Evaporation-induced assembly of biomimetic polypeptides

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We report an evaporation assisted plasma lithography (EAPL) process for guided self-assembly of a biomimetic silk-elastinlike protein (SELP). We demonstrate the formation of SELP structures from millimeter to submicrometer range on plasma-treatment surface templates during an evaporation-induced self-assembly process. The self-assembly processes at different humidities and droplet volumes were investigated. The process occurs efficiently in a window of optimized operating conditions found to be at 70% relative humidity and 8 μl volume of SELP solution. The EAPL approach provides a useful technique for the realization of functional devices and systems using these biomimetic materials. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2957992]

Nature has long been a source of amazement in creating extremely complex yet elegant and functional materials, such as silk and elastin proteins, which form fascinating structures across a multitude of length scales. Recently, the development of genetic engineering of protein-based polymers has enabled scientists to create polypeptides that mimic these remarkable natural designs. For instance, a class of biomimetic polypeptides, silk-elastinlike proteins (SELP), which consists of the repeating units of silk and elastin proteins, has been synthesized with excellent controllability and monodispersity.[1] SELP displays a set of outstanding physical characteristics and biological properties,[2–5] which generate opportunities for creating innovative smart building blocks for nanomanufacturing, energy absorbing materials, tissue engineering, and drug delivery,[6–10] as well as understanding the fundamental assembly characteristics of proteins.[11] Nevertheless, little is known about the self-assembly mechanisms and characteristics of these synthetic proteins and their natural counterparts, and there is a paucity of techniques for creating desired SELP structures across different length scales. To address these fundamental questions of biomimetic proteins, we investigate the self-assembly process of SELP on plasma-patterned surface templates during droplet evaporation.

In the evaporation-assisted plasma lithography (EAPL) approach, plasma surface treatment is applied to selectively functionalize the surface of a polymeric substrate, which serves as the template during evaporation-assisted assembly. This highly effective, batch plasma lithography process can be generally applied to a variety of polymeric materials taking advantage of the large selection of substrates and processing gases available for plasma surface treatment. Figures 1(a) and 1(b) show the procedures for surface modification. In plasma lithography, a deformable mold was employed to mask plasma treatment area of a substrate in order to spatially functionalize the surface. The surface pattern that would be generated was determined by a microscale or nanoscale master pattern used to create a polymer replica or mold. The master molds used were optical gratings obtained from Edmund Optics and Anchor Optics. Polymer molding was performed with the master pattern to create the polymer replica. The technique was carried out using polydimethylsiloxane (PDMS) (Dow Corning), as both a deformable mold material as well as a substrate. For all results shown, it was cured at room temperature for at least 24 h. After curing, the

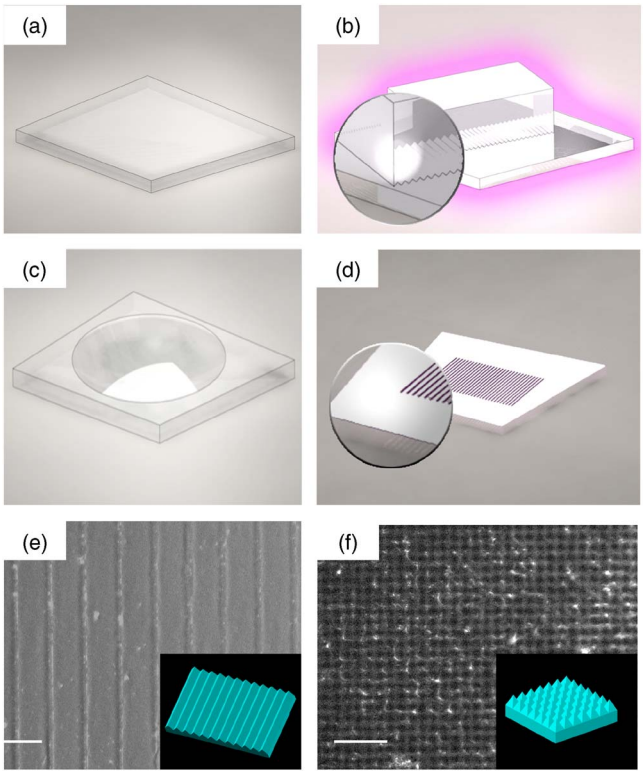

FIG. 1. (Color online) [(a)–(d)] Schematic of the EAPL process. The technique employs a deformable mold made using PDMS replication molding. The mold protects selective areas of the substrate from plasma surface treatment, which spatially alter the surface hydrophobicity of the substrate. A drop of SELP solution is allowed to evaporate on the substrate and SELP self-assembles on the hydrophobic regions of the template. [(e) and (f)] Dark-field images of SELP arranged on a line and a grid pattern. Insets: schematics of the mold used in plasma treatment. Scale bars: 25 μm.

