



Self-organization kinetics in polypeptide electrospun fibers



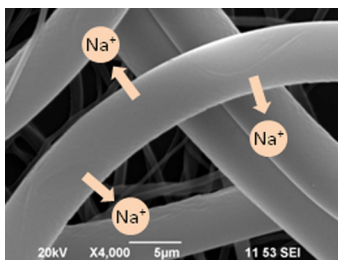
Donald T. Haynie*, Dhan B. Khadka, Gabriel Marcus, Nicole K. Le

Nanomedicine and Nanobiotechnology Laboratory, Center for Integrated Functional Materials, Department of Physics, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, United States

HIGHLIGHTS

- The self-organization of matter is currently of great interest.
- We have electrospun polypeptide fibers and analyzed the kinetics of annealing.
- Two different model polymers were studied, and annealing was done in water.
- Materials analysis was carried out by energy-dispersive X-ray spectroscopy.
- The kinetics data support structural change along two parallel pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

Self-organization processes are a major focus of current scientific interest worldwide. Novel materials research and development increasingly seeks to exploit ways in which matter self-organizes. Here, self-organization kinetics have been measured for electrospun fiber annealing. A key feature of such materials is a large surface-to-volume ratio. Fibers were spun from aqueous solutions of poly(L-ornithine) Br[−] (PLO) or co-poly(L-glutamic acid₄, L-tyrosine₁) Na⁺ (PLEY), model synthetic polypeptides. After crosslinking *in situ*, fibers were annealed in water at 22 °C. Analysis by infrared (IR) spectroscopy has revealed that annealing involved polymer restructuring within fibers on a time scale of 29 min for PLO and 63 min for PLEY. The largest changes in the distribution of polymer conformations occurred in the first 13 min of annealing. There was a substantial decrease in the amount of Na⁺ bound to PLEY fibers during annealing, according to energy-dispersive X-ray spectroscopy (EDX) analysis. Two parallel pathways provided a better account of the annealing trajectory than a single pathway with multiple transition states. This study advances current understanding of polypeptide-based materials and suggests approaches for applications development.

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1. Introduction

The self-organizing tendencies of matter are a major topic of research and technology development today. Moreover, interest is

strong across science disciplines. Engineering studies have sought to harness principles of self-organization for novel materials fabrication and device development. A sub-area of great practical significance is guided self-organization, in which human control over a feature of a process, for instance, a thermodynamic quantity in materials manufacture, can have a marked impact on the mechanical properties of a product without completely determining them. One of many guided self-organization processes is known

* Corresponding author. Tel.: +1 813 974 7793; fax: +1 813 974 5813.
E-mail address: dhaynie@usf.edu (D.T. Haynie).

as annealing. The present study concerned the annealing of polymers after crosslinking.

Annealing brings about a change in the macroscopic properties of a material, for instance crystallinity, by influencing microstructure, notably, the average conformation of the polymer backbone. Various methods are practiced. In thermal annealing, a material is heated and then cooled gradually [1]. Computational analogs are used to prepare energy-minimized molecular structures of large molecules; increasing the average kinetic energy of atoms can help to avoid a structure remaining trapped in a local free energy minimum [2]. Annealing a plastic can alter the conformations of chains and thus relieve internal stresses. Solvent uptake from the vapor phase can yield a change in material properties in solvent–vapor annealing [3]. Solvent annealing can bring about changes in material properties by way of solvent evaporation [4]. Solvent selection and dosing can markedly influence the annealing process and therefore material properties. Annealing thus can potentially enhance the structure or strength of a material. Important for fibrous materials, typical annealing processes place few restrictions on the geometry of the bulk material. Here, we investigated the annealing of crosslinked polymers upon submersion in water.

The polymeric materials of interest here are electrospun fibers. Production of nano- and microscale polymeric fibers by electrospinning is currently of broad interest for advancing basic scientific knowledge and developing materials of commercial value [5–7]. Potential applications of electrospun materials include filtration devices, high-strength, low-weight composites and scaffolds for cell and tissue culture. Electrospinning itself may be described as a guided self-assembly process. A small quantity of polymer solution leaves the feedstock as a liquid jet. Susceptible to various physical instabilities, the trajectory of the jet changes spontaneously as the solvent evaporates. The polymers within the jet then undergo spontaneous changes in conformation. Small differences in chemical structure and solvent properties can have a marked impact on polymer spinnability and fiber properties, notably, morphology. The potential for commercially successful manufacture of electrospun products could be increased by the discovery or invention of ways of increasing the predictability of materials processing or the physical properties of products.

