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Self-assembly of polylactic acid and cholesterol-modified dextran into hollow nanocapsules

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ABSTRACT

A study of the direct preparation of hollow polymer nanocapsules which composed of the biocompatible and biodegradable polymers, polysaccharide and polylactic acid (PLA), was presented. By the dialysis of a DMSO solution of cholesterol-modified dextran (Chol-Dex) and poly(D,L-lactic acid) against water, hollow polymer nanocapsules with a highly stable structure and relatively narrow size distribution were obtained. The formation mechanism and the effects of various factors such as PLA molecular weight and the weight ratio of Chol-Dex to PLA on the formation of hollow polymer nanocapsules were investigated by SEM, TEM and ^{1}H NMR analysis. The results showed that hollow capsules were obtained when the weight ratio of Chol-Dex to PLA was between 3:1 and 1:1, and when PLA of molecular weights greater than 360 Da were used. The hollow capsules with a sandwich shell structure derived from deposition of PLA and some amphiphilic polysaccharide on the internal interface of the polysaccharide-coated aggregates, which were formed through phase separation during the initial phase of the dialysis. This novel approach to hollow polymer nanocapsule formation represents a rare example of the self-assembly of two biocompatible polymers into nanometer-scale objects with interesting structures, shapes and morphology through a simple assembly process.

1. Introduction

Hollow capsules of nano to micrometer scales are generating increasing interest because of their importance in basic science and extensive applications in fields such as biology, cosmetics, medicine, catalysis, ecology and nutrition (Wang, Feng, Tong, & Gao, 2007). The methods currently used to fabricate various hollow capsules include nozzle reactor processes and sol–gel processing (Khopade & Caruso, 2004). New techniques such as lipid vesicles, suspension and emulsion polymerization around latex particles and layer-by-layer adsorption strategies are also being developed (Chen & Jiang, 2005; Decher, 1997; Decher & Hong, 1991; Guo, Yang, Deng, Wang, & Fu, 2005; Keller, Johnson, Brigham, Yonemoto, & Mallouck, 1995; Liu, Niu, Xu, Guo, & Yang, 2005; Niu, Yang, Hu, Lu, & Han, 2003; Ruths, Essler, Decher, & Riegler, 2000). Recently the self-assembly of amphiphilic copolymers such as amphiphilic coil–coil-type diblock copolymers (Harris, Rose, & Bruening, 2002; Hest, Delnoye, Baars, Genderen, & Meijer, 1995; Maskos & Harris, 2001), peptide-based coil–rod diblock copolymers (Jeroen et al., 1998; Kukula, Schlaad, Antonietti, & Förster, 2002), asymmetric ABC (Stoenescu & Meier, 2002; Yu & Eisenberg, 1998) and symmetric ABA (Chen & Jenekhe, 2000; Nardin, Hirt, Leukel, & Meier, 2000; Schillén, Bryskhe, & Mell'nikova, 1999) triblock copolymers have attracted significant attention. Intermolecular complexation has also been reported to induce polymer capsule assembly in solution (Faysal, Trent, Mark, Gilles, & Vincent, 2000; Oktay, Hao, Eunhee, Raymond, & Vincent, 2005).

Polysaccharides constitute an important class of physiological material. They are expressed in membrane cells and are involved in cell surface properties including tissue addressing and transport mechanisms. Additionally, oligo and polysaccharides are involved in active targeting and are used in specific receptors in some cells and tissues. Capsules made of saccharides or polysaccharides are attractive models of cells and organelles because of their resemblance to biological membranes (Caroline, Ruxandra, & Patrick, 2004). To the best of our knowledge, only a few reports have dealt with hollow capsules or vesicles based on polysaccharides to date (Dou, Jiang, Peng, Chen, & Hong, 2003; Uchegbu et al., 1998; Wang, Li, & Guo, 2005). In this paper, we present a new method for the preparation of hollow capsules by micelle internal phase separation and interface deposition. The amphiphilic polymers polysaccharide and polylactic acid were dissolved in DMSO. Gradual removal of the solvent by co-dialysis first led to phase separation, resulting in the formation of polysaccharide-coated colloid particles. Hollow

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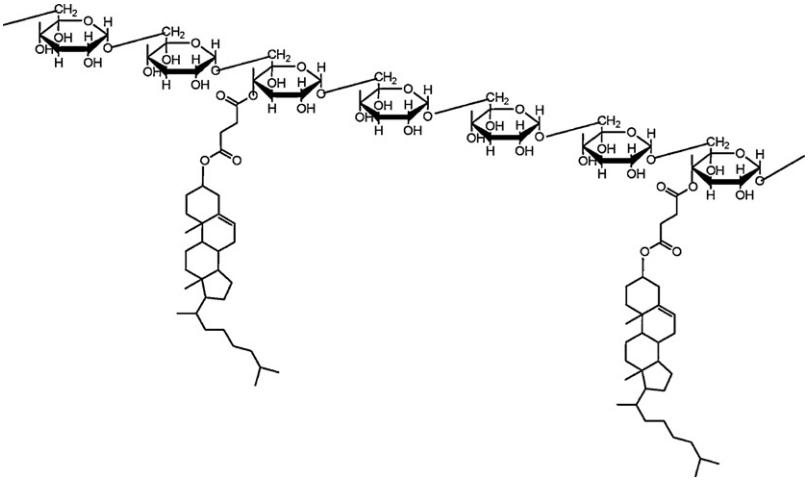

Scheme 1. The structure of cholesterol modified dextran (Chol-Dex).

capsules were then formed by the stepwise deposition of PLA and some amphiphilic polysaccharide on the internal interface of the aggregates.

