Template Mineralization of Ordered Macroporous Chitin−Silica Composites Using a Cuttlebone-Derived Organic Matrix

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One of the principal objectives of biomimetic materials chemistry is the development of new environmentally benign strategies for the synthesis of materials possessing shapes and structures analogous to biominerals such as shell, bones, and teeth.[1,2] In pursuit of this goal, self-assembled organic templates of synthetic or biological origin have been used to organize and pattern processes of inorganic deposition involving molecular or nanoparticle building blocks. For example, block copolymer lyotropic mesophases,[3,4] latex particles,[5] colloidal crystals,[6] filamentous crystals,[7] gel fibers,[8] spongelike polymer gels,[9] lipid tubules,[10] viral filaments,[11] bacterial membranes,[12] bacterial superstructures,[13] and spider silk fibers[14] have been employed in the synthesis of novel inorganic−organic composites with controlled size, shape, organization, and porosity. Such materials could have important applications in separation and purification processes, catalysis, storage and release systems, smart fillers, and small-scale magnetic and quantum devices.

In this paper we describe a biomimetic strategy for the production of ordered chamber-like macroporous silica composites by using the intact organic matrix isolated from the cuttlebone of *Sepia officinalis*, commonly known as the cuttlefish. The cuttlebone is a highly organized internal shell structure constructed from aragonite ($CaCO_3$) in association with a β-chitin organic framework.[15] The shell has a chamber-like architecture (porosity, 93 vol %; specific gravity, 0.19) in the form of mineralized sheets arranged in parallel layers and separated by S-shaped pillars (see graphical abstract), which functions as a rigid buoyancy tank that enables the animal to withstand external pressures of up to 2.4 MPa and maintain a fixed position up to a depth of 230 m.[16,17] Significantly, previous investigations have indicated that the demineralization of cuttlebone results in a well-organized β-chitin replica of the chamber-like architecture.[15] This suggests that a range of artificial materials with the cuttlebone architecture might be accessible by remineralization of the β-chitin

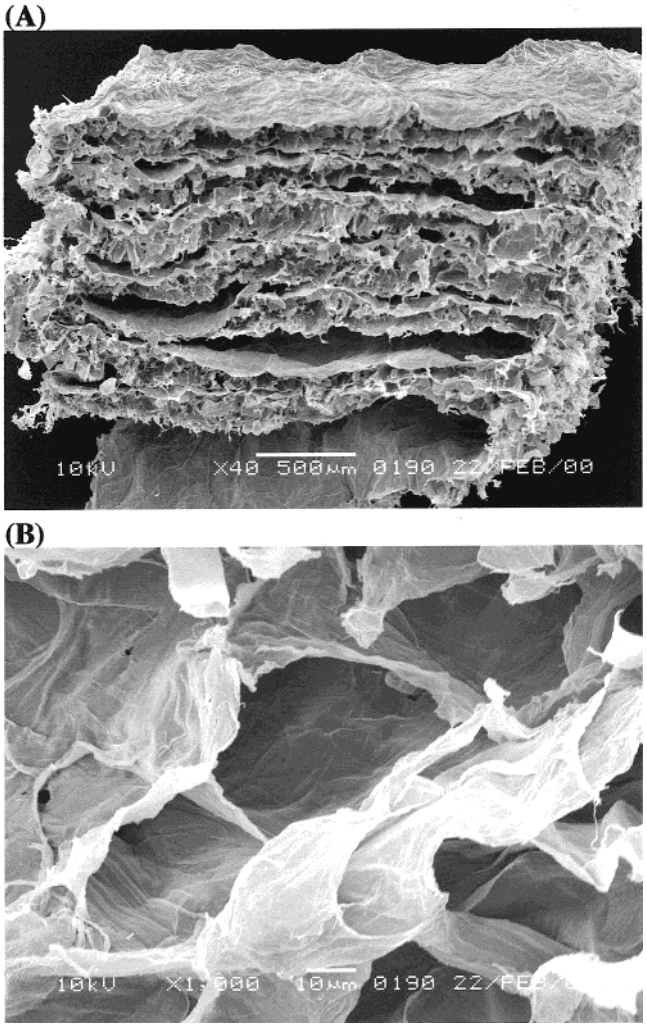

Figure 1. SEM images of (A) β-chitin organic matrix isolated from cuttlebone, scale bar = 500 μm and (B) high-magnification image showing interconnecting wall structure between adjacent organic sheets, scale bar = 10 μm.