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mold was carefully peeled off from the master and the final mold was trimmed into appropriate size. This mold was then placed on a substrate which served as the base upon which SELP 47K will self-assemble. This process worked with polystyrene, glass, and PDMS and polystyrene was used throughout this study. Plasma treatment of the substrate was carried out at 22 °C using atmospheric plasma at 150 Pa in a plasma cleaner (Harrick Plasma Inc.). For the experiments conducted, 5 min plasma treatment time was used in order to ensure that complete surface modification had taken place. During plasma treatment, uniform pressure was applied to the top of the PDMS mode in order to ensure conformal contact between the PDMS mold and the polystyrene substrate. The process shields selective areas and introduces functional groups only on desired regions of the substrate,[12–14] which modifies the contact angle from 80° to 15° (data not shown). This results in functionalized surface templates for self-assembly of SELP hydrogel structures.

SELP 47K, which contains a significant fraction of hydrophobic amino acids (22.4% valine and 12.2% alanine), is used in this work. The polypeptide has a repeating sequence of $[(GVGVP)_4GKGVP(GVGVP)_3(GAGAGS)4]_{12}$ and has been applied for various applications.[3–5] To facilitate the self-assembly of SELP 47K, the assembly process is performed by placing a droplet of SELP 47K solution (0.1 % w/v in phosphate-buffered saline), which is allowed to evaporate, on the plasma-functionalized template [Fig. 1(c)]. The sample was loaded into a full range humidity controlled chamber (Electro-tech systems Inc.). During solvent evaporation, the concentration of the SELP 47K in the droplet increases and the template provides nucleation sites for the growth of hydrogel structures [Fig. 1(d)]. We observed that SELP structures of arbitrary shapes can be self-assembled by the EAPL technique. Figures 1(e) and 1(f) show lines and grid patterns of SELP structures characterized by dark-field microscopy. We have also performed atomic force microscopy (AFM) measurements on the self-assembled SELP 47K structures (Fig. 2). The height of the hydrogel structure is on the order of 0.5 μm. SELP 47K selectively assembled on the plasma-treated, hydrophilic areas. Occasionally, small discrete aggregates were observed in the shielded, hydrophobic areas [Fig. 1(e)]. These observations are consistent with previous study on elastinlike polymers,[15,16] and indicate the importance of hydrophobic stabilization in the assembly process.

The humidity of the environment and the initial volume of the droplet are determined to be the two most critical parameters for the success of the EAPL process and provide effective handles for controlling the final structures. The total lifetime of a spherical, evaporating droplet t can be approximated by $t \approx R^2\rho/2D\Delta C$, where R is the initial radius, ρ is the density of the evaporating media, D is the diffusion constant of the material in the exterior gas, and ΔC is the concentration difference between the droplet surface and the ambient, which can be controlled by the humidity of the chamber.[17] To investigate the dependence of these parameters, the humidity and initial droplet volume were adjusted systematically. Figure 3(a) shows representative images obtained at different conditions. At low humidity (i.e., high evaporation rate), irregular or partially assembled structures are typically observed. At increasing humidity (i.e., lower evaporation rate and longer time for self-assembly), the SELP 47K begins to self-assemble on the selective regions of the template. Template-guided self-assembly of SELP 47K occurs efficiently in a window of optimized operating conditions found to be at 70% relative humidity and 8 μl volume of SELP solution. SELP 47K structures covering over 95% area of the droplet area (~5 mm in diameter) can be assembled at this condition. At higher humidity (over 80%), crystal-like structures are reproducibly observed and the structures are found in random locations with no correlation to the surface template. Mechanical probing with a micromanipulator reveals that the crystal-like assemblies are brittle, solid structures.