2. Experimental

2.1. Materials

High molecular weight poly(D,L-lactic acid) (PLA) was obtained from the ring-opening polymerization of D,L-lactide in chloroform, using tin (II) 2-ethylhexanoate ($SnOct_2$) as catalyst, followed by precipitation of the polymer by addition of ethanol. The average molecular weight and molecular weight distribution of the polymers generated by this process were determined by GPC measurement to be $n = 29{,}000$ Da with a dispersion of 1.64. PLA oligomers, with molecular weights from 210 to 2470 Da, were obtained by condensation polymerization of D,L-lactic acid. Oligomers with molecular weights from 950 to 2470 Da were purified by precipitation of the chloroform solution of the oligomer using ether. Oligomers with molecular weights less than 360 Da could not be precipitated with ether, so were used without purification. The molecular weights of the oligomers were determined by ^{1}H NMR measurement. Dextran ($M_w = 40{,}000$ Da) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Cholesterol was purchased from Tianjin Chemical Reagent Co. (Tianjin, China) and recrystallized from ethanol before use. Dimethyl sulfoxide (DMSO) was dried using 4 Å molecule sieves and redistilled under reduced pressure. Chloroform was dried over anhydrous calcium chloride and then distilled under atmospheric pressure. All other reagents and chemicals were obtained from commercial sources and used without further purification. Dialysis chambers were purchased from Green Bird Technology Debel Ltd. (Shanghai, China), and have a molecular weight cut-off of 1000 g/mol.

2.2. Synthesis of cholesterol modified dextran (Chol-Dex)

The synthesis of Chol-Dex (structure shown in Scheme 1) was performed according to procedures reported previously (Gu et al., 2007). Briefly, cholesterol was esterified with succinic anhydride in anhydrous pyridine. The resulting cholesterol 3-hemisuccinate was then reacted with $SOCl_2$ in anhydrous chloroform to form cholesterol 3-hemisuccinyl chloride. Chol-Dex was obtained by the esterification of dextran with cholesterol 3-hemisuccinyl chloride in anhydrous DMSO using triethylamine as catalyst. The structure of Chol-Dex was confirmed by ^{1}H NMR (DMSO-d_6, Varian UNITY plus 400 MHz NMR spectrometer). The degree of substitution (DS), determined via analysis of the ^{1}H NMR spectrum and defined as the percentage cholesterol content per 100 glucopyranosidic unit, was 8.2%.

2.3. Preparation of Chol-Dex/PLA aggregates

Chol-Dex and PLA were co-dissolved in DMSO in a ratio of 2:1 (w/w). The resulting solution was dialyzed against distilled water, using a dialysis membrane to form polymeric aggregates. The medium was changed every hour over the first 3 h period, and then every 3 h in the following 24 h.

2.4. Characterization of Chol-Dex/PLA aggregates

2.4.1. Scanning electron microscopy (SEM)

The surface morphology of the Chol-Dex/PLA aggregates was measured by a Hitachi S-4800 scanning electron microscopy equipped with 10 kV. A drop of nanoparticle suspension (1 mg/ml) was added to a quartz plate and dried overnight. Around the nanoparticles were coated with conducting resin before imaging.

2.4.2. Transmission electron microscopy (TEM)

TEM pictures of the Chol-Dex/PLA aggregates were taken in a transmission electron microscope (TEM, JEM-2000 FX II, JEOL, Tokyo, Japan). A drop of the aggregates suspension was placed on a copper grid coated with carbon film and dried at room temperature. Observation was performed at 80 kV.

To define the internal hydrophilic/hydrophobic microenvironment of Chol-Dex/PLA capsules, the gadolinium chloride ($GaCl_2$) loaded aggregates were prepared by stepwise dialysis. The solution of polymers were firstly dialyzed against distilled water for 12 h, followed by 5% $GaCl_2$ solution for another 12 h, and finally distilled water for 2 h until the free $GaCl_2$ had been removed from the dialysis membrane. The obtained capsules were subjected to TEM observation as mentioned above.

2.4.3. ^{1}H nuclear magnetic resonance (^{1}H NMR)

Chol-Dex and PLA were dissolved in deuterated DMSO and then dialyzed against D_2O. At defined time intervals, an aliquot of the suspension was withdrawn and the ^{1}H NMR spectrum was recorded using a 500 MHz Bruker instrument (Karlsruhe, Germany).

