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Cryo, Cryo-etch and Tandem Cryo-HRSEM Correlated with Cryo-STEM of Elastin-Mimetic Block Copolymers

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Genetic engineering of protein-based materials provides a means for the production of a diverse array of biomaterials. In order to understand some of the basic interactions of materials with biological organisms, the outer topology of the substance must be elucidated. Cryo-HRSEM methods provide a simple and accurate approach for characterization of frozen-hydrated specimens. In this study we focus on the morphological characterization of elastin-mimetic micelles and hydrogels.

Elastin-mimetic block copolymers **1** and **2** were produced via protein hyper-expression utilizing pET 24 a plasmid derivatives (Novagen; Madison, WI) and *E. coli* strain BL21-Gold(DE3) (Stratagene; La Jolla, CA) as the bacterial host [1]. The genes coding for the two polypeptides were synthesized by a revised gene concatamerization protocol [2, 3]. Bulk samples were prepared as 20 to 25% protein solutions in sterile ddH_2O. The solutions were pipetted into 3-mm gold planchets (Balzers BU 012 130T), which had been preequilibrated to 4°C. The temperature of the specimen was raised to between 20° and 25°C and allowed to stabilize. The gelled protein samples were plunge-frozen in liquid ethane at its melting point (-183°C) or high pressure frozen (Balzers HPM 010); the samples were stored in liquid nitrogen (LN_2) [4, 5]. Dilute samples were prepared as 1 to 3 mg/mL protein solutions in sterile ddH_2O. The solutions were deposited on the surface of lacey carbon coated 300 mesh copper grids, which had been preequilibrated to 25°C. The solution was allowed to settle on the grid for 60 seconds and excess was wicked away with filter paper. The grids were plunge-frozen in ethane and stored in LN_2. A sample, either planchet or grid, was mounted on the precooled Gatan CT-3500 cryo-stage held in the cryo-preparation chamber. The specimen was fractured (planchets) with a prechilled blade and/or washed with LN_2. The shutters of the stage were closed and the cryo-stage was transferred to the Denton DV-602 Cr coater. Specimens were either sputter coated with 1 to 2 nm of Cr film or cryo-etched and subsequently coated. The stage shutters were then closed and the stage was transferred to the upper stage of the DS-130 FESEM operated at 25 kV.

Three complimentary methods were employed to elucidate the structure of the elastin-mimetic block copolymers as they self-assembled into discrete structures. At high protein concentrations, microscopic phase separation of the protein blocks formed thermoplastic elastomer hydrogels above a lower critical solution temperature T_t (Fig. 1a-1d). Initial observations with cryo-HRSEM demonstrated the fairly uniform dispersion of micelles throughout the lakes of vitreous ice (Fig. 1a and 1c). Cryo-etch experiments provided a means for the removal of excess unbound water from the protein hydrogels (Fig. 1b and 1d). This provided information on how the hydrogels were organized from the aggregation of micelles in solution above the T_t. Tandem cryo-HRSEM correlated with cryo-STEM of dilute aqueous protein solutions afforded data concerning short-range aggregation

patterns and the mass density of the micelles and micellar clusters (Fig. 1e and 1f). The cryo-HRSEM/STEM methods employed enabled us to characterize the morphological features of the elastin-mimetic materials without artifacts that would be incurred with conventional specimen preparation.

