

## Template assisted synthesis of layered chitosan microcapsules

Qinghe Zhao<sup>1</sup>, Bingyun Li<sup>1,2,3</sup>

<sup>1</sup>Biomaterials, Bioengineering & Nanotechnology Laboratory  
Department of Orthopaedics, School of Medicine, West Virginia University

<sup>2</sup>WVNano Initiative, West Virginia

<sup>3</sup>Department of Chemical Engineering, West Virginia University

**Statement of Purpose:** Biocompatible microcapsules are of both scientific and technological interest and have extensive applications in areas of biotechnology and nanomedicine [1]. Chitosan, a copolymer of *N*-acetylglucosamine and glucosamine (i.e. 2-amino-2-deoxy- $\beta$ -D-glucose), was incorporated into  $\text{CaCO}_3$  microparticles, which were used as templates to fabricate microcapsules using a modified layer-by-layer self-assembly (LBL) technology [2]. Chitosan was layer-wise assembled onto as-prepared  $\text{CaCO}_3$ (chitosan) microparticles with the assistance of a biocompatible crosslinker, genipin. Successful assembly of chitosan/genipin was monitored using UV-vis spectrophotometry. Confocal laser scanning microscopy (CLSM) images showed the loading of a model drug, fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA), into the chitosan/genipin microcapsules.

**Methods:**  $\text{CaCO}_3$ (chitosan) colloidal microparticles were prepared by mixing solutions of 0.33 M  $\text{Na}_2\text{CO}_3$  and chitosan-containing 0.33 M  $\text{Ca}(\text{NO}_3)_2$ . The microparticles were rinsed twice with deionized (DI) water and then incubated in a 0.5% genipin solution for 30 min, followed by washing three times with DI water. In the next step, the microparticles were incubated in a 1 mg/mL chitosan solution for 30 min, followed by three washings. By repeating the procedure of genipin and chitosan treatments, chitosan/genipin multilayers were assembled onto quartz slides and  $\text{CaCO}_3$ (chitosan) microparticles. After forming multilayers on the  $\text{CaCO}_3$ (chitosan) microparticles, the core-shell particles were transferred to a 0.1 M disodium ethylenediaminetetraacetic acid (EDTA) solution and the templates were removed. The obtained microcapsules were washed three times in DI water prior to further use.

The deposition of chitosan, with the assistance of genipin, on quartz slides was recorded using UV-vis spectrophotometry (Biomate 3, Madison, WI, USA). The loading of FITC-BSA into chitosan/genipin microcapsules was confirmed using CLSM (Zeiss LSM 510, Thornwood, NY, USA).

**Results:** Genipin is a natural crosslinker that has been used to crosslink polysaccharides and proteins containing primary amine groups [3], and it has promising applications in drug delivery and tissue engineering. In the present work, genipin was used in the assembly process of deposition of chitosan onto flat and curved surfaces. The absorbance of chitosan/genipin on quartz slides increased with an increasing number of chitosan/genipin layers (Figure 1A).

Chitosan/genipin microcapsules were fabricated by depositing chitosan/genipin onto  $\text{CaCO}_3$ (chitosan) microparticles followed by the removal of  $\text{CaCO}_3$ . FITC-BSA was employed as a model drug to demonstrate its loading in the developed chitosan/genipin microcapsules, as shown in the CLSM image (Figure 1B).

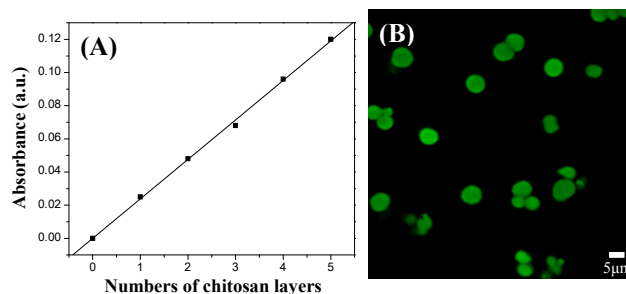


Figure 1. (A) Assembly of chitosan/genipin on quartz slides, and (B) loading of FITC-BSA into chitosan/genipin microcapsules.

Moreover, the chitosan/genipin microcapsules were found to be stable in harsh pH solutions, i.e., when immersed in pH 1.0 HCl and pH 10.0 NaOH solutions. In the two pH solutions, FITC-BSA could be loaded into the microcapsules, according to the strong fluorescence intensities inside the microcapsules (data not shown).

**Conclusions:** A novel microcapsule containing a single polysaccharide, chitosan, was fabricated. We integrated chitosan molecules into  $\text{CaCO}_3$  microparticles during the preparation of the particles. Chitosan was subsequently adsorbed onto  $\text{CaCO}_3$ (chitosan) particles using a modified LBL technology with genipin as a crosslinking agent at each deposition interval. After dissociation of the  $\text{CaCO}_3$  templates, biocompatible microcapsules were obtained. A model protein drug, FITC-BSA, was loaded into chitosan/genipin microcapsules. The biocompatible and biodegradable microcapsules appear promising for controlled drug delivery and other applications.

### References:

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