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Low Voltage Electron Microscopy of Silk Fibers and Films

Lawrence F. Drummy, David M. Phillips, Hilmar Koerner, Richard A. Vaia, B. L. Farmer and Rajesh R. Naik
Materials and Manufacturing Directorate
Air Force Research Laboratories, WPAFB, OH 45433

We have investigated native *Bombyx mori* cocoon silk and regenerated thin films using a relatively new technique, low voltage transmission electron microscopy (LVTEM) [1], with the goal of improving understanding of silk processing-structure relationships. Using accelerating voltages of approximately 5 kV, the LVTEM generates extremely high contrast for small changes in sample density or thickness. We have also used various microscopy and diffraction techniques to characterize recombinant silk elastin like protein (SELP). Fibers, thin films and foams of silk are of current interest for biomedical applications [2].

Due to their small size, low density and extreme sensitivity to electron beam irradiation, direct images of beta sheet crystallites in silk fibers are rare. The size and spatial distribution of these structural elements are expected to have significant effects on fiber and thin film properties. Our images of silk fiber beta sheet crystallites show relatively good correlations with crystallite sizes determined from x-ray diffraction [3] and mechanical property models [4]. Some new insights are obtained using LVTEM, as these other techniques are more indirect methods for determination of crystallite sizes. Figure 1 shows example LVTEM images from a longitudinal thin section (<30 nm) of a cocoon fiber. Density fluctuations on a ~10 nm length scale can be seen, and these arise from the mass-thickness contrast between the more dense beta sheet regions and the less dense amorphous regions in the fiber.

While natural *B. mori* silk is abundant, silk produced recombinantly (i.e. *E. coli*) allows for the possibility of genetic fusions to other proteins of interest for tissue engineering applications. However, the mechanical properties of silk materials produced from recombinant sources are often inferior to natural silk fibers and regenerated silk films. We have investigated the structure and properties of a recombinant fusion protein based on both silk and elastin amino acid motifs (SELP). Highly exfoliated silk nanocomposite materials were produced by mixing aqueous solutions of SELP with Na^+ Montmorillonite (MMT). The mechanical properties of the composites showed marked improvements compared to the protein material alone. Fig. 2a shows a LVTEM image of the plate-like MMT filler material. Fig. 2b shows the morphology obtained when this material is processed into SELP at 5% loading. This work is intended to show the evolution of microstructures that can be obtained when processing both natural and synthetic silk into thin films.

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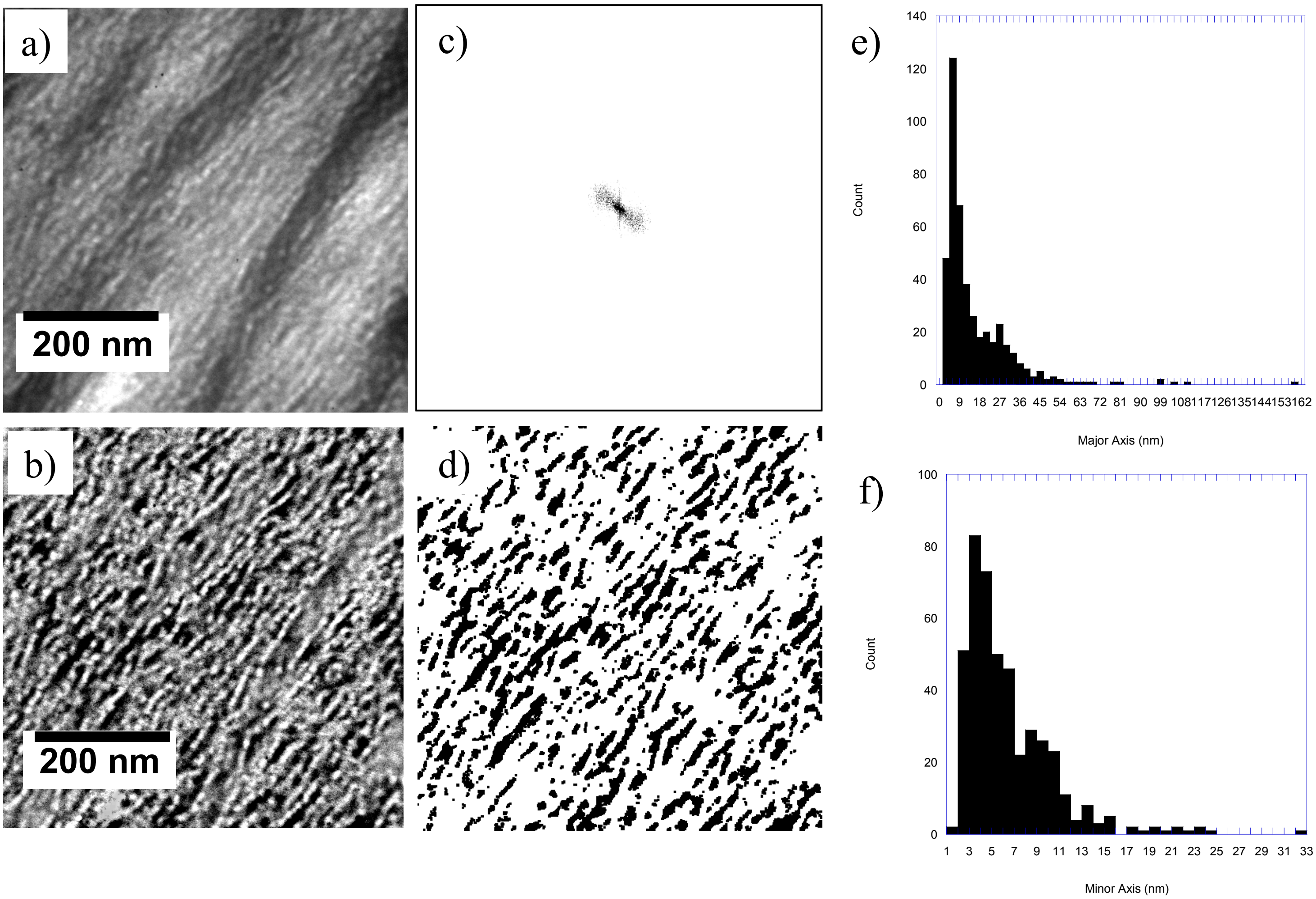