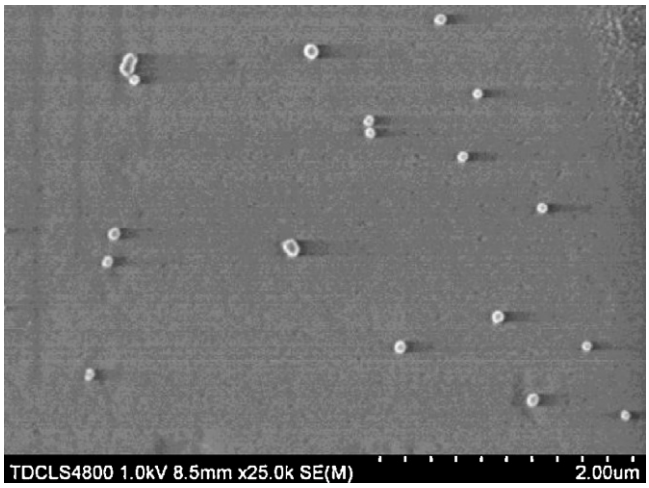

Fig. 1. Scanning electronic microscopy micrograph of Chol-Dex/PLA aggregates.

2.4.4. Differential scanning calorimetry (DSC)

The glass-transition temperatures (T_g) of PLA and Chol-Dex/PLA hollow capsule samples were determined using DSC. Thermograms were recorded using a DSC 92 apparatus (Setaram, Lyon, France). The heating rate was 10 °C/min under a nitrogen atmosphere. All the samples were dehydrated by heating at 40 °C under vacuum for 20 h.

3. Results and discussion

3.1. Self-assembly of Chol-Dex and PLA into hollow nanocapsules

Polysaccharide surface modified nano-scale carrier systems have attracted the attention of numerous researchers in the field of polymeric carrier materials because of their useful properties, such as non-toxicity, biodegradability, non-immunogenicity, biocompatibility and other biological properties (Aumelas, Serrero, Durand, Dellacherie, & Leonard, 2007; Rouzes, Gref, Leonard, De Sousa Delgado, & Dellacherie, 2000). Polysaccharide surface modified nanoparticles with a hydrophobic biodegradable polyester core material, such as polylactic acid, have attracted particular interest. Nouvel et al. (2009) prepared polysaccharide-coated polyester nanoparticles via the emulsion/solvent evaporation process, using water-soluble polylactide-grafted dextran as a polymeric stabilizer during the emulsification step. Employing the same method, Coombes et al. (1997) elaborated polysaccharide surface-modified poly(D,L-lactide-co-glycolide) particles using POE-dextran conjugates as surfactants. Previously, we have synthesized cholesterol modified dextran conjugates and prepared polysaccharide-coated PLA nanoparticles using the co-dialysis method (Gu et al., 2007). However, little has been reported on the formation of hollow capsules composed of polysaccharide and polyester. Here, we present our synthesis of cholesterol modified dextran conjugates and polysaccharide-coated PLA hollow nanocapsules prepared by the co-dialysis method.

To examine the morphologies of the self-assembled Chol-Dex/PLA aggregates, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) measurements were performed. As shown in Fig. 1, spherical particles were obtained after dialysis against distilled water. When subjected to TEM observation, however, a clear luminance contrast between the center and the periphery of the Chol-Dex/PLA aggregates was observed (Fig. 2a), indicating that the aggregates are hollow capsules. To determine the location of polysaccharide and PLA in the hollow capsule structure, double bonds were introduced into both Chol-Dex and PLA, through an acryloyl chloride reaction. Acryloyl Chol-Dex/PLA and Chol-Dex/acryloyl PLA aggregates were then prepared respectively. Before TEM imaging, these aggregates were stained by dropping a droplet of OsO_4 solution on the copper line. Fig. 2b shows the TEM micrograph of hollow capsules composed of acryloyl Chol-Dex and PLA, while TEM micrographs of samples with double bonds appear darker after staining with OsO_4 compared to those of samples without double bonds. A sharp contrast between acryloyl Chol-Dex/PLA capsules and Chol-Dex/acryloyl PLA capsules (Fig. 2c) seemed indicate that hollow capsules have a shell of two layers, a polysaccharide layer located on the external surface and an inner PLA layer. However, these double layer structures seem unfavorable because PLA is very hydrophobic. Otherwise the capsules would possess a hydrophobic core with kinetically unstable high interface energy. In consideration of the OsO_4 solution may be difficult to penetrate the PLA layer to stain the possible interior acryloyl Chol-Dex, and to assess the interior hydrophilic/hydrophobic microenvironment of hollow capsules, aqueous solution of $GdCl_2$(a precursor for the preparation of contrast agent of Magnetic Resonance Imaging) was used to replace distilled water to dialyze capsules. The obtained capsules were subjected to TEM, and it was found that, in contrast to the original hollow capsules, most of the microcapsules showed a darker core than shell (Fig. 2d). As gadolinium was a high atomic number element (64) and had a higher mass-thickness contrast than other elements of samples, the darker core should be assigned to $GdCl_2$, which meant the capsules possessed a hydrophilic inner core. Thus a hydophilic inner layer which is most probably formed by amphiphilic polysaccharide should be existed. Therefore we deduced that the capsules should consist of a sandwich shell structure, *i.e.*, inside the Chol-Dex layer and PLA layer mentioned above, there was still another innermost layer of hydrophilic Chol-Dex.

Table 1
Effect of PLA molecular weight on the morphologies of Chol-Dex/PLA aggregates.

$M_{W_{PLA}}$	Solubility	Morphology
210[a]	Ethanol	Solid particles
360[a]	Chloroform	Hollow capsules
940[a]	Chloroform	Hollow capsules
2400[a]	Chloroform	Hollow capsules
29,000[b]	Chloroform	Hollow capsules
48,000[b]	Chloroform	Hollow capsules

[a] Obtained from the analysis of ^{1}H NMR spectra of PLA synthesized by condensation polymerization of lactic acid.
[b] Obtained from GPC measurements of PLA synthesized through ring-opening polymerization.

