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Nanoassembly of Biodegradable Microcapsules for DNA Encasing

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The development of layer-by-layer (LbL) self-assembly technology[1] promises a solution to many problems of modern biotechnology. Encapsulation of DNA molecules in micro- and nanovolume is an important goal. Although considerable progress has been made in gene therapy and DNA vaccine technology, the problem of DNA degradation upon delivery still remains.[2] It is important to develop a carrier system that can penetrate the cell shell and protect plasmid DNA from degradation on the way to the cell nucleous. Depot and delivery systems such as polymer microparticles prepared using emulsion method, solvent-diffusion nanospheres, and liposomes provided an essential progress in DNA delivery.[3] However, a lack of high enough efficiency of encapsulation and needs to engineer the structure and properties of protective shell on nanometer scale provide an avenue for further efforts in this direction. In this work, we propose a new "vehicle" for DNA delivery based on microcapsules with a 40 nm thick molecularly organized biocompatible shell.

Polyelectrolyte capsules, first reported in ref 4a, are based on application of LbL assembly of nanometer-thick polymeric films with controlled composition and properties onto the surface of tiny, decomposable cores. A variety of materials (synthetic and natural polyelectrolytes, proteins, multivalent ions, inorganic and organic nanoparticles, and lipids) were used as components of the capsule shell providing versatile properties.[4] Macromolecules can be introduced inside the capsules by two ways: first, using encapsulated material as template core, and second, loading macromolecules into a preformed polyelectrolyte shell. Adjusting shell permeability by changing a solvent (water−alcohol−acetone), ionic strength, or pH allows controlling release of the encapsulated compound.[5]

In this study, a novel process of microencapsulating DNA in biocompatible poly[β-glucuronic acid-(1 → 3)-*N*-acetyl-β-galactosamine-6-sulfate-(1 → 4)] (known as chondroitin sulfate, Sigma) (PG)/poly(-L-arginine) (PA) capsules of 4 μm diameter was developed. DNA molecules were deposited by controlled precipitation of DNA/sperimidine (Sp) complex onto a surface of template microparticles followed by LbL assembly of PA and PG protective biocompatible shell. Large 4 μm capsules were used as a model to develop DNA encapsulation procedure and to understand DNA behavior in capsule volume.

$MnCO_3$ particles of 4 μm diameter (from PlasmaChem Gmbh, Germany) were used as template cores. $MnCO_3$ (0.5 mg/mL) particle suspension (30 mL) was mixed with 1 mL of 1.5 mg/mL DNA solution (highly polymerized DNA sodium salt from Calf Thymus, Sigma). Precipitation of water-insoluble DNA/Sp complex on template particles was made adding dropwise 2 mL of 1 mg/mL sperimidine solution into stirred $MnCO_3$/DNA solution (Figure 1a,b). Further alternated LbL assembly of biocompatible PA/PG shell was carried out with 1 mg/mL PA or PG solutions (Figure 1b,c). After each deposition step, microparticles were washed out

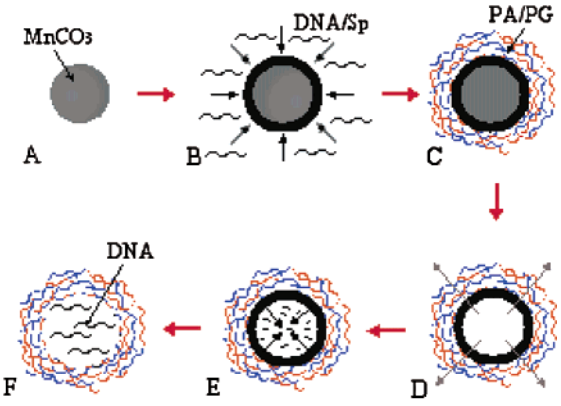

Figure 1. Schematic illustration of the DNA encapsulation process. (A,B) Controlled precipitation of DNA/sperimidine (Sp) complex on the surface of template particles. (B,C) LbL assembly of protective biocompatible shell. (C,D) Template dissolution. (D,E) DNA/Sp complex dissolution.

3 times. A layer-by-layer assembly of polyelectrolyte layers was monitored by electrophoretic mobility measurements (ZetaPlus Zeta Potential Analyzer, Brookhaven Instrument Corp.). For each sample, four PA/PG bilayers were deposited with PA as the last monolayer, and the surface potential of the capsule regularly changed from +40 mV for PA to −35 mV for PG, indicating formation of the planned wall composition. The assembly step for PA/PG was found to be 5 nm from parallel PA/PG assembly on QCM electrode (Quartz Crystal Microbalance, USI-System, Japan). Therefore, the total capsule wall thickness was 40 nm. The outermost layer was chosen to be PA because of its potential to increase translocation activity through the cell membranes.[6]

At the final step, $MnCO_3$ template particles were dissolved in deaerated 0.01 M HCl. As a result, biocompatible PA/PG capsules containing DNA/Sp complex were obtained. After removal of the core, the capsules were studied with confocal fluorescence microscopy (Leica DM IRE2 confocal fluorescence microscope). Figure 2a illustrates a typical fluorescence image of (DNA/Sp)PA/PG capsules immediately after dissolution of template. The fluorescence signal is caused by presence of Rhodamine-labeled DNA[4c,7] in the capsule interior. The cross-sectional profile of fluorescence intensity along the capsule diameter gives the DNA distribution. There are two peaks demonstrating that initially DNA was confined to the inner capsule walls. Then these capsules were treated with 0.1 M HCl for 10 min. Such treatment led to the decomposition of DNA/Sp complex formed in aqueous solution at neutral pH.[4d] Thus, low-molecular weight sperimidine was released and removed from the capsule interior. The capsule was now filled with freely floating DNA molecules, which was proved by fluorescence signal from the whole capsule volume (Figure 2b). The fluorescent signal is distributed evenly over the capsule interior.

