Atomic force microscopy of electrospun organic-inorganic lipid nanofibers

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An organic-inorganic hybridization strategy has been proposed to synthesize polymerizable lipid-based materials for the creation of highly stable lipid-mimetic nanostructures. We employ atomic force microscopy (AFM) to analyze the surface morphology and mechanical property of electrospun cholesteryl-succinyl silane (CSS) nanofibers. The AFM nanoindentation of the CSS nanofibers reveals elastic moduli of 55.3 ± 27.6 to 70.8 ± 35 MPa, which is significantly higher than the moduli of natural phospholipids and cholesterol. The study shows that organic-inorganic hybridization is useful in the design of highly stable lipid-based materials. © 2011 American Institute of Physics. [doi:10.1063/1.3635783]

Lipid-based materials (LBMs) that resemble key properties of plasma membranes are appealing biomaterials for applications in biosensors, drug delivery, and functional coatings.¹ For instance, liposomes capable of encapsulating both hydrophilic and hydrophobic drugs have been explored and clinically used as carriers for drug delivery.² However, naturally derived LBMs often display limited morphological and mechanical stabilities, hampering their vigorous applications. A number of polymerization methods have been proposed to enhance the stability of LBMs. Among them, an organic-inorganic hybridization strategy was developed to create liposomal cerasomes with outstanding morphological stabilities against water and surfactants.³ In the design of cerasomes, the phosphate heads of natural phospholipids is replaced by triethoxysilyl moieties, which can be hydrolyzed and polymerized to form highly stable polysiloxane networks, rendering cerasomes morphologically stable. Using this hybridization strategy, polymerizable cholesteryl-succinyl silane (CSS) was synthesized and engineered into stabilized nanovesicles for drug delivery.⁴ Nevertheless, the stabilities of the organic-inorganic LBMs such as cerasomes and CSS are still often evaluated by morphological analysis. In this letter, atomic force microscopy (AFM) is used to analyze the surface morphologies and mechanical properties of CSS nanofibers.

We employed electrospinning as a versatile mechanism^{5,6} for creating nanofibers. Following a procedure detailed in supporting materials,⁷ CSS powder was dissolved in a mixed tetrahydrofuran (THF) and hydrochloric acid (HCl) solvent (1 ml THF with 10 μ l 37% HCl). Under acidic conditions, the triethoxysilyl moieties of the organic-inorganic hybrid CSS were hydrolyzed and polymerized [Fig. 1(a)]. In a fashion similar to the micelle formation proposed by McKee *et al.* for phospholipid,⁶ the hydrolyzed and polymerized CSS molecules formed entangled micelles, enhancing the solution elasticity. The negatively charged

alkoxysilane groups of the hydrolyzed CSS molecules further rendered the solution electrospinnable. At a concentration of 69% w/w, the CSS solution elasticity suppressed the Rayleigh instability and the breakup of the solution jet into droplets.⁸ Continuous CSS nanofibers of 300–800 nm in diameter were then electrospun [Fig. 1(b)].

A high-resolution imaging analysis of surface morphology was performed on the CSS fibers using AFM. Briefly, the CSS fibers were deposited on silicon wafers and examined using a Digital Instrument Nanoscope IIIA AFM in air at room temperature (25 ± 1 °C). Both the AFM deflection [Fig. 2(a)] and three-dimensional (3D) images [Fig. 2(b)] of the CSS fibers were obtained in the contact mode. The AFM sectional analysis showed that the CSS fibers displayed undulated diameters ranging from 370 nm to 600 nm. Bead-like structures 800 nm in width and 900 nm in height were also observed. Unlike high-molecular-weight polymers, the electrospinning of small organic molecules like lipids into nanofibers is challenging because of their weak chain entanglement in solutions. Increasing the solution concentration can enhance chain entanglement, critical to fiber formation.⁶ However, fast solvent evaporation from concentrated solutions presents another challenge to obtain perfect fibers [Figs. 2 and S1].

