

SOL-GEL PREPARATION OF SILICA-BASED NANO-FIBERS FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Silica nanotube sponges and solid silica fiber mats were fabricated using a collagen and electrospun poly(vinylalcohol) as the templates via the sol-gel route. In vitro reassembled collagen fibrils were treated in the Stöber sol-gel precursor mixture of tetraethoxysilane (TEOS), ethanol, water, and ammonia to produce the silica-coated collagen fibrils. The electrospun PVA fiber mats were soaked in the Stöber type as well as acid-catalyzed silica oligomer sol from TEOS. Those were calcined to yield apparently silica nano- or micro-fibers, but transmission and scanning electron microscope observations showed that the Stöber sol treatment resulted in silica nano- or micro-fibers with hollow structure, or nanotubes (silica NTs), while the acid-catalyzed sol yielded solid fibrous mats.

INTRODUCTION

After Iijima et al. [1], carbon nanotubes and inorganic nanotubes have attracted much attention in development of novel electronic, thermal, and photo-catalytic materials because of their unique one-dimensional tubular structures and high surface-to-volume ratios. Recent researches have indicated that those nanotubes exhibit non-toxicity or low toxicity and have been used as drug delivery and tissue regeneration matrix. Their one-dimensional tubular structure not only mimic the fibrous structure of extracellular matrix (ECM) to support cell attachment and proliferation [2], but also provide the void space for accommodation and release of drugs or biological factors to stimulate cell functions [3].

Silica nanotubes are typical inorganic nanotubes and have been explored as carriers for anti-cancer drugs, DNA, and enzymes [4-5]. Compared with other kinds of nanotubes such as carbon and titania nanotubes, silica nanotubes exhibit better biocompatibility because of their hydrophilic silanol groups (Si-OH) and silicate networks (Si-O-Si) [4-5]. They have been fabricated using various templates including carbon nanotubes [6], and reverse microemulsion [7]. However, those routes are not suitable for a large-scale fabrication of the silica nanotube.

The present study employed the reassembled collagen fibrils and electrospun poly(vinylalcohol) (PVA) as the templates to fabricate the silica nano- and micro-tubes and solid silica fiber mats on a large scale. The reassembled collagen fibrils were firstly coated with silica layer in the Stöber-type sol-gel system [8] consisting of tetraethoxysilane (TEOS), ethanol, water, ammonia and subsequently calcined to produce the silica nanotube sponge. The microstructure of those silica mats was discussed in terms of the base- and acid-catalysis of TEOS.

EXPERIMENTAL PROCEDURE

Silica nanotube sponge was in situ fabricated using the reassembled collagen fibrils as template. Figure 1 schematically presents a procedure for fabricating the silica nanotube sponge. In brief, type I porcine collagen was dissolved in 1 mM of HCl to produce 0.5%(w/v) collagen solution. The collagen solution was mixed with the phosphate buffer saline (PBS, pH=7.4, 1370 mM NaCl, 27 mM KCl, 80 mM Na₂HPO₄, and 15 mM KH₂PO₄) at volume ratio of collagen/PBS of 9/1 and then incubated at 37 °C for 2 h to produce the reassembled collagen fibrils. Subsequently, those collagen fibrils were then immersed in a Stöber-type sol-gel precursor mixture [8] of ethanol (9 mL), water (9 mL), ammonium hydroxide (0.5 mL, 25%), and TEOS (1 or 2 mL): in the molar ratio 1: 23.0: 12.3: 0.7 (TEOS: EtOH: H₂O: NH₄OH). After 24 h, the silica-coated collagen hybrid fibrils were obtained and then calcined at 600 °C for 2 h to in situ produce the silica nanotube sponge. Electrospinning of PVA was conducted for 8 mass% aqueous solution of PVA with degree of polymerization 2000, using a standard set of spinning apparatus, consisting of a syringe (anode) and a sheet of Al foil (cathode) that were 15 cm apart and loaded with 15kV acceleration. For those PVA mats, a weaker alkaline Stöber type system with the mixing molar ratio TEOS: EtOH: H₂O: NH₄OH / 1: 23.0: 12.3: 0.5 was employed as well as the HCl-catalyzed system of TEOS: EtOH: H₂O: HCl / 1: 5: 4: 0.5. The mats were soaked in both sols for 2 h.