3.2. Effect of PLA molecular weight on assembly morphologies of Chol-Dex and PLA

PLA oligomers were synthesized through direct condensation polymerization of lactic acid to assess the influence of PLA molecular weight on the assembly morphologies of Chol-Dex and PLA. The Chol-Dex/PLA oligomer aggregates were prepared using the same dialysis method used to prepare the other aggregates described here. Table 1 and Fig. 3 show the relationship between the molecular weight and solubility characteristics of PLA oligomers, and the morphologies of the resulting aggregates. As can be seen from the Table 1, the assembly morphologies of Chol-Dex and PLA were found to correlate with the hydrophobicity of the PLA. Hollow capsules were obtained in the case of PLA oligomers dissolved in chloroform. In contrast, oligomers with molecular weights of less than 210 Da, which are insoluble in chloroform but soluble in ethanol, formed solid particles when co-dialyzed with Chol-Dex. These results suggest that the morphologies of Chol-Dex/PLA aggregates depend on the hydrophobicity of PLA. In other words,

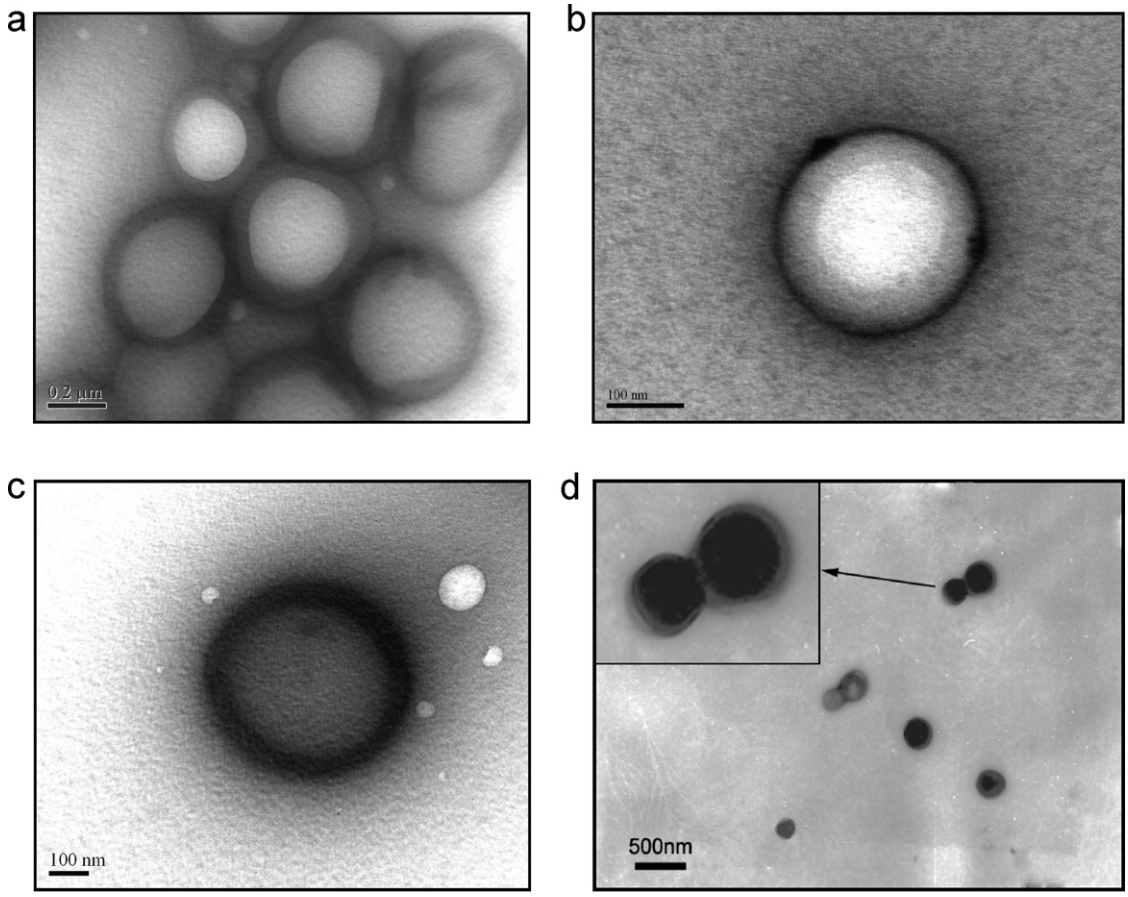

Fig. 2. Transmission electron microscopy micrographs of Chol-Dex/PLA aggregates (the weight ratio of Chol-Dex/PLA was 0.5). (a) Chol-Dex/PLA hollow capsules; (b) acryloyl Chol-Dex/PLA hollow capsules stained with OsO_4; (c) Chol-Dex/acryloyl PLA hollow capsules after stained with OsO_4; (d) Chol-Dex/PLA hollow capsules after treatment by 5% $GaCl_2$ solution during the formation of the capsules.

