvantages are discussed of functional hollow capsules, to understand what specific opportunities there are for LbL hollow capsules in the DDS field. This review is expected to be of interest to scientists in multidisciplinary fields focusing on DDS research using multilayered hollow capsules fabricated by LbL assembly.

## 2. Fabrication of multilayered hollow capsules

### 2.1 Layer-by-layer assembly

Layer-by-Layer assembly was discovered at the beginning of the 1990s by Decher and Hong [12,13]. This technique is based on the alternate adsorption of oppositely charged polymers on a substrate. A charged substrate, for example, a silicon wafer or glass, is immersed into an aqueous solution of a charged polyelectrolyte. After immersion into the solution, excess polymers adsorbed on the substrate are removed by washing with water. For the next step, the substrate with the adsorbed polymers is immersed into the second polyelectrolyte solution, which has an opposite charge to the first polyelectrolyte solution. This second polyelectrolyte adsorbs onto the first polymer layer, and the washing process is performed to remove the excess polyelectrolyte. The whole procedure can be repeated as many times as one desires. In this way, multilayered films with tuneable physicochemical properties such as surface charge and the number of layers can be easily prepared on the substrate. It is easy to regulate the film thickness at the molecular level by changing the step sequence.

The LbL hierarchic construction of films can be achieved by introducing heterogeneous polymers, resulting in new polymer blend systems [22]. Almost any type of charged species, including synthetic polymers, proteins [23-27], nucleic acids and DNA [28,29], viruses [30], polypeptides [31-33], polysaccharides [34-37], nanoparticles [38], nanotubes and nanowires [39,40] and nanoplates [41], can be successfully used as components to prepare LbL films. The formation force of LbL films is not limited to electrostatic interactions only. Assemblies based on hydrogen bonding [42], charge transfer [43], covalent bonding [44], biological recognition [45-47] and hydrophobic interactions [48] have also been investigated. The authors also focused on the fabrication of LbL films by van der Waals interactions [49-52]. One of the most eye-catching applications of LbL assembly involves polyelectrolyte capsules fabricated by LbL coating on colloidal particles, and the application of these multilayered capsules as drug carriers has extended LbL research in the field of DDS [53]. The following sections describe further information on multilayered hollow capsules.

### 2.2 Multilayered hollow capsules

Polyelectrolyte multilayered hollow capsules were first reported in 1998 by LbL assembly on a colloidal template, followed by the dissolution of the template particles shown schematically in **Figure 1** [9,10]. The physicochemical [19] and mechanical [54] properties of multilayered capsules composed of polyelectrolyte have been evaluated by several groups. In general, multilayered hollow capsules have been fabricated using polyelectrolytes based on electrostatic interactions, except for a few cases reported by Zhang *et al.* [55], Sukhishvili and co-workers [56,57] and Quinn *et al.* [58], where hollow capsules were prepared using the hydrogen-bonding interactions between uncharged polymers. Much less attention has been paid to multilayered hollow capsules composed of non-ionic multilayers constructed through their van der Waals interactions, probably because these hollow capsules were believed to be easily decomposed after the removal of the template particles. Recently, the authors' group demonstrated the successful fabrication of hollow capsules composed of poly(methyl methacrylate) (PMMA) stereocomplex LbL films through van der Waals interactions [59]. They believe that weak interactions, such as van der Waals interactions, can also be useful to fabricate multilayered hollow capsules. In the case of template particles, the initial hollow capsules were prepared using organic template particles such as polystyrene (PS) or melamine formaldehyde (MF) particles, which were dissolved after the LbL coating using both organic solvents and acidic solutions. However, a serious drawback during the template removal is the stability and integrity of the capsule wall [60,61]. Organic solvents create pores in the polyelectrolyte multilayers, allowing the polystyrene to diffuse from the capsule [62]. The oligomers generated from the MF particles during the dissolution process and the polymers with a high molecular mass from the PS particles have difficulty passing through the capsule shell [63]. The remaining MF oligomers in the capsule wall lead to a rather undefined structure of the capsules, and may also be toxic. Thus, the MF particles were used less frequently as templates. On the other hand, the fast dissolution of PS in organic solvents induces an osmotic pressure that may destroy the polyelectrolyte shell [62]. To dissolve these inconveniences, inorganic materials, such as calcium carbonate ($CaCO_3$) [64], manganese carbonate ($MnCO_3$) [65] and metal particles [66] have recently been used as a template particle for the fabrication of hollow capsules. These inorganic templates are easily and completely dissolved by ethylenediaminetetraacetic acid (EDTA) or low pH conditions. The authors have focused on silica particles as a template because of their broad sizes (nanometer to micrometer sizes) and the fact that they are easily dissolved in hydrofluoric acid (HF) solution [67]. Recently, therapeutic crystals [68], hydrogels [69,70] and living cells [71] have been reported as template materials

