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Capsules with Concentric Biopolymer-Nylon Shells Imaged by Cryo-FIB/SEM

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Capsules can be easily formed by contacting droplets of a cationic biopolymer (such as chitosan) with a solution of an anionic polymer or surfactant. These capsules have a thin shell of polymer hydrogel encompassing an aqueous interior [1]. Such capsules can be loaded with drugs and proteins for applications in pharmaceuticals, consumer products, drug delivery, and tissue engineering [2]. However, the capsules tend to allow the encapsulated solutes to leak out because of their large pore sizes when compared to solute sizes. Accordingly, to contain solutes for longer periods, researchers have looked at encasing the above capsules with synthetic polymers formed by chain-growth polymerization [1] or by waxes [3]. Here, we introduce a new method by which a biopolymer capsule can be covered by an outer layer formed by step-growth polymerization. Examples of synthetic polymers created by step-growth polymerization include polyamides such as nylon. Our approach yields capsules with an aqueous core, surrounded first by a biopolymer shell and thereafter by a concentric nylon (polyamide) layer. To our knowledge, such a multilayered capsule has never been created before. This paper presents a novel approach to investigate the internal microstructure of capsules with biopolymer-nylon shells by Cryo-FIB/SEM method.

To synthesize these capsules, we employ an 'inside-out' strategy [1]. First, a biopolymer capsule is made in the usual way and it has a shell of biopolymer hydrogel surrounding an aqueous core. The capsule is then loaded with an aqueous diamine monomer, and this is placed in an organic solution of an acid chloride monomer. This begins the interfacial step-growth polymerization, which results in a polyamide layer around the biopolymer shell. The polymerization process is completed in less than a minute because of the fast polymerization rates of polyamides. The polyamide layer is typically quite thin (~ 200 nm), which is much less than the biopolymer shell (~ 10 μm). To image each layer distinctly, we have resorted to cryo-SEM and moreover, the focused ion beam (FIB) technique. The sample for cryo-SEM was prepared by rapidly freezing a 2-mm-diameter capsule by submerging into liquid nitrogen (Leica EM VCM). The frozen sample was then fractured and coated with gold in a high vacuum coater (Leica EM ACE600), and then transferred onto a FIB/SEM cryo-stage using a cryo-transfer system (Leica EM VCT500). Cryo FIB/SEM was performed using Tescan GAIA FIB/SEM with the accelerating voltage of 30 keV for Ga ion beam and 5 keV for electron beam. The entire process including sample preparation, transfer, SEM observation, and FIB milling was carried out at cryo-temperature (-150 oC).

A low-magnification image of a frozen capsule of 2-mm diameter is shown in Fig. 1A. The aqueous-core and the biopolymer shell are clearly evident from this image. However, to visualize the thin nylon layer, a close-up view is required. A magnified section of the capsule (Fig. 1B) shows its different regions: (1) the liquid core, (2) the highly crosslinked inner shell of biopolymer, and (3) the thin outer layer of polyamide (nylon). A different section of the capsule clearly captures the separate biopolymer layer and the outer nylon layer with a gap in between them (Fig. 1C). Figure 1D shows the thickness of the polyamide layer (224 nm) and the layer of porous biopolymer network. This thickness is consistent with the thickness reported for polyamide layers typically used in thin-film composite membranes for nanofiltration and reverse osmosis water purification technologies. To validate these findings, cryo-FIB was employed. Due to the high energy ion beam and the "soft" nature of the hydrated materials, the FIB milled surface tends to be rather rough by strong curtain effect as compared to the smooth knife fractured surface (Fig. 2A). Nevertheless, when the FIB mills a hole through an intact capsule (shown on the top corner of Fig. 2B), it again shows the three different regions of the capsule (Fig. 2B), which validates the previous images. In conclusion, we have demonstrated the synthesis of capsules with an unusual morphology: i.e., they have an inner layer of biopolymer hydrogel followed by an outer layer of a polymer formed by step-growth polymerization (polyamide or nylon). We have also employed state-of-the-art cryo-microscopy techniques to obtain insight into the above structures. The capsules described here are likely to be of interest to a range of scientists and engineers.

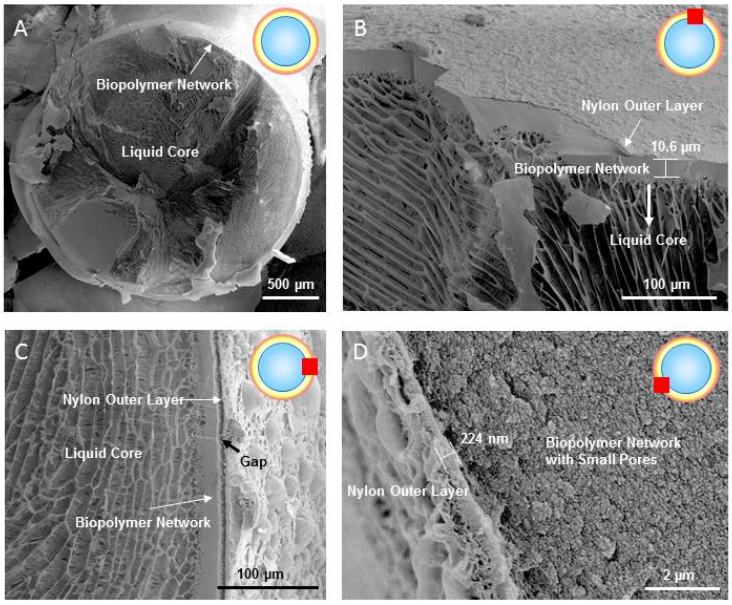

Figure 1. Fig. 1. (A) Low magnification image of multilayered capsule, (B) Close-up view of the multilayered capsule (red region) showing the liquid core, biopolymer shell and, the thin nylon outer layer along with the thickness of the biopolymer network, (C) Close-up view of the capsule showing the gap between the layers and, (D) SEM image showing the thickness of the outer nylon layer and porous biopolymer network.

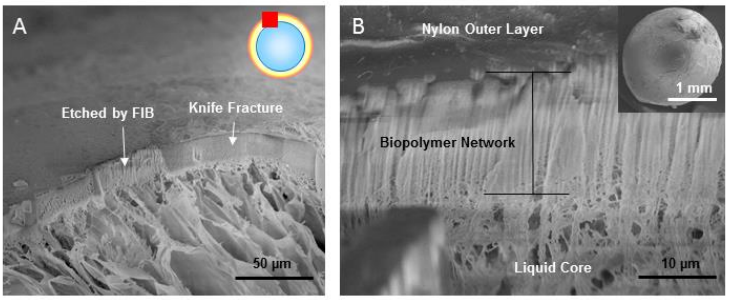

Figure 2. Fig. 2. (A) SEM image showing the strong curtain effect of FIB milled surface and, (B) SEM image showing the three different regions of the multilayered capsule using cryo-FIB technique along with the intact capsule (on the top right) which was milled using FIB.

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