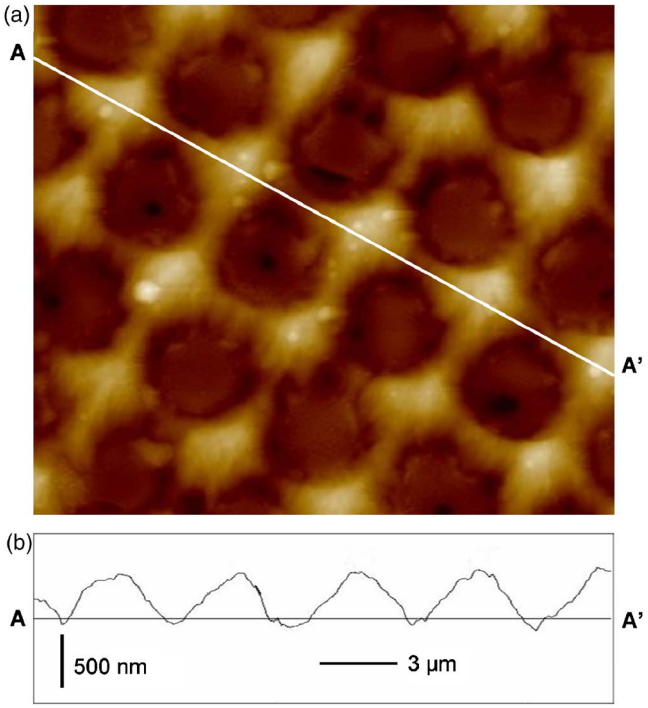

FIG. 2. (Color online) (a) AFM characterization of the self-assembled SELP structure as in Fig. 1(f). (b) Section analysis of the structure.

These observations could be understood by the thermodynamic and kinetic competition during the assembly

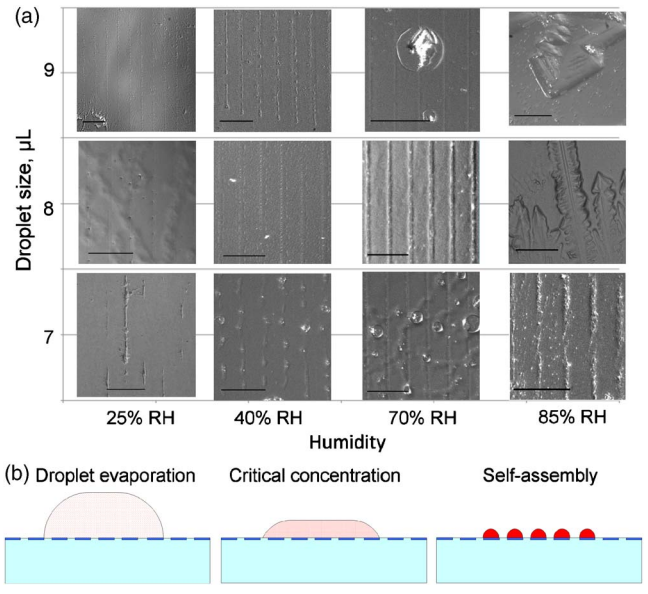

FIG. 3. (Color online) (a) EAPL of SELP at different humidity and initial droplet volume. Scale bars: 50 μm. The droplet covers an area of ~17 mm^2. (b) Schematics illustrating the evaporation-induced assembly process.

process.[18] It is known that a critical micelle concentration exists for many amphiphilic molecules and polymers composed of hydrophobic and hydrophilic parts.[19,20] In our experiment, assembly of SELP 47K structures was only observed with the evaporation of the solution. It indicates that a critical concentration may be required for template-guided self-assembly of SELP 47K. It is likely that the self-assembled hydrogel structure on the surface template and the crystal-like structure represent thermodynamically favorable equilibrium states of SELP 47K. At a small droplet volume or low humidity, the droplet reaches the critical concentration in a late stage during the evaporation process and little time is available for the SELP 47K to arrange into thermodynamically favorable structures. Therefore, irregular structures are assembled randomly at low humidity and small droplet volume. At an appropriate humidity and droplet volume, the surface template serves as nucleation sites facilitating the SELP hydrogel growth into patterned structures. At larger droplet volume or high humidity, the droplet reaches the critical concentration for self-assembly in an early stage during the evaporation process. This provides a relatively long time for self-assembly and rearrangement of SELP 47K into crystal-like structures, which is likely to be the most energy favorable state.

In conclusion, the biomimetic SELP 47K can be assembled into different structures using EAPL. The EAPL approach provides a means to directly link the bottom-up building blocks to other top-down fabricated systems and enables SELP assembling into arbitrary two-dimensional structures that span across multiple length scales over orders of magnitude (from submicrometer to millimeter). In addition, the creation of crystal-like structures at high humidity can potentially be useful for studying the structure of SELP. We believe that the EAPL technique can be extended to other genetic engineered proteins for the investigation of the protein assembly process and for the creation of a new generation of synthetic peptide-based nanosystems.

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