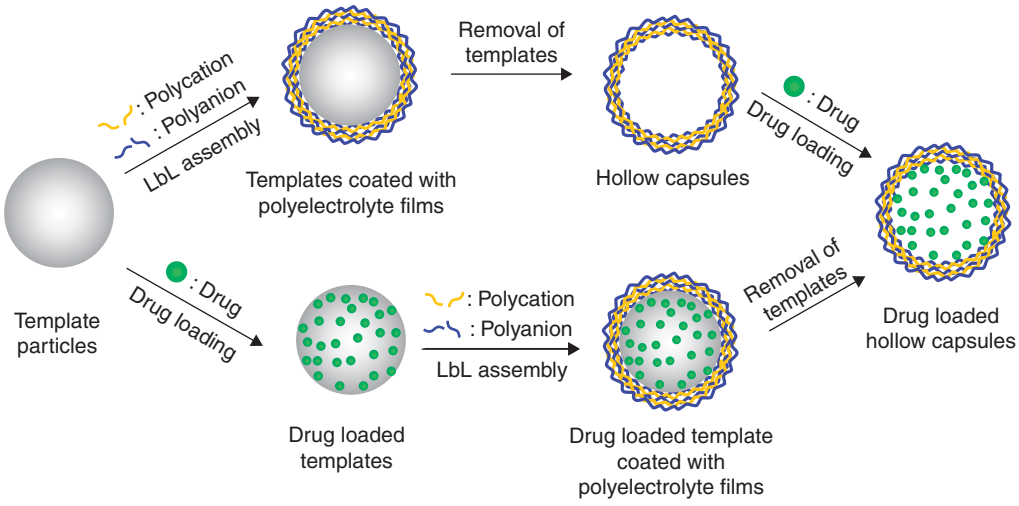


**Figure 1. Schematic illustration showing the fabrication of multilayered hollow capsules containing drugs.** Typically, two methods are used for encapsulation of drug molecules in the hollow capsules, encapsulation after (upper) and before (lower) fabrication of hollow capsules.
LbL: Layer-by-layer.

for the development of multilayered hollow capsules in the DDS field.

### 2.3 Encapsulation of drugs in multilayered hollow capsules

In general, two techniques have been reported for the loading of macromolecules into multilayered hollow capsules: encapsulation in template particles or in hollow capsules (**Figure 1**). In the case of the first method, macromolecules (e.g., proteins) are loaded into the template particles, such as $CaCO_3$ and mesoporous silica particles, during or after their preparation. The mild dissolution conditions for the $CaCO_3$ templates do not destroy the encapsulated proteins [64]. Although the HF solution required to dissolve the silica templates seems to be extreme because of the acidic pH, the loaded proteins maintain their activities sufficiently [72,73]. As described above, the use of therapeutic crystals of enzymes or proteins as a template is an effective method to avoid the dissolution step of the template cores [68]. For the second method, loading drug molecules into hollow capsules, the physicochemical properties of the multilayer films are controlled by varying the solvent polarity [74], salt concentration or pH [75] of the medium in order reversibly to create pores. Subsequently, the pores are closed by dispersing the capsules in their original solution. This method has been used mainly for PS or MF template particles. A variation of this route is filling the capsules with drugs followed by crosslinking of the shell, leading to entrapment of the drugs. Although various macromolecules have been loaded into the multilayered hollow capsules in these ways, one kind of molecule has generally been used for encapsulation.

Recently, the authors reported the encapsulation of two kinds of protein in a single hollow capsule at separate positions for the time-modulated individual release of two proteins by enzymatic degradation [76]. Rhodamine-labeled albumin was incorporated into multilayers composed of chitosan and dextran sulfate by using it as a third component, and the multilayers were fabricated on the surface of mesoporous silica microparticles containing fluorescein isothiocyanate (FITC)-labeled albumin. Subsequent immersion into HF solution allowed the first report for fabrication of the hollow capsules with two kinds of protein, which were located separately in the LbL films and inside the capsules (**Figure 2**). The individual controlled release of these proteins was successfully performed by the selective use of enzymes for the degradation of the multilayers. The next sections review the controlled release of encapsulated macromolecules from the multilayered hollow capsules for local delivery to target tissues or cells.