During optimization of DNA adsorption we found that 100% of DNA could be deposited on the template surface from the water

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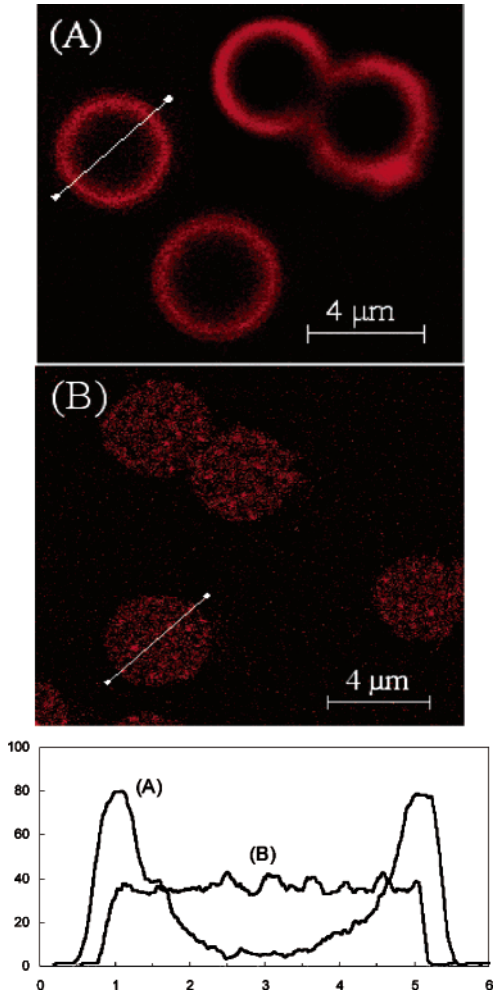

Figure 2. Fluorescence confocal microscopy images of the DNA-containing capsules composed of 4 PA/PG bilayers just after decomposition of template core (A) and after dissolution of the inner DNA/Sp complex (B). Areas under the curves are similar. The inset demonstrates the fluorescence profile for both cases.

solution as DNA/Sp complex. Judging from precipitation yield, quantity of the capsules in solution ($\sim 10^8$), and mass of DNA captured in one capsule (12 pg as measured using Quartz Crystal Microbalance), one can estimate the average concentration of DNA in the capsule volume. The average concentration of DNA encapsulated via DNA/Sp complex is 0.4 mg per 1 mL of capsule volume.

Due to the helical structure of double-stranded DNA, its circular dichroism (CD) spectrum has a strong signal in the 230−350 nm range.[8] In Figure 3, the CD-spectra of initial DNA, DNA treated with 0.1 M HCl, and DNA captured in capsule volume are compared (Jasco J-810 spectropolarimeter). The spectrum of initial DNA in water solution is typical of the double helix conformation of DNA. Adding HCl to DNA solution results in the disappearance of the negative band at 230−260 nm caused by denaturation of double-stranded DNA.[8] However, after decomposition of the DNA/Sp complex, the CD spectrum of DNA captured inside PA/PG microcapsules reveals minor changes as compared to initial DNA. As compared with DNA mixed with 0.1 HCl, the encapsulated DNA exhibits more stability against denaturation or more ability to refold, thus preserving ~90% of the helicity. Apparently, sperimidine and

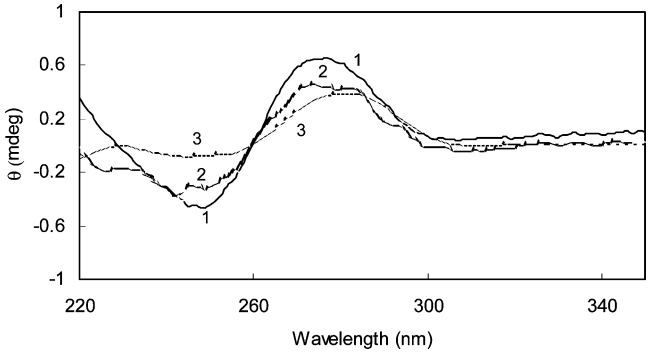

Figure 3. Circular dichroism spectra of initial DNA (1), DNA captured in capsule volume (2), and DNA in 0.1 M HCl (3).

polyarginine partially compensate in capsule volume the negative effect of low pH forming pH gradient across the capsule shell. This was previously observed for polyelectrolyte capsules containing polyamines inside.[9] Encapsulated DNA can be released from capsule at pH <3.

In conclusion, we developed a versatile approach for DNA encapsulation inside biocompatible polyelectrolyte microshell retaining natural double-helix structure of DNA. This approach can be applied for fabrication of DNA containers of smaller size (up to 100 nm). In the future, this technique will be employed for targeted delivery of plasmid DNA in living cells using capsules of a smaller diameter (200−300 nm).

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