References

[1] H. Daniell et al., *Methods Mol. Biol.* 63 (1997) 359.
[2] R.A. McMillan et al., *Macromolecules* 32 (1999) 3643.
[3] E.R. Wright et al., *Adv. Funct. Mater.* 12 (2002) 149.
[4] K.P. Ryan et al., *J. Microsc.* 145 (1987) 89.
[5] R.P. Apkarian, *Acta Microscopia* 1 (2000) 20.
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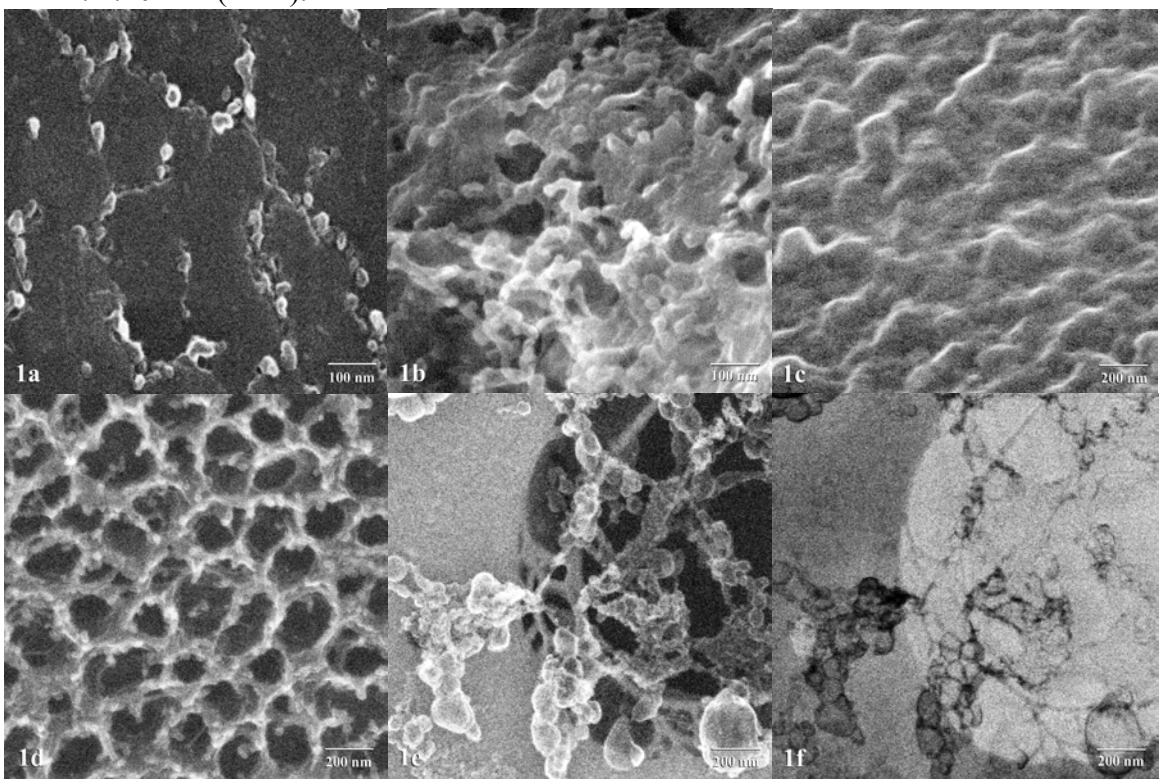

FIG. 1. Cryo, cryo-etch and tandem cryo-HRSEM correlated with cryo-STEM of elastin-mimetic block copolymers. (a) cryo-HRSEM of **1** (25%), (b) cryo-etch HRSEM of **1** (25%), (c) cryo-HRSEM of **2** (25%), (d) cryo-etch HRSEM of **2** (25%), (e) cryo-HRSEM of **1** (2 mg/mL), (f) cryo-STEM of **1** (2 mg/mL).

TABLE 1: Amino acid sequence of protein-based block copolymers **1** and **2** derived from elastin-mimetic protein sequences.

$\{VPAVG[(IPAVG)_4(VPAVG)_{16}IPAVG\}$-[X]-$\{VPAVG[(IPAVG)_4(VPAVG)]_{16}IPAVG\}$
1: [X] = $VPGVG[(VPGVG)_2VPGEG(VPGVG)_2]_{30}VPGVG$
2: [X] = $VPGVG[(VPGVG)_2VPGEG(VPGVG)_2]_{48}VPGVG$

A: alanine; E: glutamic acid; G: glycine; I: isoleucine; P: proline; V: valine

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