## 3. Local delivery of encapsulated drugs from multilayered hollow capsules

### 3.1 pH-Responsive release

Multilayered capsules generally consist of weak polyelectrolytes that are responsive to the pH of the environment. When the environmental pH becomes higher (in the case of a polybase) or lower (in the case of polyacid) than the $pK_a$ of the polyelectrolytes used in the capsule membrane, the permeability of the capsule membrane should be changed. Antipov *et al.* reported the permeability control of poly(styrene sulfonate) (PSS)/poly(allylamine hydrochloride) (PAH)-based hollow capsules [77]. When the hollow capsules were treated with acidic solutions, pores in the capsule walls were clearly observed by atomic force microscopy (AFM), although alkaline treatment did not affect the capsule membrane. It was shown that the capsules are closed at pH > 8, but at pH < 6 the macromolecules permeate into the

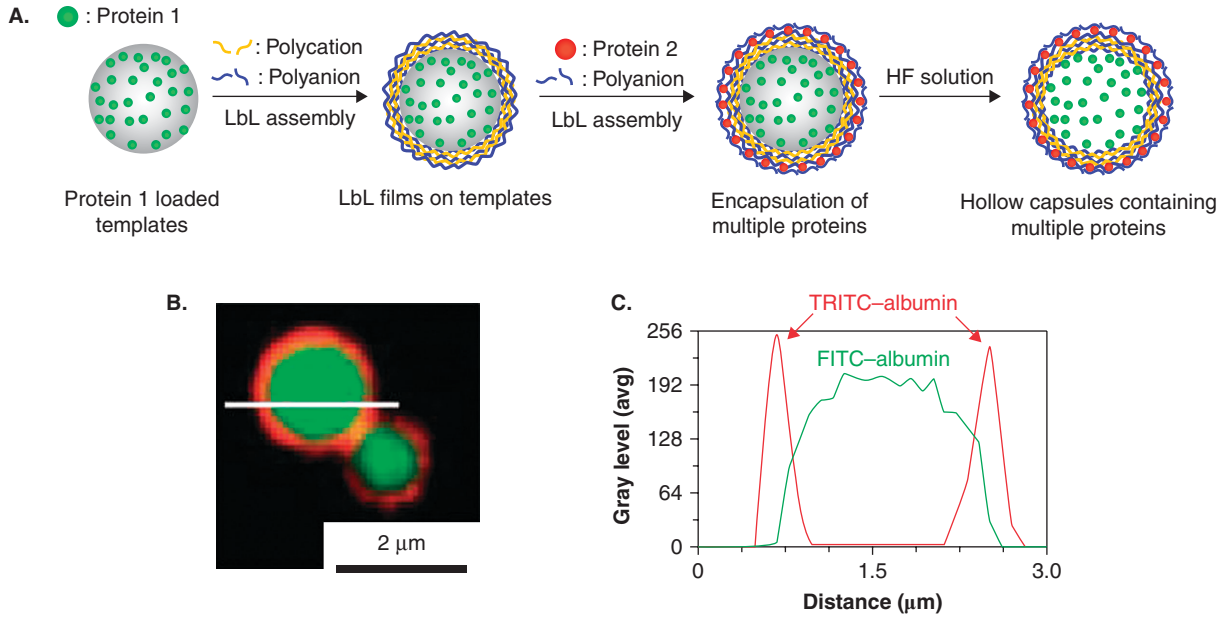


**Figure 2. A.** Schematic representation of individual encapsulation of two kinds of drug in membrane and core cavity. Confocal microscope image **(B)** and line scan image **(C)** of the capsules containing FITC-albumin inside the capsule, and rhodamine-albumin in the capsule membrane.

Reproduced with permission from [76].