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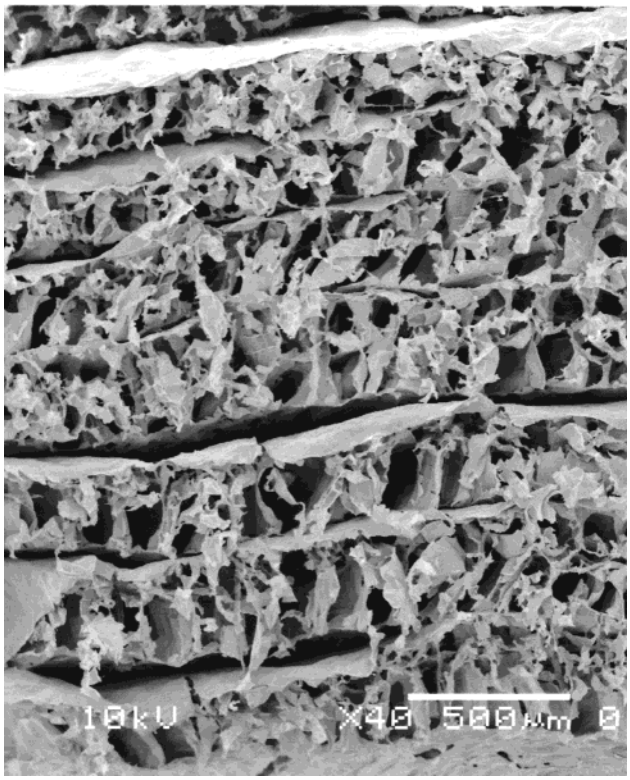

Figure 2. Low-magnification SEM image of ordered β-chitin−silica macroporous replica of the cuttlebone organic matrix. The sample was prepared using alkaline solutions containing 15 vol % ethanol. Scale bar = 500 μm.

framework if complementary interfacial interactions can be achieved. In this regard, a recent study showed that the β-chitin matrix of squid pen was only active in calcium carbonate mineralization when certain shell macromolecules were present in the crystallization medium.[18] Here, we show that high-fidelity silica replicas of the cuttlebone structure can be produced from condensation reactions of silicate ions in alkaline solutions without the addition of auxiliary molecules.

Demineralization of cuttlebone was carried out according to previous work[15,19] with some modifications.[20] The resulting white organic matrix consisted of a three-dimensional (3-D) spongelike monolith that was confirmed by FTIR spectroscopy and XRD to be pure β-chitin.[21] Optical and SEM images showed that the organic matrix was structurally continuous and retained the 3-D chamber-like architecture of the native bio-

