6212 *Chem. Mater.* **2010,** *22,* 6212–6214
DOI:10.1021/cm102129k

CHEMISTRY OF MATERIALS
Communication

Strategy for the Modification of Electrospun Fibers that Allows Diverse Functional Groups for Biomolecular Entrapment

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Received July 31, 2010
Revised Manuscript Received October 18, 2010

We report a method to functionalize surfaces of electrospun (ES) fibers under relatively mild, aqueous conditions. We illustrate the utility of this method by immobilizing biomacromolecules while retaining their activities on surfaces of these fibers. Although recent development in surface chemistry has enabled many applications of ES fibers and other polymeric interfaces,[1−5] there is still no promising approach for functionalizing surfaces of polymers[6] in aqueous conditions at room temperature. Many research groups have attempted at generalized solutions to this problem;[7] each one, however, has its limitations. Some strategies of surface modification of polymer coating require high-temperature treatment, over 250 °C, to reinforce the coating layer by cross-linking. This treatment will certainly damage or deform most polymers, since such high temperature is beyond most of glass transition temperature of polymers.[7a] Others, such as the "graft from" method to functionalize surfaces through radical polymerization, do not accommodate reactive groups like NH_2 and SH due to the fact that they quench radicals.[7b] Thus we wish to develop a method to easily introduce reactive groups on polymeric surfaces, in particular ES fibers, at room temperature and under relatively mild conditions. To demonstrate the utility of our method, we immobilize biomacromolecules on the ES fiber and maintain their activities even under extreme environments.

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With this set of requirements in mind, we took inspiration from nature for most of biochemical processes occur in aqueous conditions and at room temperature. Here we employ the well-developed biosilicification[8] of certain marine organisms that catalyze silica acid polymerization under physiological conditions via proteins called silaffins.[9] We hope to introduce this polymerization onto surfaces of ES fibers and obtain different functional groups by using commercially available and fully developed organosilane precursors[10] to avoid further complex organic synthesis. Because this silica polymerization is based on nuclei formation, which is chemically orthogonal to radical polymerization, reactive groups like NH_2 and SH can be directly introduced onto surfaces without worrying about their quenching radicals in "graft-from methods". In addition, a number of peptides, small molecules, and polymers have been developed to mimic the effect of silaffin to initiate this polymerization, and to yield nanostructured silica and titania formation on surfaces.[11−14] To initiate homogeneous polymerization of silica acid on fibers, we introduced the initiator, 2-(dimethylamino) ethylmethacrylate (DMAEMA, a molecule that mimics silaffins),[15] onto surfaces via atom transfer radical polymerization (ATRP), which can result in a high coverage of DMAEMA. This strategy can functionalize ES fibers with tunable chemical groups under aqueous environment and at room temperature (Scheme 1).

To demonstrate this design, we fabricated polystyrene (PS) ES fibers as a model material with the device shown in Figure 1a. A solution of water/EtOH (v/v, 3:1) is used to receive and disperse hydrophobic ES fibers in pure water after copious rinses to remove EtOH.[16] As all following processes are under aqueous conditions, this treatment

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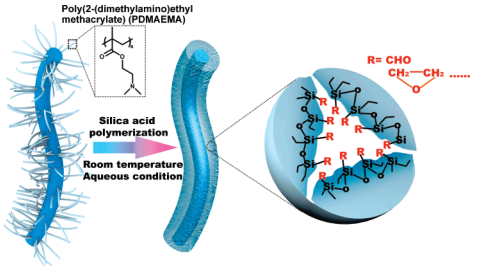
 Published on Web 11/03/2010

Scheme 1. PDMAEMA, a Molecule That Mimics Silaffins, Was Introduced onto ES Fibers via ATRP; PDMAEMA Initiates the Polymerization of Organosilane Precursors to Obtain Various Functional Groups on Surfaces



is essential to maximize surface exposure to solutions (Figure 1b, right) for further chemical reactions. Additionally, this treatment enlarges the distance between fibers, which prevents the space between fibers from being filled up by coating silica layers. By contrast, fibers without this treatment aggregated as a film in an aqueous solution (Figure 1b, left).