HF: Hydrofluoric acid; LbL: Layer-by-layer.



capsule interior. The open and closed states of the capsule wall are reversible. Sukhorukov *et al.* reported the diffusion of FITC-labeled dextrans into the PSS/PAH capsules at a low pH, and subsequent increment in the pH allowed the entrapment of the FITC-dextran [75]. The authors applied this permeability control of the capsule membrane for the entrapment and local release of basic fibroblast growth factor (bFGF) [78]. The multilayered hollow capsules were fabricated by means of the LbL assembly of chitosan and dextran sulfate. As the capsule membrane tightened at pH values < 8 owing to the electrostatic interactions between the amine group of chitosan and the sulfate group of dextran sulfate, the bFGF could not diffuse to the capsule wall. However, the bFGF easily entered the capsules at pH values > 8, which can be attributed to the electrostatic repulsion of the dextran sulfate caused by the deprotonation of the amine group in chitosan. After treatment with the acetic acid buffer (pH 5.6), bFGF was successfully encapsulated. The bFGF-encapsulating capsules adsorbed well onto the cell surface, and the cells proliferated even in serum-free culture medium owing to the sustained release of encapsulated bFGF for 2 weeks. This was the first report of the controlled release of cytokines from biodegradable multilayered hollow capsules. Recently, Geest and co-workers reported that polyelectrolyte capsules containing an enzymatically or hydrolytically degradable polycation, poly-L-arginine (pARG), spontaneously degraded in VERO-1 cells after lipid-raft-mediated uptake [79]. The encapsulated FITC-dextran was specifically released from the capsules inside the cells by the degradation of the pARG/dextran sulfate capsules. This pH-responsive controlled release system via the permeability control of the capsule membrane is applicable to *vivo* local delivery in response to a specific environmental pH in the target tissues or cells, such as the stomach (pH 1 – 2), intestine (pH 8.4) or endosome (pH 6.0 – 6.5), and so on.

### 3.2 Light-responsive release

Optical-sensitive hollow capsules were first reported by Tao and co-workers [80]. They reported on the use of the azo dye Congo Red (CR) as a component of the polyelectrolyte multilayers. Irradiation of the hollow capsules with visible light for 120 min slightly distorted the membranes, enhancing their permeability for fluorescently labeled dextrans with a molecular mass of up to 464 kDa. However, a long irradiation time and visible light are not suitable for the *in vivo* use of light-responsive capsules as DDS carriers. When using lasers with biological objects, it is important to minimize the absorption of laser light by cells and tissues. Near infrared (NIR) laser light is interesting for DDS, as most tissues show negligible adsorption in the 800 – 1200 nm region. The IR-laser light induces structural changes in drug-containing capsules injected into tissues located at the surface of the body. Polyelectrolyte hollow capsules incorporating gold nanoparticles in the membrane have also been fabricated. The gold nanoparticles absorb the energy and transform it into heat, which locally disturbs the integrity of the capsule walls, by irradiation with IR light. Radt and co-workers were

the first to demonstrate the release of macromolecules on the IR irradiation of polyelectrolyte capsules functionalized with gold nanoparticles [81]. Skirtach and co-workers reported the intracellular release of encapsulated fluorescently labeled dextran from the hollow capsules containing silver nanoparticles by NIR laser irradiation (**Figure 3**) [82,83]. As NIR light is less harmful and has a much deeper penetration depth in tissues (e.g., 8 mm in the liver at a wavelength of 1070 nm [17]) as compared with the shorter wavelength, light-responsive hollow capsules have potential applications for *in vivo* drug delivery.

### 3.3 Magnetic field-responsive release

Multilayered hollow capsules can also be functionalized by magnetic nanoparticles for enhancing their therapeutic performance or to impart a recognition property with functional molecules to perform targeted delivery. PSS/PAH-based hollow capsules containing one layer of ferromagnetic gold-coated cobalt nanoparticles 3 nm in diameter in the multilayers were fabricated as a magnetic field-responsive capsule [84]. External alternating magnetic fields of 100 – 300 Hz and 1200 Oe were applied to rotate the embedded nanoparticles, which subsequently disturbed and distorted the capsule wall and drastically increased its permeability to FITC-labeled dextran. Hu and co-workers reported the fabrication of magnetic-sensitive microcapsules using $Fe_3O_4$/PAH polyelectrolyte and the rupture of the capsules was controlled by the magnetic field (**Figure 4**) [85]. A cell culture study indicated that these magnetic-sensitive capsules allowed for a rapid uptake by A549 cancer cells, suggesting a feasible and controlled drug release from the capsules via rupturing of the walls by a magnetic field exposure. 'Switching on' of these microcapsules using a magnetic field makes this method a good candidate for controlled drug delivery. However, the long exposure time and the strong magnetic field required to permeabilize the hollow capsule wall led to a 30°C increase in temperature of the capsule solution [84], which is problematic for loading sensitive or unstable proteins into the capsules. Furthermore, controlled drug release from capsules injected into the body by a magnetic field would also have a risk owing to the same problem. It was believed that the vibrating magnetic particles created pores in the capsule wall, facilitating the release of encapsulated drug molecules. Recently, Babincova and co-workers reported the site-specific release of encapsulated doxorubicin from magnetic liposomes with a static magnetic field, which induces a local increase in temperature up to 42°C triggered by melting of the liposome [86]. This method may be useful for magnetic field-responsive hollow capsules.