Figure 1. (a) LVTEM image of a longitudinal thin section of *B. mori* cocoon fiber. The fiber axis is diagonal from bottom left to top right. (b) Flattened image, with large scale intensity fluctuations in the image subtracted. (c) FFT from the raw LVTEM image. (d) Thresholded image. (e) Histogram of the major axis length of the crystallites measured from the thresholded image. (f) Histogram of the minor axis length of the crystals.

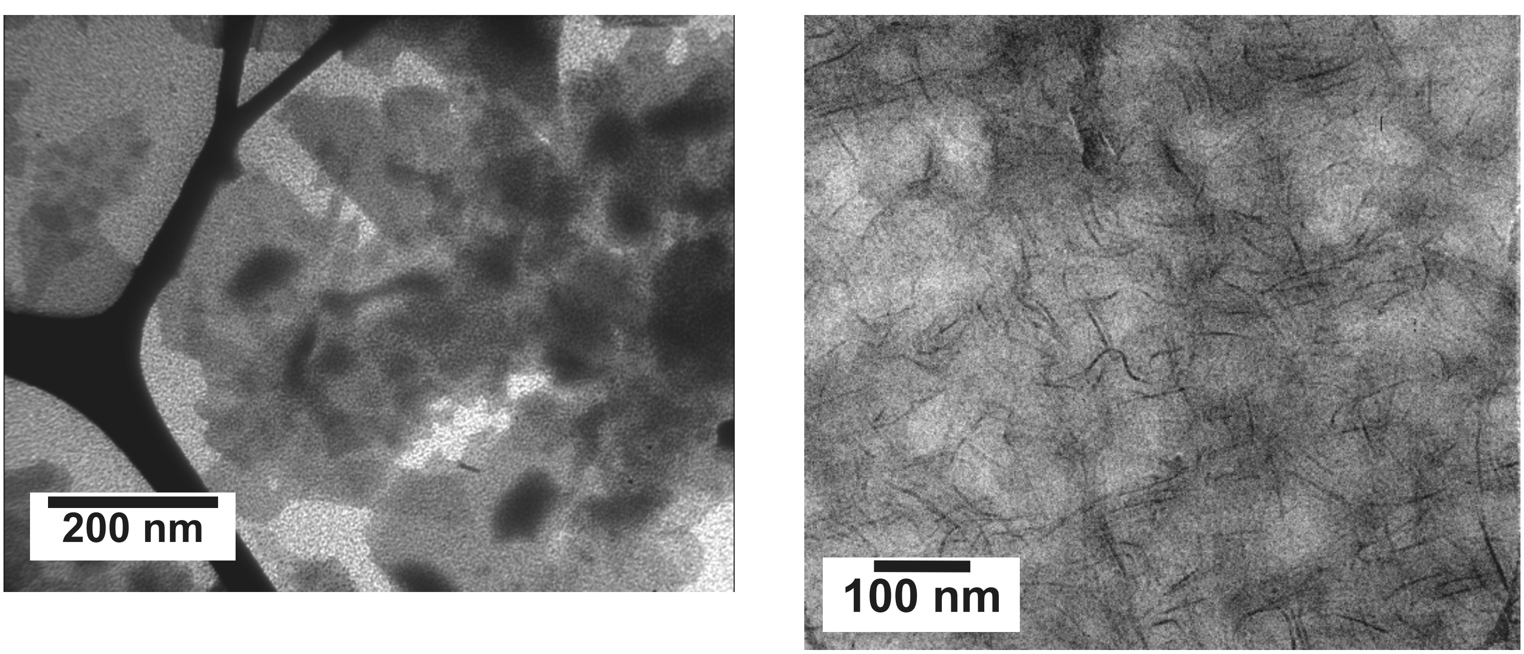

Figure 2. (left) LVTEM image of Na+ MMT cast from solution onto an amorphous carbon substrate. The MMT plates lie down flat on the substrate. (right) TEM image of a microtomed thin section of 5 wt% MMT in SELP.

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