In addition to imaging analysis, AFM also offers a powerful tool for analyzing unfolding and refolding of single polymer chains and for probing local properties of surfaces.^{9,10} For instance, AFM nanoindentation has been used for characterizing the mechanical properties of single poly(L-lactic acid) (PLA) nanofibers¹¹ and collagen fibrils.¹² Here, the elastic modulus of the CSS nanofibers was assessed using AFM nanoindentation. Specifically, after the AFM images of the electrospun CSS nanofibers were obtained, surface forces between the AFM probe and a fiber were consecutively measured in the force mode. The spring constants of the AFM probes were calibrated using the resonant frequency technique, as detailed elsewhere.¹³ A representative force-distance curve for the CSS fibers is shown in Fig. 3(a).

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FIG. 1. (Color online) (a) The chemical structure of CSS and schematic of CSS hydrolysis, polymerization, and micelle formation. A CSS molecule consists of a triethoxysilyl head, steroid tail, and amide link. In acidic THF solutions, the non-polar triethoxysilyl heads of the CSS molecules are hydrolyzed into negatively charged alkoxy groups, which can be further polymerized, creating highly stable polysiloxane networks. The hydrolyzed and polymerized CSS forms entangled micelles prior to electrospinning. (b) A typical scanning electron microscopic (SEM) image of the electrospun CSS nanofibers.

A striking feature of the force-distance curve is that the approach curve does not show any adhesion while the retraction curve displays a large attractive force of 364 nN. When the AFM tip approaches a deformable surface, attractive forces often cause it to jump onto the surface.⁹ The jump-to-contact process was observed in the AFM nanoindentation studies of PLA nanofibers¹¹ and polypyrrole (PPy) nanotubes.¹⁴ This phenomenon is largely attributed to van der Waals interactions and capillary forces induced by water condensation on the sample surface.^{11,14} The CSS nanofibers are more deformable than PLA nanofibers and PPy nanotubes. Thus, the lack of adhesion forces from the approach curve is due to the unique fiber surfaces. As illustrated in Fig. 1(a), the alkoxy groups of the hydrolyzed and polymerized CSS molecules constitute the cores of CSS micelles in THF solution, while the highly hydrophobic steroid tails form the shells. The CSS micelles are then entangled, stretched, and solidified into nanofibers during the electrospinning process. Consequently, the hydrophobic steroid tails with methyl groups, functioning as non-adhesive brushes, form the surface layer of the CSS fibers, preventing capillary condensation and adhesion of the AFM tip to the surface.

When the AFM tip penetrates the fiber surface, it is able to access the amide links and alkoxy groups of the hydrolyzed CSS molecules. The surface of the AFM tip in air is predominately composed of silicon dioxide with silanol and silylamine groups.¹⁵ The silanol groups of the AFM tip can interact with the amide links and alkoxy groups of the CSS fibers via hydrogen bonding that is responsible for the tip-fiber attraction. During the retrieving process, the hydrogen bonds will be gradually broken. As a result, the adhesion that is the maximum attractive force occurs before the retrieving tip reaches the contact point [Fig. 3(a)].

FIG. 2. (Color online) AFM characterization of the CSS nanofibers. (a) A representative AFM deflection image with a data scale of 50 nm. (b) The 3D AFM image. Section analysis shows that the fiber widths and heights are 594 nm and 434 nm at A', 375 nm and 330 nm at B', and 750 nm and 913 nm at C'.

This explanation is supported by an AFM nanoindentation study of the CSS fibers that were electrospun from a less-hydrolyzed solution. When the CSS solution was hydrolyzed less, the CSS molecules would be charged and polymerized less, leading to the electrospinning of less-robust fibers [Fig. S2]. Compared to the fibers shown in Fig. 2, these fibers would have more triethoxysilyl groups but fewer alkoxy groups. Because a triethoxysilyl-silanol interaction is weaker than an alkoxy-silanol interaction, weaker adhesion is expected between the AFM tip and the less-hydrolyzed fibers. Indeed, the fibers displayed an adhesion of only 8.5 nN [Fig. S3].