To introduce the initiator of ATRP on surfaces, we used both layer-by-layer (LbL) absorption and the blending method. For LbL, fibers were etched with sulfuric acid (9.8 M) for 2 min to impart charges on surfaces, followed by several rinses with a phosphate buffer saline (PBS, pH 7.5). Three bilayers of poly(diallyl-dimethylammonium chloride)-poly(acrylic acid-g-alkyl bromide) were adsorbed. An alternative method that completely avoids the use of sulfuric acid achieves similar results: by adding ethane-1,2-diyl bis-(2-bromo-2-methylpropanoate) into the polystrene via blending,[6] we also obtain the initiator-functionalized ES fibers. We polymerized DMAEMA via ATRP directly on the fibers and initiated silica polymerization in a buffered solution (pH 7.5) of silica acid, hydrolyzed from tetramethoxysilane (TMOS) for 10 min. Characterization with a scanning electron microscope (SEM) and a transmission electron microscope (TEM) demonstrated the successful deposition of silica on fibers and their core/shell structure (Figure 1c−f). Electron diffraction demonstrated the amorphous structure of silica layer. We used element analysis of the surfaces of ES fibers via X-ray photoelectron spectra (XPS) to monitor the chemical reactions on fibers. Figure 1g shows XPS surveys of the fibers by silica modification (curve A), fibers after ATRP (curve B), and the pure PS fibers (curve C). The peaks at 399 eV in curves A and B are attributed to the tertiary amino groups of DMAEMA grafted onto PS fibers via ATRP. The peaks at 103 and 154 eV in curve A belongs to Si2p and Si2s respectively and demonstrated the deposition of silica on fibers. As fibers are covered with silica, both of N1s and C1s peaks in curve A are much less intense than those in curve B. The XPS results further confirmed the successful polymerization of silica acid on surfaces of ES fibers.

From our examination of the amount of protein adsorption by a bicinchoninic acid (BCA) kit, we found the amount of protein adsorbed on silica modified ES fibers is 151.2 mg/g which is six times that adsorbed on the bare ES fibers (25.2 mg/g), for the largely expanded surface area (from 8 to 98 m^2/g as a result of the mesoporous silica

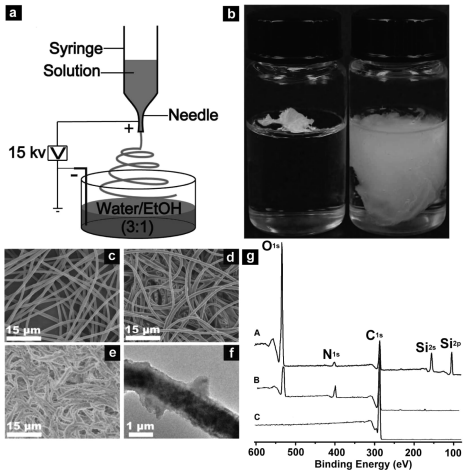


Figure 1. (a) Schematic illustration of the apparatus for fabrication of ES fibers. (b) Comparison of aggregated fibers without water/EtOH treatment (left) and dispersed fibers after such treatment (right). (c) SEM images of PS fibers, (d) fibers after ATRP, and (e) fibers coated with silica. (f) TEM image of fibers coated with silica. (g) XPS of (curve A) silica-modified fibers, (curve B) DMAEMA grafted fibers, and (curve C) PS fibers without any modification.

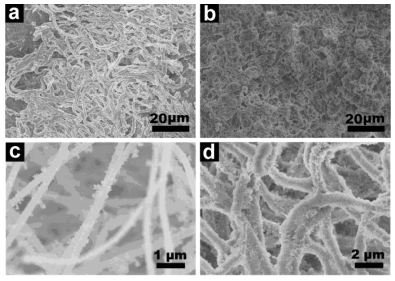


Figure 2. SEM images of (a) silica deposition on dispersed ES fibers after the treatment of water/EtOH (v/v, 3:1), (b) silica deposition on aggregated fibers, (c) silica deposited on ES fibers of PEI-g-PSMA, and (d) DMAEMA grafted via ATRP.

outerlayer, measured by Brunauer−Emmett−Teller (BET) nitrogen absorption). As our method could disperse fibers in aqueous conditions after the treatment of water/EtOH (v/v, 3:1), which enlarges the distance between fibers, preventing the pores of the mesh from being blocked by silica (Figure 2a), while the pores of aggregated fibers are almost completely filled up by the silica coating (Figure 2b). Thus we maintained the microfibrillar porous network in this approach. Compared to other methods of silica deposition, such as ES fibers of polyethyleneimine-grafted poly(styrene-co-maleic anhydride) (PEI-g-PSMA) (Figure 2c), ATRP allows higher efficiency of deposition of silica, most likely due to a high coverage of DMAEMA (Figure 2d). Thus highly dense DMAEMA could be easily achieved to precipitate silica layer with thickness of 100 nm within 10 min. Also the blending method to introduce ATRP initiators on fibers allowed other surfaces not suitable for LbL to be modified with silica coating.