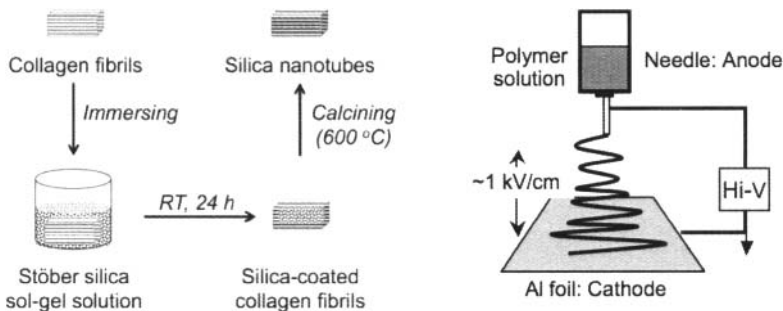


Figure 1. Schematic presentation of (left) fabrication procedure for the silica nanotube using collagen fibrils as a template via a sol-gel process, and (right) the electrospinning system. A similar sol-coating was applied to the electrospun PVA mats, for which silica sol from acid-catalysis of TEOS was also employed.

Microstructure of the reassembled collagen fibrils was scanned with an atomic force microscopy (AFM; SPM-9600, Shimadzu, Kyoto, Japan), while that of the silica nanotube

sponges and fibrous mats was observed under a scanning electron microscope (FE-SEM; JSM-6500F, JEOL, Japan) and under a transmission electron microscope (TEM, Hitachi H-800, Japan).

RESULTS AND DISCUSSION

Silica-Coated Collagen and PVA Fibril Mats

Figure 2 (a) shows an AFM image of the reassembled collagen fibrils. The appearance of fibrils indicated that the collagen monomers were highly self-assembled into the collagen fibrils in the presence of PBS and the reassembled collagen fibrils were successfully obtained. Those fibrils had an average fibril diameter of 80 ± 10 nm (ranging from 70 nm ~ 90 nm). The SEM image (b) shows the electrospun PVA fiber mats were composed of 0.5 ~ 1 μ m fibrils, which are fused to be connected each other in places.

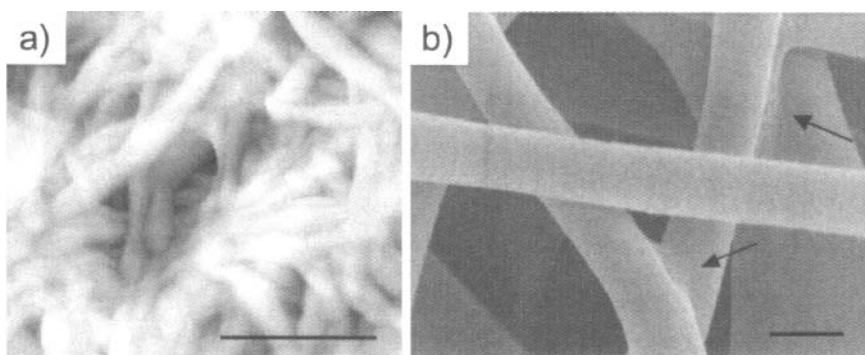


Figure 2. The microstructures of (a) the reassembled collagen fibrils (an AFM image) and (b) the electrospun PVA (a SEM image). Bar: 500nm.

Figure 3 shows the SEM images of the silica nanotube sponges derived from the Stöber systems containing (a) 1 mL and (b) 2 mL of TEOS, while (c) is a TEM image of a fibril shown in image (a). At low amount of TEOS, Figure 3(a) shows that the samples were only composed of silica nanofibers with an average fibril diameter of 133 ± 7 nm (ranging from 125 to 137 nm). At high amount of TEOS, Figure 3(b) shows that the mat involved both silica nanofibers and silica nanoparticles (arrows). The fibril diameter for silica nanofibers in (a) ranged from 130 to 170 nm (147 ± 20 nm in the average). The increase in the TEOS content not only resulted in an increase in fiber thickness but also led to the coexistence of silica nanoparticles. A TEM image in Figure 3(c) shows a hollow microstructure, i.e., the fibrils were nanotubular (silica NT). The average inner diameter was 81 ± 4 nm (70 ~ 90 nm), being coincident with the collagen fibril diameter (Figure 2(a)). In addition, the wall, with the average thickness 42 ± 3 nm (40 ~ 50 nm), indicates some roughness, probably introduced by the granular aggregation of the Stöber silica colloids. Figure 3(d) demonstrates a TEM microstructure for the NT from the Stöber system with 2ml TEOS. Though Figure 3(b) shows fibrous morphology, 3(d) indicates the presence of