## 4. Targeting of multilayered hollow capsules for cellular uptake

The functionalization of the capsule wall to obtain specific interactions with target tissues or specific uptake properties to target tumors or immune cells is important for the *in vivo* applications of multilayered hollow capsules. This section reviews the biofunctionalization of capsule walls to achieve targeting properties for specific tissues and cells. Ai and co-workers reported the effect of polyelectrolyte hollow capsules with different outermost layers such as lipids, poly(ethylene glycol) (PEG), albumin, poly(ethyleneimine) (PEI) and poly(L-lysine) (PLL) on cellular uptake using human MCF-7 tumor cells [87]. Different surface compositions led to a wide range of electrostatic potentials from -46 to +47 mV in buffer. Capsules with different shells were taken up into the cell cytoplasm differently, and the highest uptake percentage (> 80%) was noted for shells coated with lipid bilayers and the lowest uptake for capsules with PLL shells (< 50%). The reasons for these varied uptake properties were not clarified, but the present information should be helpful for designing the multilayers of hollow capsules. The modification of the capsule membrane with PLL-*g*-PEG and biotin-conjugated PEG (PEG-biotin) was reported to control nonspecific and biospecific protein adsorption of hollow capsules [88]. Fischlechner and co-workers demonstrated an engineered capsule wall with Rubella-like particles (RLPs) to achieve binding and fusion properties of virus [89]. The RLP-decorated hollow capsules clearly showed binding to the cell surface, the induction of endocytosis, and subsequent fusion with the late endosome membrane without any cytotoxicity. This technique can be applied for other viruses, and thus the specific binding properties attributed to the virus could be obtained for the capsule walls.

One of the most certain methods to obtain targeting specificity is the immobilization of an antibody on the capsule multilayers. Cortez and co-workers reported the immobilization of a humanized A33 monoclonal antibody, which binds to a transmembrane glycoprotein, the human A33 antigen, on hollow capsules [90,91]. This antigen is expressed by 95% of human colorectal tumor cells. The findings showed that the antibody-immobilized capsules had a potential for targeting colorectal cancer cells with high specificity. Asialoglycoprotein receptors, which are expressed exclusively on the surface of liver parenchymal cells, were targeted by D-galactose-branched polyelectrolyte hollow capsules [92]. The hollow capsules composed of poly(vinyl galactose ester-*co*-methacryloxyethyl trimethylammonium chloride) (PGEDMC) and PSS had specific recognition abilities with peanut agglutinin lectin rather than concanavalin A lectin, suggesting a specific recognition for hepatic cells.

Rose and co-workers first reported the uptake of polyelectrolyte multilayered capsules into immune cells such as dendritic cells and monocytes, important antigen-presenting cell (APC) populations, within fresh human blood (**Figure 5**) [93]. These capsules were internalized by blood APCs. By encapsulating the model HIV vaccine peptide KP9 within the capsules, KP9 was internalized into the APCs and intracellularly trafficked for presentation with MHC-I to responding primate lymphocytes to elicit an immune response. These vaccine peptide-loaded capsules will be highly efficient for the stimulation of immune responses to a wide range of