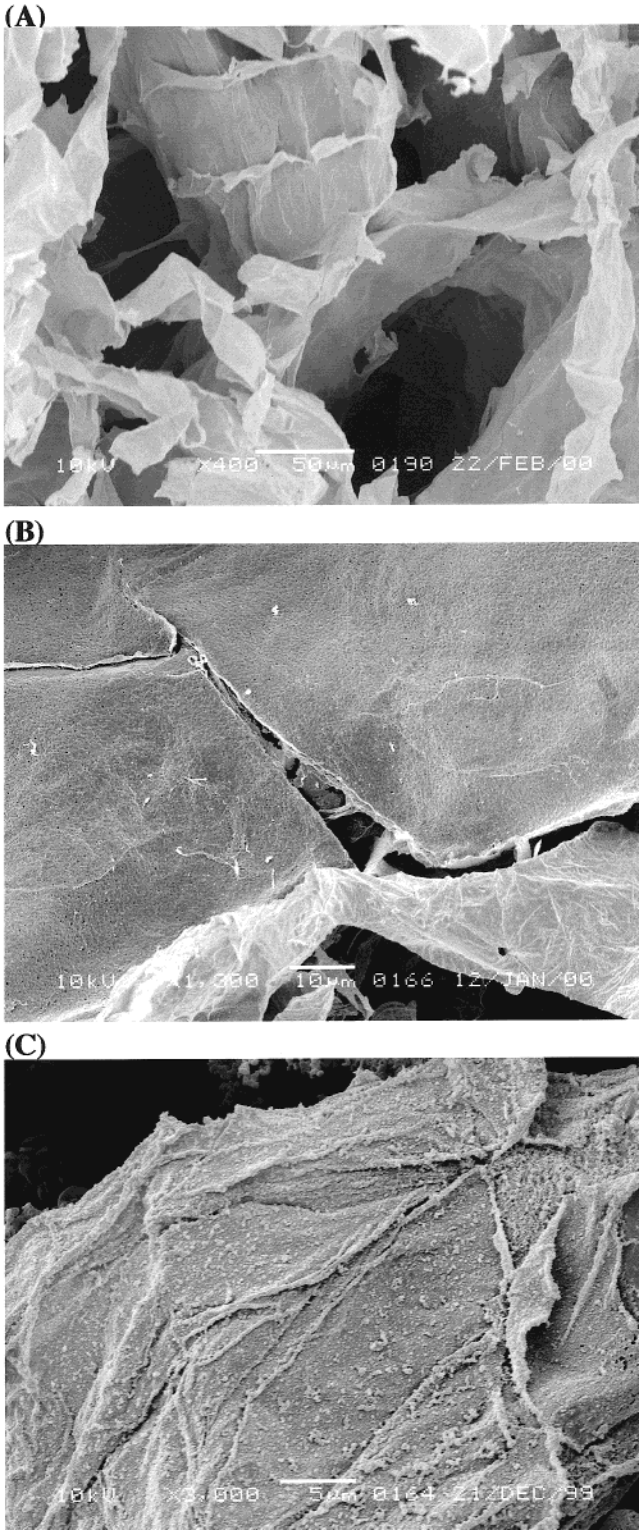

Figure 3. High-magnification SEM images of β-chitin−silica structure shown in Figure 2. (A) Mineralized pillars in interlayer space, scale bar = 50 μm, and (B) silica-coated sheets, scale bar = 10 μm. (C) Mineralized sheets with increased silica loading formed at higher silicate supersaturation (25 vol % ethanol), scale bar = 5 μm.

mineral observed prior to decalcification. Although the samples collapsed to some extent in the vacuum of the SEM, a well-organized lamellar structure in which parallel stacks of thin organic sheets were spaced at regular intervals of 100−300 μm could be readily observed (Figure 1A). The individual sheets were sup-

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(20) A cuttlebone (≈15 g) was cut into 1 × 1 cm pieces and soaked for 12 h in 500 mL of 2 M HCl under low vacuum. A low vacuum was used to exclude CO_2 bubbles, which formed during dissolution of the aragonite component, from the internal chambers of the organic matrix. The procedure was repeated overnight in fresh aqueous 2 M HCl. The pieces were then washed with distilled water until neutral pH and added to 500 mL of 1 M NaOH at a boiling temperature for 4 h to remove associated proteins. The samples were filtered, washed with copious amounts of water, and added to 95% ethanol for 3 h, followed by further washing with distilled water. The organic matrix was stored in water at 4 °C and freeze-dried for SEM analysis.

(21) FTIR data for organic matrix (β-chitin): ν(OH) 3450, 3095 cm^{-1}; ν(NH, H-bond) 3270 cm^{-1}, ν(C=O) 1620−1620 cm^{-1}, δ(NH) 1560 cm^{-1}. (Reference data: Kurita, K.; Tomita, K.; Tada, T.; Ishi, S.; Nishimura, S.; Shimoda, K. *J. Polym. Sci. A* **1993**, *31*, 485. Focher, B.; Naggi, A.; Torri, G.; Cosani, A.; Terbojevich, M. *Carbohydr. Polym.* **1992**, *17*, 97.). XRD data: broad bands at 1.100, 0.453, and 0.33 nm (weak). (Reference data: Gardner, K. H.; Blackwell, J. *Biopolymers* **1975**, *14*, 1581).

ported by organic wall structures that spanned and divided the interlayer space into air-filled chambers, 50−100 μm in size (Figure 1B). The sheets and interlamellar pillars were ca. 0.5 μm in thickness and continuous and smooth in texture.