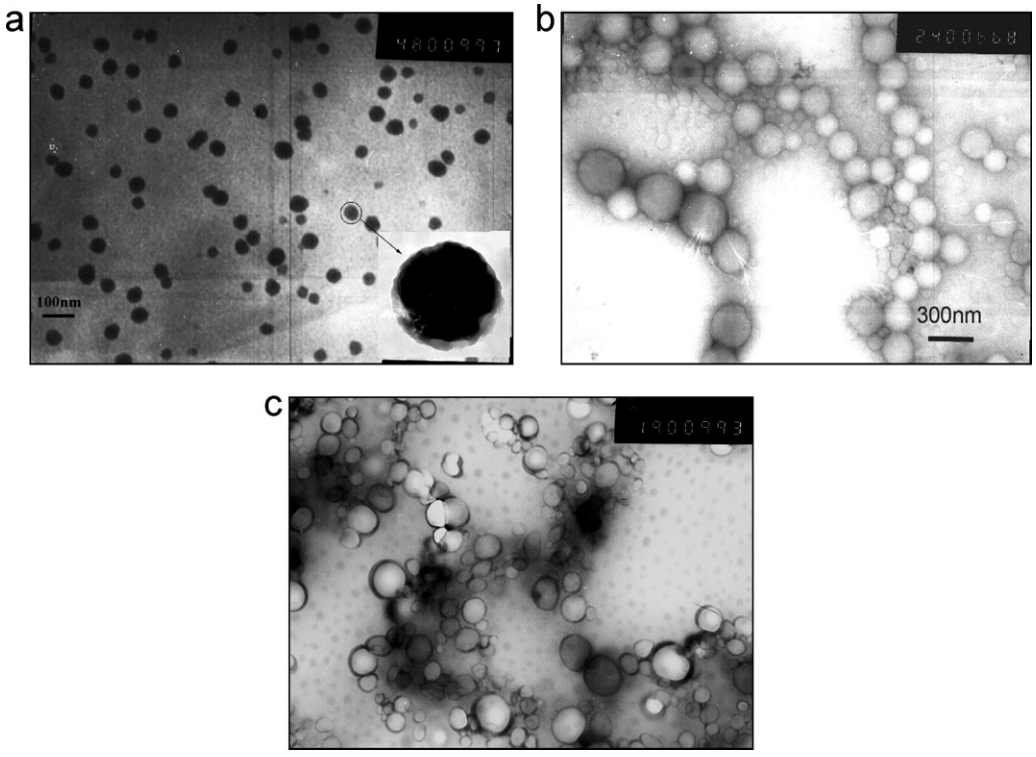

Fig. 3. TEM micrographs of aggregates prepared from Chol-Dex and PLA oligomers of different molecular weights. The molecular weight of PLA: (a) 210 Da; (b) 360 Da; and (c) 940 Da.