Electrospinning research in our laboratory is focused on the relationship between material properties and polypeptide design. Polypeptides constitute about half of the dry mass of living organisms; every protein molecule comprises at least one polypeptide chain. Polypeptides are potentially useful for a wide range of materials applications in biotechnology, medicine and other areas. A comprehensive review of protein-based electrospun fibers has appeared recently [8]. Investigators who have contributed to the growth of knowledge in this area include Wnek and colleagues [9], Sotzing and colleagues [10] and Guthold and colleagues [11]. PLO and PLEY have simpler structures than do proteins. Here, PLO and PLEY were adopted as model polymers. All polymer feedstocks were aqueous solutions. PLO is a polycation at neutral pH, PLEY a polyanion. These polymers are readily crosslinked *in situ* with glutaraldehyde (GTA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), respectively, making the fibers insoluble in aqueous solution under mild conditions [12–15]. PLO fibers and PLEY fibers are cytocompatible ([8] and unpublished results from the D.T.H. laboratory). Predicting the physical, chemical and biological properties of fibers from peptide structure alone is a formidable challenge. The results of focused experimental studies on model systems could help to improve the predictive value of existing empirical models and further reveal the strengths and weaknesses of competing explanations of physical properties.

2. Materials and methods

2.1. Experiment

2.1.1. Polymers

PLEY (20–50 kDa by viscometry) and PLO (153.5 kDa by viscometry) were obtained from Sigma (USA) as lyophilized polydisperse salts. The counterions were Br[−] for PLO and Na⁺ for PLEY.

2.1.2. Fiber sample preparation

Aqueous feedstocks of the polymers were prepared by dissolving as-received peptide in DI water (Marlo MRO-3600-4V reverse osmosis system, USA). The conductivity of water was 0.9 $\mu\text{S cm}^{-1}$ prior to polymer dissolution. The nominal final polymer concentration was 40% (w/v) for PLO and 50% for PLEY. Fibers were spun from these preparations as described previously [12–15]. Briefly, polymer feedstock was taken up into a 1-mL syringe outfitted with a 0.5-mm metallic blunt-end needle (Jensen Global, USA). A positive potential in the 5–20 kV range was applied to the needle with a Glassman High-Voltage PS/FX20P15.0-11 power supply (USA). Fibers were collected 5–15 cm from the spinneret on pieces of 60 $\Omega\text{ in}^{-2}$ indium tin oxide-coated poly(ethyleneterephthalate) (ITO-PET; Sigma-Aldrich) or glass cover slips, as indicated. The feedstock flow rate was not regulated.

2.1.3. Crosslinking

Fibers were crosslinked *in situ*, PLO with GTA in the vapor phase [25% (w/v) in water; Sigma] and PLEY with 50 mM EDC (Thermo Scientific, USA) in 90% ethanol/10% water [12–15]. A schematic diagram of the chemistry of the crosslinking reactions is provided in Supporting information. 10 mL of crosslinking solution was deposited in a petri dish for each reaction. PLO fiber-coated substrates were suspended over the liquid phase; PLEY fiber-coated substrates were submerged. Each dish was covered, sealed with parafilm and maintained at 22 °C overnight. Next day, fiber samples, now crosslinked, were rinsed 4 times with 5 mL of deionized (DI) water. The duration of each rinse was 5 s. The final rinse was for 1 min on an orbital shaker. Fiber samples were blotted gently with porous paper for <5 s to remove solvent and dried thoroughly with N₂ gas within 30 s prior to annealing. Fiber-coated ITO-PET substrates were then divided into 12 pieces of equal area for kinetics determination. The final size of each piece, 8 cm × 6 cm, matched the size of the attenuated-total-reflectance (ATR) crystal for IR spectral analysis (see below).

2.1.4. Annealing

Crosslinked fibers on ITO-PET were annealed *in situ* by submerging each sample in 3 mL of DI water for 0, 1, 2, 3, 4, 5, 8, 13, 21, 34, 55, 89 or 144 min at 22 °C, or for 0, 15, 45, 75, 300 or 3600 s at 45 °C followed by rapid cooling to 22 °C. Immediately thereafter, each fiber sample was blotted gently with porous paper for <5 s to remove excess water, dried thoroughly with N₂ gas within 30 s and analyzed by IR spectroscopy. A different fiber sample was analyzed for each time point.

2.1.5. Hydrogen exchange

Crosslinked fiber samples on ITO-PET were nominally completely annealed *in situ* for 4.5 h, blotted and dried with N₂ gas as described above. Each sample was then submerged in 3 mL of D₂O (Sigma) for 0, 5, 10, 15, 20, 25, 40, 65, 105, 170, 275, 445 or 1200 min at 22 °C, blotted, dried with N₂ gas and maintained in a desiccator for 24 h prior to IR analysis. A different fiber sample was analyzed for each time point.

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