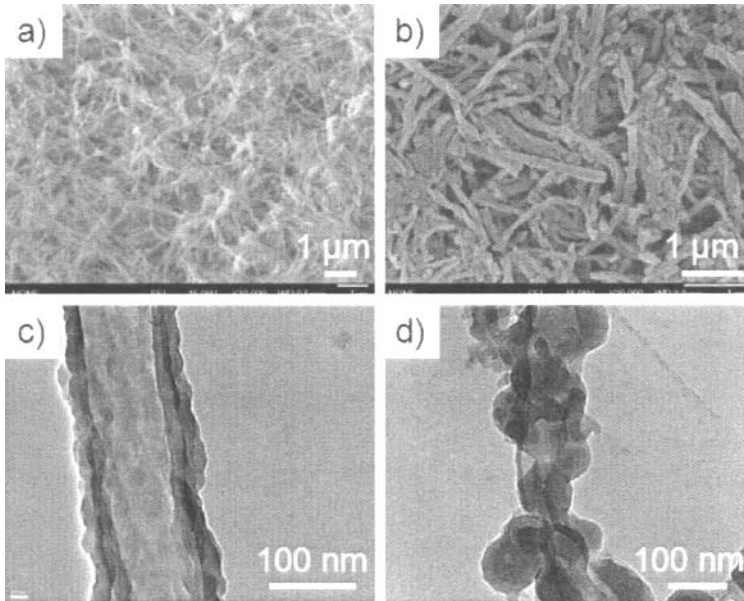


Figure 3. SEM (a, b) and TEM (c, d) images of silica nanotube sponges derived from (a, c) 1 mL and (b, d) 2 mL of TEOS.

larger particles (arrows), about a few tens of nm in diameter, barely forming fibrils. The significant increase in the constituent silica particles was attributed to the increase in the TEOS content in the Stöber system.

Figure 4 shows the surface microstructure of the PVA fibrils dependent on the soaking sols: the Stöber sol gave rough gnarled surface with silica colloid granules (a), but the granules seemed more independent than in Figure 3. Smooth surface (b) was obtained from the silica sol derived by the acid-catalysis of TEOS. The dark and bright contrasted area in (b) (arrow) was caused by drying the sol layer. It was noticed that longer soaking in the HCl-catalyzed sol gave webbing-like regions at the fiber-crossing area. Since gelation reaction kept proceeding during the soaking, the increasing viscosity with the condensation was responsible for such regions. In addition, the longer soaking the PVA fiber mats in the Stöber type sol resulted in degradation of the substrate PVA fibrils and silica particle-coated fibrils were not obtained.

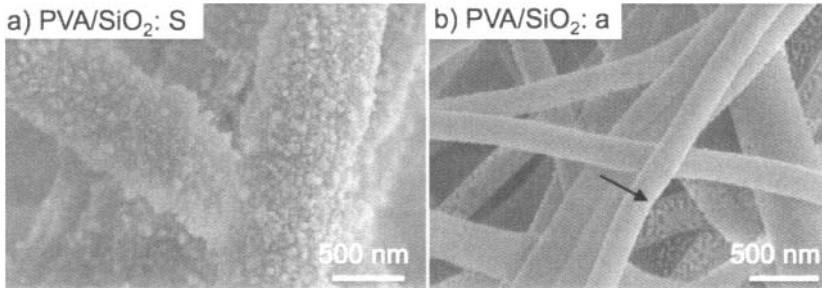


Figure 4 Surface microstructures of electrospun PVA fiber mats, soaked in (a) Stöber type colloid sol and (b) HCl-catalyzed silica sol.

Silica Nanotubes and Solid Fiber Mats due to Calcination

Figure 5 indicates the microstructure of the PVA-templated silica mats obtained by calcining (a) the Stöber colloid-coated and (b) the HCl-catalyzed sol-coated at 650°C for 1h the precursor mats. The silica colloid particles compose hollow silica fibrils in (5a), while the fracture surface of the PVA-templated fiber mats (5b) soaked in the HCl-catalyzed silica sol indicated solid silica fibrils. The microstructure of the silica mats from collagen templating was similar to that shown in (5a). For comparison, titania nanotubes were fabricated using collagen via a route similar to the present one, where titania sol was prepared by hydrolyzing tetraethoxytitanate (TEOT) in the system EtOH (40 mL)/ H₂O (0.5 mL)/ TEOT (1 mL). The collagen mat was soaked in the titania sol for 3 h and the titania coated mats were calcined at 600 °C for 1 h to obtain titania nanotubes (Figure 6). They seemed sausage-like, and consisted of units with length comparable to that the silica NT in Figure 3. Close inspection indicates the presence of some longitudinal cracks (arrows) or a funnel-like shaped mouth (circle), showing that the fibrils are hollow.