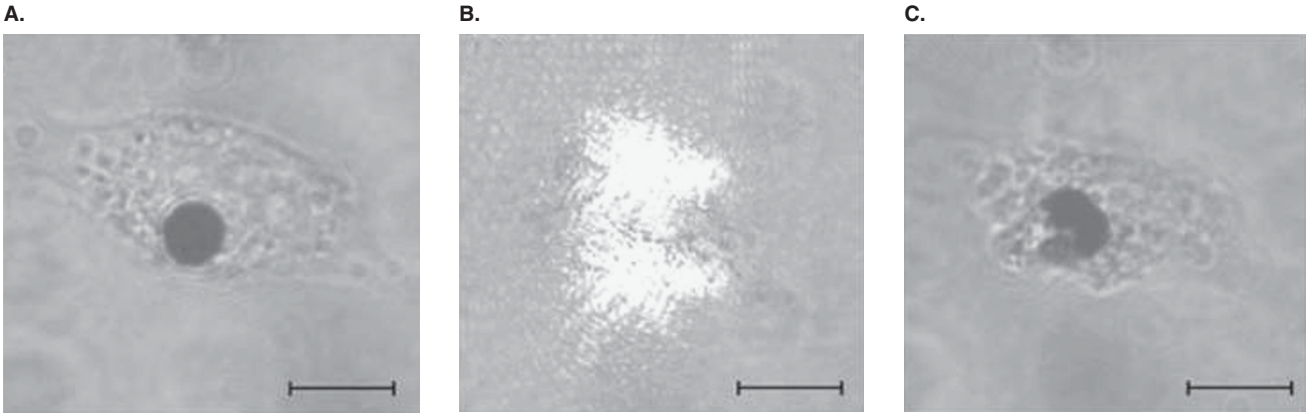


**Figure 3. Remote activation of a capsule containing silver nanoparticles in its walls.** The capsule was ingested by a living MDA-MB-435S cancer cell. The images show the cell before **(A)**, during **(B)** and after **(C)** illumination with a laser (830 nm, 50 mW). The scale bars correspond to 10 μm.



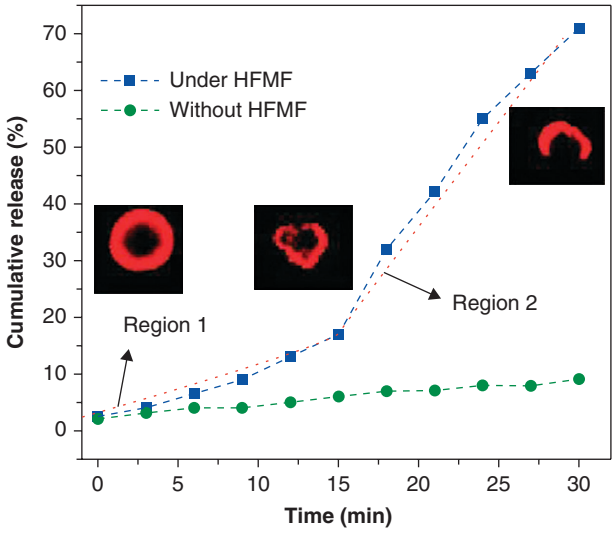


**Figure 4. The drug, doxorubicin hydrochloride (Dox) release behavior and morphologies of $(Fe_3O_4/PAH)_4$ capsules under continuous HFMF.**



important diseases, and will not be limited to the delivery of peptide antigens. The specific trapping and accumulation of multilayered hollow capsules for increasing cellular uptake have been reported by Zebi and co-workers [94]. In regions where capsules containing magnetite ($Fe_3O_4$) nanoparticles in the membrane were trapped by a magnetic field, a drastically increased uptake of capsules by breast cancer cells (MDA-MB-435s) was observed. These results suggest the feasibility of the magnetic targeting of multilayered hollow capsules loaded with pharmaceutical agents to pathogenic parts of a given tissue.

On the other hand, for the successful targeted delivery of drugs or proteins using hollow capsules, it is necessary that the capsules have bioinert surfaces, which can inhibit the nonspecific adsorption of serum proteins and hemocytes in order to maintain their stable circulation and property in the blood without being scavenged non-selectively [95,96]. Recently, Ochs and co-workers reported biofunctionalized capsules with stealth property by depositing heterobifunctional PEG [97]. Wattendorf and co-workers reported the stealth function of polyelectrolyte hollow capsules functionalized with PEG on their surface [98]. The PSS/PAH-based hollow capsules were coated with adlayers of PLL-*g*-PEG and PEG grafted poly(L-glutamic acid) (PGA-*g*-PEG). Phagocytosis experiments using human monocyte-derived dendritic cells and macrophages against capsules with PGA-*g*-PEG showed no significant effect on cellular recognition, whereas PLL-*g*-PEG effectively blocked the phagocytosis of the hollow capsules. This may be due to insufficient PEG density on the surface. The PLL-*g*-PEG coatings functioned as an efficient adlayer for at least 3 weeks, thus this coating technology is suggested as one of the possibilities as a DDS that escapes fast clearance by the mononuclear phagocytic system.