Remineralization of the cuttlebone matrix was achieved by a multistep process involving incubation of the isolated β-chitin monoliths in a sodium silicate solution at pH 11.5 followed by reaction in a series of ethanol/water mixtures at pH values between 10 and 10.5.[22] This water-based (non-alkoxide) route provided good control over the rate of silica precipitation. In particular, low levels of supersaturation were reproducibly achieved by using successive alkaline solutions differing in only 1.0−1.5 pH units less than the original silicate solution. Under these conditions, the supersaturation level could be effectively controlled by changes in the ethanol content of the alkaline solutions. Increases in ethanol concentration reduced the silicate solubility and hence increased the supersaturation, presumably by a dehydration-induced mechanism accompanying changes in the dielectric constant of the solvent. In addition, when a series of three successive ethanolic alkaline solutions was used, nonspecific silica deposits adventitiously associated with the β-chitin matrix were selectively removed to produce high-fidelity silica replicas of the organized chamber-like architecture of the β-chitin matrix (Figure 2). EDXA, and FTIR spectroscopy,[23] confirmed the presence of silica throughout the macroporous composite and high-magnification SEM images showed that the lamellar sheets and interlamellar wall structures were coated in a smooth thin film of silica (Figure 3A and B). Corresponding TGA profiles[24] showed a rapid weight loss at ≈280 °C due to removal of the β-chitin template and indicated a silica loading of ≈65 and 75 wt %, for macroporous monoliths prepared from silicification reactions in alkaline solutions containing 15 and 25 vol % ethanol, respectively. Although the intact chamber-like architecture was not retained in the calcined material, interconnected sheets of amorphous silica resembling aspects of the inorganic−organic composite were observed.

The close association and high degree of structural integrity between the inorganic and organic constituents in the remineralized cuttlebone matrix suggest that silicate ions and silica oligomers preferentially interact with glucopyranose rings exposed at the β-chitin surface, presumably through polar and H-bonding interactions. The template activity of the chitin matrix was predominant at relatively low levels of silica formation, and raising the supersaturation by increasing the concentration of ethanol in the alkaline solutions from 15 to 25 vol % produced coatings with a significantly rougher texture (Figure 3C). Thicker coatings were also obtained by increasing the level of deacetylation in the β-chitin matrix prior to silicification by pretreatment in a boiling NaOH solution (pH 14) for 3 h.[25] At high supersaturation levels (over 40 vol % ethanol), nonspecific silica aggregation was observed.

In conclusion, we have shown that cuttlebone β-chitin can be used as a highly organized organic template with macroscopic porosity to prepare analogous silica−polysaccharide replicas with 3-D interconnected box structures. Our results indicate that the inorganic content and fidelity of replication can be controlled by using alkaline ethanolic solutions with variable levels of silicate supersaturation. The template specificity may depend in part on partial deacetylation of the chitin matrix, although no harsh chemical treatments were required. Further experiments with other mineral phases are in progress.

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(22) A piece of the β-chitin cuttlebone matrix was soaked for 5 h in 5 mL of sodium silicate (1.72 M, pH 11.5) at room temperature, then removed, and immersed for 30 min in 3 mL of an ethanol/water mixture with variable ethanol concentration (100, 50, 40, 35, 30, 20, 15, 10, or 0 vol %, final pH 10). The sample was then transferred to a fresh ethanol/water mixture for an additional 30 min (final pH ≈10), after which it was left to stand for 1 h in 3 mL of an ethanol/$NH_4OH_{(aq)}$ mixture ([ethanol] = 100, 50, 40, 35, 30, 20, 15, 10, or 0 vol %, [NH_4-OH] = 30 mM, pH = 10.5). The intermediate ethanol/water stages were omitted from some experiments. The mineralized matrix was washed twice with 10 mL of distilled water and freeze-dried for SEM analysis.

(23) FTIR data for β-chitin-silica composites (30 vol % ethanol): ν(OH) 3450, 3095 cm^{-1}; ν(NH) 3270 cm^{-1}, ν(C=O) 1620−1620 cm^{-1}, δ(NH) 1560 cm^{-1}, δ(Si−O−Si) 468 cm^{-1}, ν(Si−O−Si) 795 cm^{-1}.

(24) Calcination of remineralized β-chitin was carried out at 5 °C min^{-1} in flowing air at 1-min intervals up to a temperature of 800 °C using a NETZSCH thermal analysis STA 409EP.

(25) Partially deacetylated β-chitin was prepared by immersing pieces of pure β-chitin in 200 mL of NaOH (5 M, pH 14) for 3 h at 100 °C. The matrix was filtered using glass wool and washed with water until the pH was neutral. Under these conditions, FTIR spectra showed a marked reduction in the intensities of the δ(NH) deformation at 1560 cm^{-1} and intermolecular H-bonded acetamide band at 3270 cm^{-1}, consistent with partial deacetylation but not chitosan formation. Method according to Kurita, K.; Sanman, T.; Iwakura, Y. *Makromol. Chem.* **1977**, *178*, 3197.