To demonstrate that ES fibers thus treated can readily accommodate different surface functions, we immobilized β-galactosidase (Gal), an enzyme widely used in the food industry and acid phosphatase (Acp), an enzyme found in many biochemical assays, respectively on ES fibers. In addition to silica acid, we polymerized (3-glycidyloxypropyl)

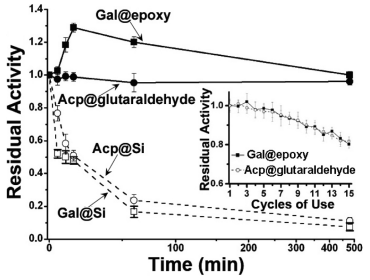


Figure 3. Long-term stability of enzymes in solutions with high ionic strength and after cycles of use (inset). Gal@epoxy, Gal encapsulated in silica functionalized by epoxy; Gal@Si, Gal encapsulated in silica alone; Acp@glutaraldehyde, Acp immobilized by glutaraldehyde-activated silica; Acp@Si, Acp entrapped in silica alone.

trimethoxysilane (containing the epoxy group) on ES fibers for the immobilization of Gal. Because Acp has smaller molecular weight and less accessible residuals,[17] we polymerized (3-mercapto propyl)trimethoxysilane activated by glutaraldehyde, which is more reactive than epoxy groups. The residue glutaraldehyde is washed away by rinsing with PBS, and there is no detectable leaching for over two weeks (see the Supporting Information). Because the silica acid and organosilanes polymerization takes place under ambient conditions, the process of TMOS and organosilanes polymerization and enzyme immobilization can proceed within one step, in a process termed coprecipitation.[18] We assayed the activities of these enzymes after their immobilization on the functionalized ES fibers. The enzymes entrapped with bare silica from TMOS comprise control experiments. We use residual enzymatic activities under high ionic strength (1 M NaCl solution) to measure the degree of success of our strategy (Figure 3). For enzymes immobilized on ES fibers with organosilanes via coprecipitation, neither Gal nor Acp had any obvious reduction in activity after 8 h of incubation, whereas the enzymes in pure silica had only 10% residual activity left. The increased activity of Gal immobilized with the epoxy group at the first 15 min is likely due to the conformational change of enzymes induced by chemical confinement of epoxy groups.

We next examined the functions of enzymes immobilized on the functionalized ES fibers after many cycles of use. After 15 cycles of use, the enzymatic activities of both Gal and Acp still stand at around 80% (Figure 3, inset). The resulting mesh of ES fibers kept as an integral piece after 15 cycles of use. We attribute this superior performance to the strong coating of silica on PDMAEMA. Precursors of organosilane are likely to strongly adsorb on the PDMAEMA via electrostatic interactions and polymerize to form networks to further concrete on them.[11c] Another motivation for enzyme coprecipitation with organosilane in one step is to adjust the reactivity of immobilized enzymes through the polycations on ES fibers for them to function under different environment, such as different pH values.

We reasoned that the optimal pH of Gal can be adjusted through changing the pK_a of carboxylic acid in the catalytic site by tertiary amino groups,[19] and the stability of Acp in extreme pH can be improved by the interaction of cationic tertiary amino groups with the negative parts of the enzyme,[20] which will expand their utility under different conditions. Our results confirmed this hypothesis as the optimal pH of Gal shifted from 4.5 to 11 (see Figure 2S in the Supporting Information). Acp has a broader profile of pH-activity and exhibited an activity 60 times more than that of the free enzyme at pH 11 (see Figure 3S in the Supporting Information). After immobilization, therefore, these enzymes can both function under extreme pHs and high ionic strength conditions. To further prove our rationale, we attempted to immobilize enzymes with excessive charges using this approach. Because α-chymotrypsin (too strongly positive) will electrostatically repel polycations on ES fibers, and glucose oxidase (too strongly negative) will block the interactions between polycations and silica acid to form silica layer, neither can coprecipitate with silica on ES fibers.[21] These enzymes, however, can be immobilized on ES fibers after organosilane polymerization within two steps for use under high ionic strength environment (see Figure 4S in the Supporting Information).

In conclusion, this approach for surface modification of ES fibers, which proceeds at ambient conditions (i.e., at room temperature and in water), readily introduces reactive groups within 10 min and enlarges the specific surface area of ES fibers, at the same time overcoming the disadvantages of existing approaches, such as the quenching of radicals when introducing certain chemical groups. As this approach is generally suitable for the introduction of biomacromolecules (i.e., growth factor) on surfaces of polymers, it may have potentials for immobilization of enzymes[16] and cells,[22a−c] tissue engineering, and molecular sensors. And we will attach bioactive molecules[23] on fibers to examine the adhesion and migration of cells.[24]

Acknowledgment. We thank Prof. Yongming Chen (ICCAS) for the help with ATRP experiments, as well as Mr. Di Cai (Sichuan University) for the design of the Table of Contents figure. This work was supported by the Chinese Academy of Sciences (KJCX2-YW-M15), the National Science Foundation of China (50902025, 90813032 and 20890020), and the (Ministry of Science and Technology (2009CB930001, 2007CB714502 and 2011CB933201).

Supporting Information Available: Materials and methods and additional figures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org/.

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