To examine whether viscoelasticity induces hysteresis under loading (approach) and unloading (retraction), AFM nanoindentation tests were repeated three to six times at each location. Fig. 3(b) shows six successive force-indentation curves under loading and unloading. Notably, repeated force-indentation curves were obtained after two cycles of loading and unloading. Additionally, the difference between the first and third loading-unloading curves was small, suggesting minimal, irreversible changes in the fiber structures induced by indentation. Intuitively, the CSS fibers behave like rubber and have no observable plastic deformation, completely recovering their shapes after full indentation. Therefore, the hysteresis of the CSS fibers is largely due to viscoelasticity rather than deformation-induced, irreversible structural changes.

FIG. 3. (Color) (a) A representative force-tip sample distance curve of the CSS fibers. The arrows mark the contact (i.e., the starting point of nanoindentation) of the tip to and the separation of the tip from the fiber. The adhesion that is the maximum attractive force is denoted as F^* . (b) The force-indentation curves were obtained from six consecutive measurements, represented by different colors. Black, red, blue, pink, dark green, and cyan represent the 1st, 2nd, 3rd, 4th, 5th, and 6th cycle of loading and unloading, respectively. Solid and dotted lines represent the approach and retraction curves, respectively.

The elastic modulus of the CSS fibers (E_{sample}) was determined by fitting the classical Hertz model into the load-indent data¹⁶

$$F = 0.7453 \cdot E_{\text{eff}} \cdot \tan \theta \cdot \delta^2. \quad (1)$$

Here, F is the applied force, θ ($= 20^\circ$) is the semi-included angle of the four-sided pyramid tip of the AFM probe, ν_{sample} is the Poisson ratio of the CSS fibers, δ is the penetration depth, and E_{eff} is the effective modulus, which can be related to E_{sample} as follows:

$$\frac{1}{E_{\text{eff}}} = \frac{1 - \nu_{\text{sample}}^2}{E_{\text{sample}}} + \frac{1 - \nu_{\text{probe}}^2}{E_{\text{probe}}}. \quad (2)$$

Here, E_{probe} ($= 200$ GPa) and ν_{probe} ($= 0.25$) are the elastic modulus and Poisson ratio of the AFM probe, respectively. Because ν_{sample} for LBMs is unknown, we take its value in the range of 0.2–0.5 for the calculation of E_{sample} . Taking ν_{sample} as 0.2 and 0.5, the elastic modulus of the CSS fibers was determined to be 70.8 ± 35 MPa ($n=36$) and be 55.3 ± 27.6 MPa, respectively. The less-polymerized fibers displayed an elastic modulus of 1.05 ± 0.32 MPa and 0.82 ± 0.25 MPa, respectively [Fig. S3]. The large standard deviations for the values of E_{sample} observed here are mainly due to the inability to precisely determine the contact point between the AFM tip and a fiber surface, as discussed in details in supporting materials [Figs. S4 and S5].

The AFM nanoindentation reveals that the mechanical properties of the CSS fibers are greater than the values found for both natural cholesterol ($E \sim 0.27$ MPa) and phosphatidylcholine ($E \sim 0.03$ MPa).¹⁷ Compared to cholesterol and phospholipids, the alkoxy silane groups of the hydrolyzed CSS molecules can be partially polymerized, leading to the formation of highly stable polysiloxane networks in the CSS fibers. It was previously reported that such polysiloxane networks substantially enhance the detergent resistance of liposomal cerasomes.¹⁸ This study shows that the polysiloxane networks also contribute to the mechanical properties of the electrospun CSS fibers. It is not surprising that the elastic modulus of the less-polymerized fibers is comparable to that of the natural cholesterol. By altering the degrees of hydrolysis and polymerization, the CSS nanofibers may display a wide range of mechanical properties.

In summary, AFM nanoindentation provides direct evidence that the mechanical properties of polymerizable LBMs such as CSS nanofibers can be enhanced by organic-inorganic hybridization. The elastic modulus of the nanofibers is further modulated by altering the hydrolysis and polymerization of CSS molecules. The results from this study may provide a basis for the design of mechanically stable, organic-inorganic hybrid LBMs.

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⁷See supplementary material at <http://dx.doi.org/10.1063/1.3635783> for CSS nanofiber fabrication, original SEM images of CSS nanofibers, additional AFM images and nanoindentation data.

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