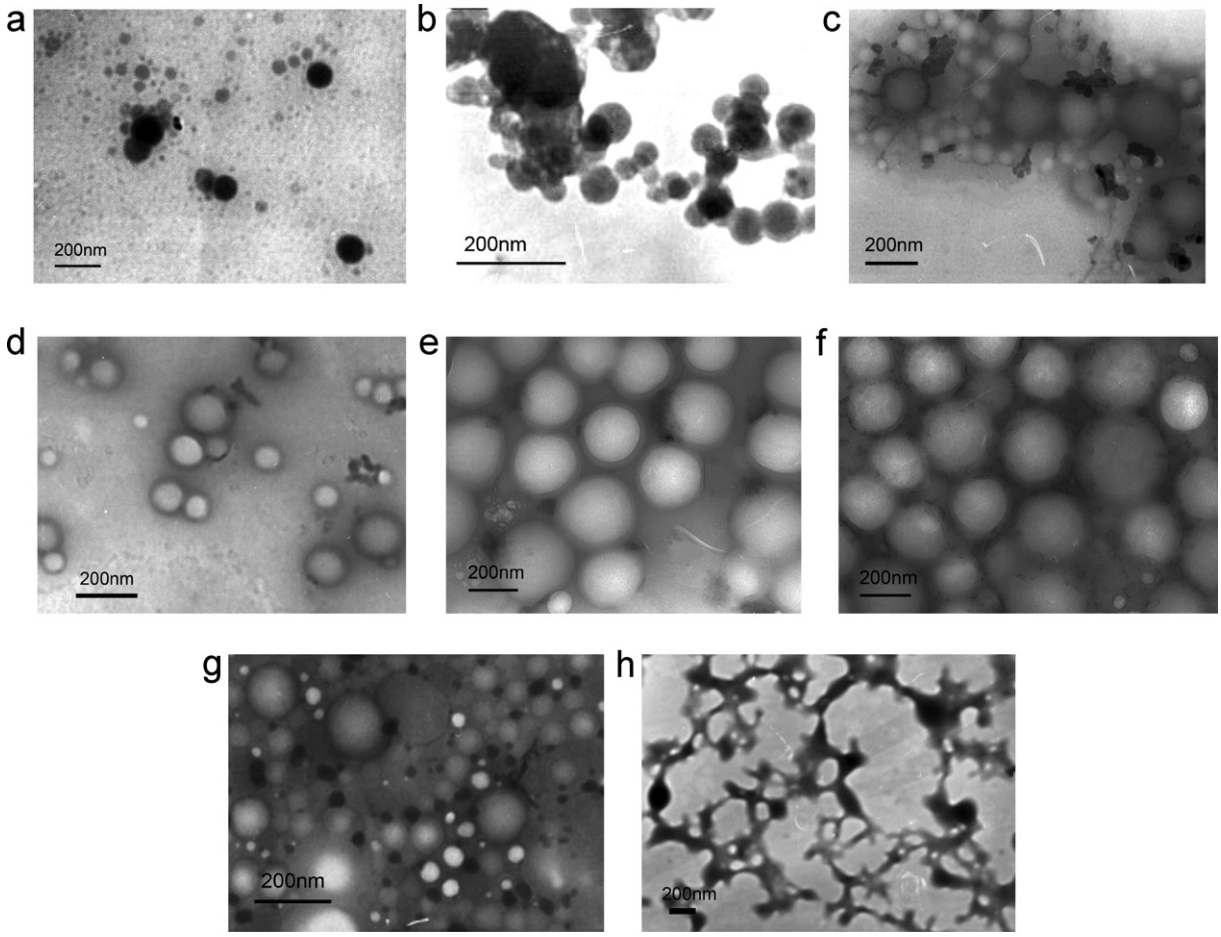

Fig. 4. TEM micrographs of Chol-Dex/PLA aggregates with different weight ratios of Chol-Dex to PLA. (a) Self-aggregates of pure Chol-Dex; (b) Chol-Dex/PLA = 7:1; (c) Chol-Dex/PLA = 5:1; (d) Chol-Dex/PLA = 3:1; (e) Chol-Dex/PLA = 2:1; (f) Chol-Dex/PLA = 1:1; (g) Chol-Dex/PLA = 1:2; and (h) Chol-Dex/PLA = 1:3.

the formation of hollow capsules relies on the polarity differences between cholesterol modified dextran and PLA.

3.3. Effect of the weight ratio of Chol-Dex/PLA on assembly of Chol-Dex and PLA

The weight ratio of Chol-Dex to PLA was found to affect the formation of Chol-Dex/PLA hollow capsules. The morphologies of PLA/Chol-Dex aggregates were examined under TEM after the complete removal of solvents (Fig. 4). Pure Chol-Dex formed solid particles with sizes of about 50 to 80 nm in aqueous solution (Fig. 4a). Spheres with a core-shell structure were observed for Chol-Dex/PLA aggregates when a ratio of 7:1 was used (Fig. 4b). A vesicular morphology was observed for weight ratios of Chol-Dex/PLA of between 3:1 and 1:1 (Fig. 4d–f), and beyond this ratio (ratio of 1:2 and 1:3, Fig. 4g and h) a phase transition of Chol-Dex/PLA complexes into solid particles occurred. Fig. 4d–f presents representative TEM images with $W_{\text{Chol-Dex}}/W_{\text{PLA}} = 3$, 2 and 1, respectively. The vesicular structure does not appear to be an incidental phenomenon, because in these cases vesicles were nearly the sole morphology observed under the visual field of TEM. Aggregates were also prepared by blending Chol-Dex and PLA with molecular weights ranging from 11,000 to 67,000 g/mol. In all cases a similar trend was observed, and the optimum weight ratio for capsule formation was consistently $W_{\text{Chol-Dex}}/W_{\text{PLA}} = 2$, with the exception that the onset of micelle–capsule transition varied from 5 to 3 depending on the molecular weight of PLA.

3.4. Formation of Chol-Dex/PLA hollow nanocapsules

The morphology transition of the Chol-Dex/PLA aggregates during the course of the dialysis process was monitored by TEM observation. As shown in Fig. 5, while DMSO diffused out and water penetrated into the dialysis bag, particles appeared quickly after 15 min (Fig. 5a). A low density region then appeared inside the particles (Fig. 5b) and gradually expanded, resulting in an increasingly large central cavity (Fig. 5c). The preliminary hollow spheres were formed within 4 h (Fig. 5d). At this point the hollow microspheres are unstable and easily broken. Regular hollow capsules were finally obtained after 24 h (Fig. 5e).

By tracking the process of self-aggregation, we propose a mechanism for the formation of hollow nanocapsules, as shown in Fig. 5. Initially, Chol-Dex and PLA are dissolved in DMSO and interpenetrated with each other (Fig. 5a). As dialysis proceeded, Chol-Dex tended to be increasingly dissolved in the water phase, while PLA tended to aggregate and precipitate by desolvation. Therefore, phase separation occurred. This process can be monitored by ^{1}H NMR and Fig. 6 shows the ^{1}H NMR spectra obtained during capsule formation. It can be seen that signals assigned to PLA molecules (1.5 ppm for $-CH_3$ groups and 5.3 ppm for –CH groups) quickly lost intensity, indicating the desolvation of PLA molecules. In contrast, the peaks attributed to the resonances of main-chain protons of Dex (3.0–4.0 ppm) became sharp, showing their good affinity to the aqueous media. Chol-Dex/PLA micelles were formed because of the entanglement of the hydrophobic cholesterol pendant groups of Chol-Dex with the PLA molecules (Fig. 5b). Most of the