## 5. Conclusion

Recent developments in functional hollow capsules specific for cellular and tissue-targeted delivery and stimuli-responsive controlled release have been reviewed. The first report on polyelectrolyte hollow capsules was only 10 years ago, but research into functional hollow capsules, with surprising speed, deserves special mention. The practical applications of multilayered hollow capsules in DDS or biomedical fields are an issue in the future, and *in vivo* experiments to clarify the availability of the capsules as functional carriers should be performed. Further studies on hollow capsules should be focused on the

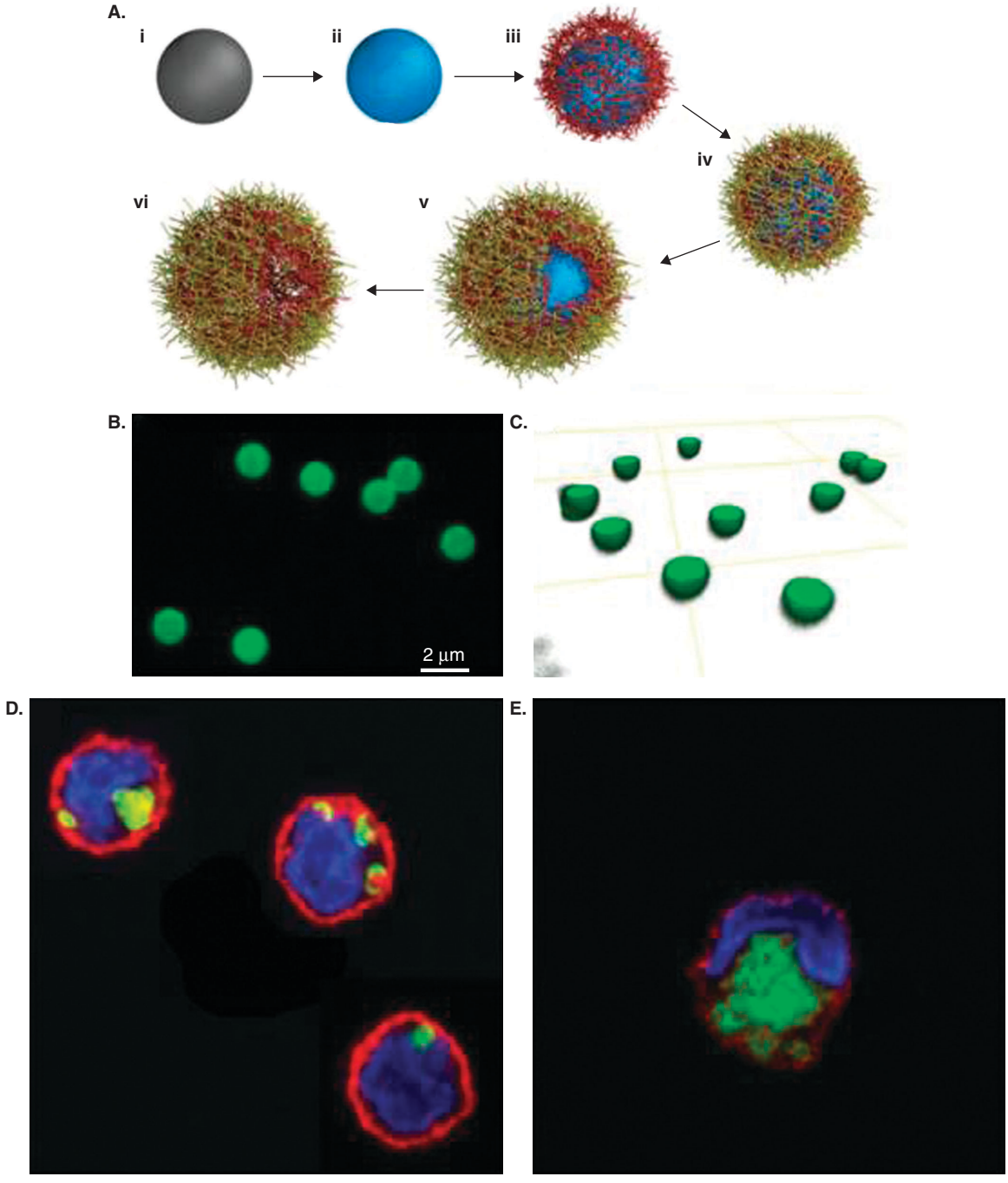


**Figure 5. A.** Schematic of capsule assembly onto a sacrificial colloidal template. The colloidal template (i) is incubated in a solution containing a peptide that is electrostatically deposited on the surface (shown in blue) (ii). The capsule is assembled by the alternate deposition of interacting polymers (iii and iv) to form a multilayered structure (v). The colloidal template is then removed to form a capsule containing the peptide cargo (vi) (the peptide (blue) is omitted for clarity). A confocal microscopy cross-section **(B)** and three-dimensional confocal reconstructed **(C)** from the capsules containing fluorescently labeled peptide, showing the uniform size and loading of the peptide within the capsules. Confocal cross-section of capsules internalized into dendritic cells **(D)** and monocytes **(E)**; capsules are labeled green, the cell membrane is labeled red and the nuclei are labeled blue.

dynamic state of capsules inside the cells and body, such as accumulation in specific organs, degradation in the tissues or cytosol, and the cytotoxicity of degraded compounds.

## 6. Expert opinion

Multilayered hollow capsules fabricated by LbL assembly on template particles have great potential as new carriers for DDS and other biomedical applications owing to their ability to be modified, their capacity to encapsulate a wide range of chemicals, and the variety of functionalities for targeting the delivery of these encapsulated macromolecules. Furthermore, the capsules are interesting in other application such as nanometer- or micrometer-sized reactors of chemicals or polymers, as diagnostic particles, and as catalyst carriers. However, the distinct advantages of these hollow capsules as compared with other carriers, such as polymeric particles, polymeric micelles, liposomes, nanogels or microgels and dendrimers, have not been clarified yet, although it is a very interesting material for scientific physicochemical interests. Recently, hollow capsules have attracted much attention for their applications in the biological and DDS fields, but fundamental research on these hollow capsules at a molecular level can be important for understanding their scientific or physicochemical characteristics. Furthermore, basic studies are expected to elucidate the specific properties or advantages of hollow capsules as compared