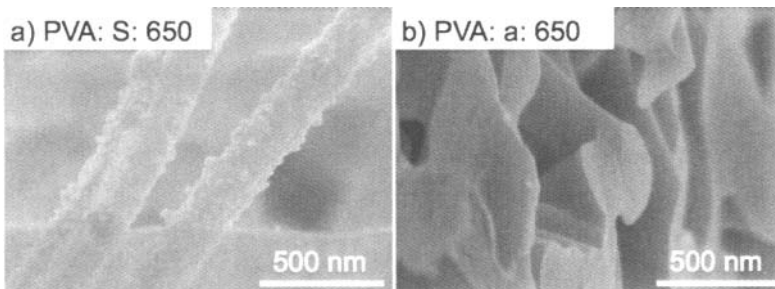


Figure 5 The calcined silica mats from (a) Stöber sol and (b) acid sol treatments: 650°C, 1 h. Silica granules were found in (a) while the fractured mat (b) shows solid fibrils.

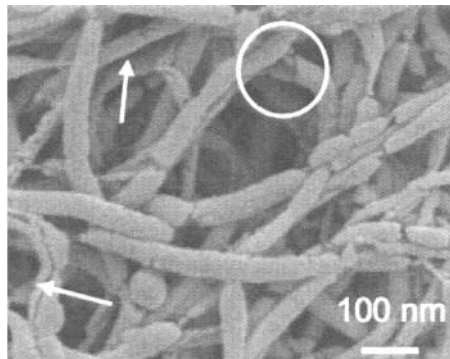


Figure 6 Titania nanotubes obtained by calcining titania sol-coated collagen fiber mat at 600 °C for 1h.

Nanotubes and Solid Fibrils

When soaked in the Stöber sol, the surface of the collagen fibrils attracted those silica colloid particles and they grew to produce silica-coated collagen fibrils. It is then reasonable that the collagen and PVA templated mats coated with colloid particles would yield ceramic mats made of nano-tubes on calcining the precursor fiber mats at lower temperature: the temperature was high enough to burn out the organic templates, but low enough not to fully sinter the colloids or to collapse the hollow spaces. In contrast, the linear and small oligomers in the HCl-catalyzed sol would be present in the intra-molecular spaces in the PVA fibrils. Thus, elimination of the template by calcination should leave the solid fibrils, found in Figure 4(b). Few studies have employed collagen as template to fabricate the hollow silica fibers. Ono et al. [9] applied a neutral mixture of collagen fibrils and TEOS to fabricate the hollow silica nanofibers, while Eglin et al. [10] used a neutral mixture of collagen gel and tetramethoxysilane (TMOS) to produce the silica-collagen hybrids and silica nanotubes. They pointed out that collagen had a strong affinity to silicate species because of their hydrogen-bonding and electric interactions. Chen et al. [11] already pointed out that the collagen derived silica NT mats doped with calcium ions more stimulated proliferation of osteoblast-like cells than Ca-free ones. Therefore, the present PVA-templated silica NT mats or solid silica fibril mats would exhibit better bone tissue compatibility when doped with Ca.

CONCLUSIONS

In the present study, two types of template sol-gel routes were developed to produce the silica nano-fibers with a controllable microstructure. Natural collagen fibrils were reassembled through neutralization of collagen monomers with phosphate buffer saline and employed as template for the production of silica hollow nano-fibers, while PVA fibers were obtained via an electrospinning technique and employed as template for the production of silica solid nano-fibers. The collagen fibrils firstly were soaked in the conventional Stöber sol-gel precursor mixture of tetraethoxysilane, ethanol, water, and ammonia and then calcined at 600 °C to produce the silica hollow nano-fibers, while the electrospun PVA nanofibers were immersed in a sol-gel precursor mixture of tetraethoxysilane, ethanol, water, and hydrogen chloride and then calcined at 650 °C to

produce the silica solid nano-fibers. It was found that the interaction between silica and templates was crucial to the microstructure of silica nano-fibers. A simple silica coating on the surface of collagen fibrils resulted in the hollow structure, while a diffusion of silica oligomers into PVA fibers resulted in the solid structure.

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