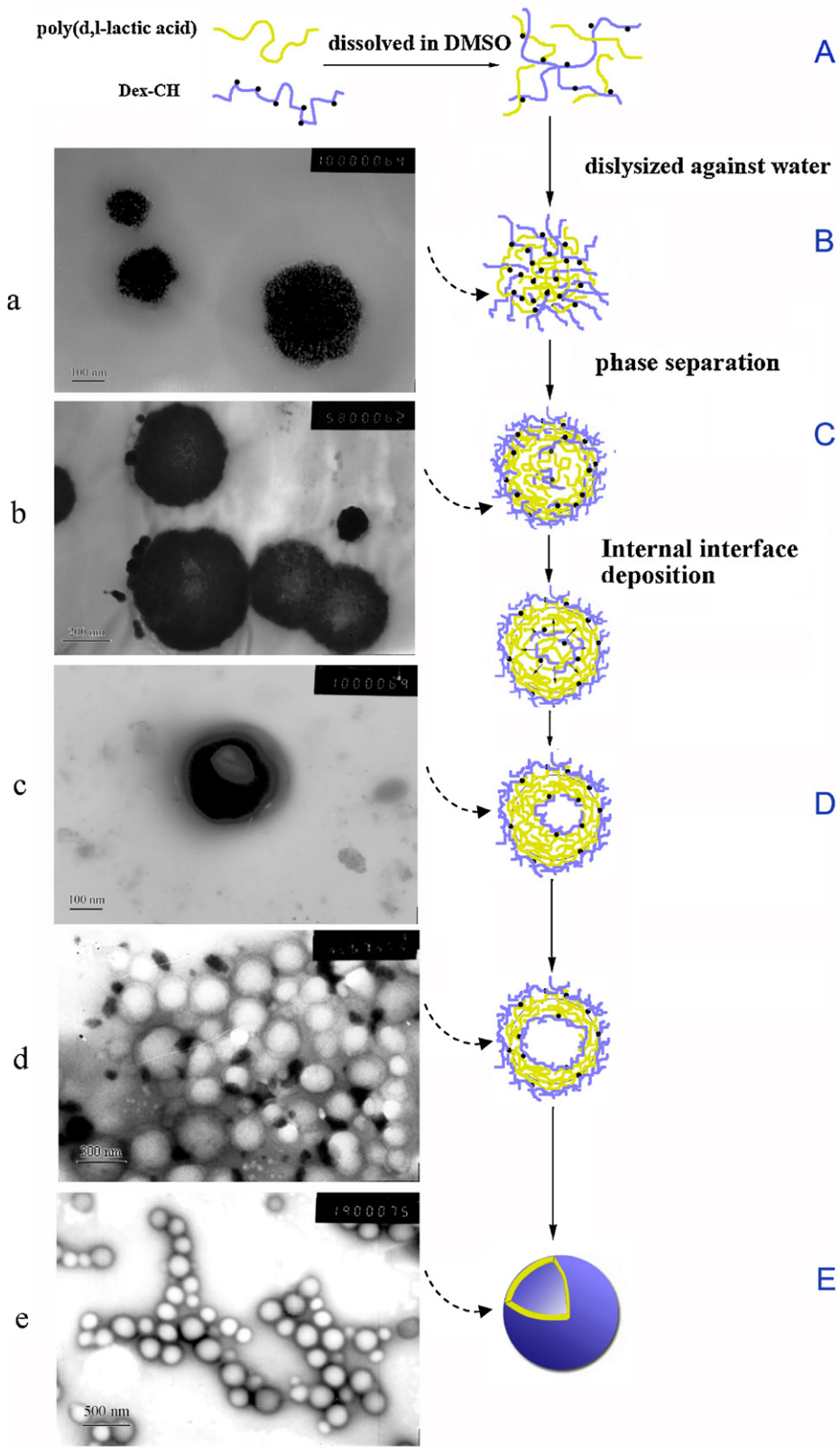

Fig. 5. Process of Chol-Dex/PLA hollow capsule formation, as tracked by TEM observation, and a schematic representation of the formation of capsules by internal interface deposition. The sampling times of the observations were: (a) 15 min, (b) 30 min, (c) 1 h, (d) 4 h and (e) 24 h.

Chol-Dex acted like a surfactant, and was "anchored" onto the swelled PLA because of its appended cholesterol groups. These amphiphilic macromolecules therefore entwined with PLA, forming the shell of the micelles. Some of the Chol-Dex, however, might be difficult to penetrate from the interior to the surface of the micelles because of their entanglement with the PLA chains. As dialysis proceeded, PLA molecules that interacted with the hydrophilic Dex may have desolvated more quickly than those inside the core,