with other types of carrier. The hasty or impatient application of research into hollow capsules may blind one to their real importance. The authors believe that the potential of multilayered hollow capsules as a drug carrier has been well documented as compared with the other carriers, such as polymeric particles and micelles, in this decade. For the next step of LbL hollow capsules, fundamental studies into the characteristic applications of the capsules in the biomedical field are now desired.

The authors consider that one of the characteristic properties of multilayered hollow capsules is the multiple and individual encapsulation of macromolecules such as proteins, peptides and polysaccharides. Although typical particles, micelles, liposomes and dendrimers can encapsulate one kind of drug molecule in the core, LbL hollow capsules can encapsulate individually at least two kinds of drug in their cavity and in their LbL multilayers. Furthermore, the separately encapsulated drugs can be released individually by controlling the degradation rate of the multilayers, as shown in recent research [76]. The authors believe that this characteristic property of the hollow capsules supports a great possibility for next-generation drug delivery systems, the gradual controlled release of two or more kinds of drug from a single carrier depending on the conditions of the affected area. Recently, the release of multiple drugs and genes from a single carrier has attracted attention because of the induction of strong synergistic effects [99]. A few studies have reported multiple drug release systems from a single carrier, but in a conventional release system it was not possible to control the individual release rate of multiple drugs from a single carrier because their release rates depended only on their diffusion properties from the carrier. To construct individually controlled release systems for multiple drugs, new methodologies such as the incorporation of multiple drugs into separate sites within a single carrier and the setting of an independent driving force for the release of each drug are required. Städler and co-workers reported a new class of hollow capsule, capsosomes, which can compartmentalize loading drugs into liposomes within the membrane [100]. This new type of capsule would also be useful for the multiple drug release system. The authors successfully prepared biodegradable hollow capsules incorporating two kinds of protein in separate positions of the capsule via LbL assembly, and achieved the time-modulated release of these two proteins by controlled enzymatic degradation of the membrane. However, the precise control of the release rate of multiple drugs has not yet been achieved. The development of individually controlled release systems for multiple drugs from a single hollow capsule *in vivo* will be an issue for next-generation drug delivery systems.

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## Declaration of interest

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