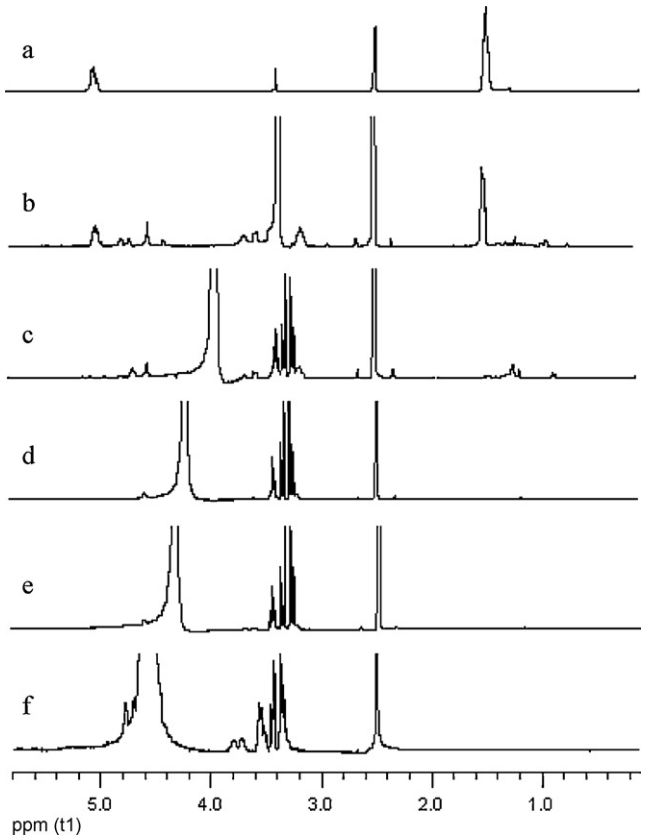

Fig. 6. ^{1}H NMR spectra obtained during hollow capsule formation. Chol-Dex and PLA were dissolved in deuterated DMSO and then dialyzed against D_2O. The sampling times for the measurement were (b) 0 min, (c) 5 min, (d) 15 min, (e) 30 min and (f) 4 h, respectively. (a) ^{1}H NMR spectra of PLA in deuterated DMSO.

leading to a firm shell (Fig. 5c). Once formed, the shell restricted the "collapse" or "shrinkage" of the micelles. Swelled PLA molecules inside the micelles would deposit on the inner surface of the shell. Meanwhile, the remaining Chol-Dex inside the aggregates assembled into the innermost hydrophilic layer (Fig. 5d). Finally, hollow spheres with a sandwich shell structure were formed (Fig. 5e).

After analysis, we believed that the formation of hollow spheres was influenced by two physical factors: (a) intermolecular forces occurring between hydrophobic cholesterol and PLA and (b) phase separation induced by the polarity difference between Chol-Dex and PLA. Differential thermal analysis results established the existence of intermolecular forces. Compared to that of pure PLA, the glass-transition temperature (T_g) of PLA decreased after the formation of hollow capsules with Chol-Dex, demonstrating the intermolecular interaction and entanglement effects of Dex segments with PLA, mediated by the hydrophobic pendant cholesterol groups. This finding is consistent with that of Rouzes et al. (2000), who found that the T_g of PLA decreased when hydrophobically modified dextran was added, while no significant changes in the T_g of PLA was observed in the presence of unmodified dextran. It was postulated that the hydrophobic groups acted as blend compatibilizers and favored phase mixing. The solubility of PLA and its oligomers had a notable influence on self-aggregation morphology, suggesting that the polarity difference between Chol-Dex and PLA plays a crucial role in the formation of hollow capsules. As shown in Fig. 3, when the molecular weight of PLA oligomers was less than 210 Da, they were insoluble in chloroform but were soluble in ethanol. A strong intermolecular interaction is therefore unlikely because of the large differences in polarity between cholesterol pendant groups and PLA molecules. This prevents the formation of core–shell micelles. Consequently, no stable solidified shell could be formed and the aggregates gradually collapsed inwards giving solid spheres.

In addition to intermolecular interactions and phase separation, we found that the weight ratio of Chol-Dex to PLA was an essential factor in determining the assembly morphology of Chol-Dex/PLA aggregates. The optimal weight ratios for the generation of hollow capsules were 3:1 to 1:1 (Chol-Dex:PLA). When weight ratios of greater than 3:1 were used, the Chol-Dex/PLA complex assembled into swelling aggregates and then gradually collapsed inward to form solid spheres. This is proposed to be because insufficient PLA was available to form a firm shell at the interface during desolvation. At weight ratios of less than 1:1, the Chol-Dex/PLA complex was not able to "pack" all the hydrophobic PLA inside, resulting in sticky, non-regular spheres. The optimum conditions for hollow capsule formation occurred with a weight ratio of Chol-Dex to PLA of 2:1, and using PLA with a molecular weight of 29,000 Da.

It is worth noting that preparation method played a decisive role in the self-assembly of Chol-Dex and PLA. When compared with emulsion/solvent evaporation methods described previously, the dialysis method is a relatively slow and progressive process. Preparing hollow capsules by gradual addition of water or using the standard organic/water emulsion method failed, suggesting that a long equilibrium time is necessary to ensure capsule formation. The long aggregation time facilitates the interaction of PLA molecules with Chol-Dex, allowing the parallel packing of PLA molecules and thus the formation of hollow structures.

4. Conclusion

Chol-Dex and PLA have been shown to self-assemble into hollow capsules by dialysis of their DMSO solution against water. The hollow capsules were thought to be formed preliminarily by anchoring of most amphiphilic polysaccharide onto the surface of swelled aggregates because of the phase separation of amphiphilic Chol-Dex and hydrophobic PLA, followed by the deposition of PLA on the inner interface of polysaccharide layer due to the "deswelling" of the aggregates, and finally the remaining Chol-Dex inside the aggregates formed the innermost hydrophilic layer. Thus a new approach to polymeric hollow capsules has been developed, and the Chol-Dex/PLA hollow capsules obtained have potential application as drug carriers or biomembrane models.

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