Aligned 3D Amino Acid Nanofiber Biomaterials

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Statement of Purpose: Self-assembled synthetic materials are typically disordered, and controlling the alignment of such materials at the nanometer scale may be important for a variety of biological applications.[1-3] In this study, **our objective** was to apply directional freeze-drying to develop well aligned three dimensional (3D) nanofiber biomaterials using amino acid like phenylalanine (Phe), dipeptide like diphenylalanine (Phe-Phe), and Phe-based composites.

Methods: Solutions of Phe were prepared at concentrations of 10, 25, 50, 100, and 150 mM. A 2 mM Phe-Phe solution was prepared by dissolving Phe-Phe in distilled water with vigorous stirring. Phe-poly(vinyl alcohol) or Phe-PVA and Phe-polyethylenimine or Phe-PEI solutions were obtained by adding PVA or PEI, respectively, into 100 mM Phe solution at a Phe/PVA or Phe/PEI weight ratio of 5/1. The solutions were used to develop Phe and Phe-based materials with two approaches: Conventional drop-casting and directional freeze-drying.

Results: A typical freezing process followed by freeze-drying (so called directional freeze-drying) was explored to develop aligned 3D architectures of Phe (**Fig. 1**). Phe nanofibers with diameter of ~150 nm and length of tens of micrometers were self-assembled and formed a highly aligned nanofibrous architecture. The nanofibrous structures obtained with liquid nitrogen freezing became denser with increasing Phe concentration (data not shown). The solution pH was also found to play an important role in the formation of Phe nanofibrous materials. Well aligned Phe nanofibers were formed at pH 5.5 and pH 9 while no nanofibers were observed at pH 11 (data not shown). In addition, significant differences were observed in the structures of freeze-dried Phe 3D nanofibrous materials prepared at different freezing temperatures (**Fig. 2**).

The same strategy (i.e. directional freeze-drying) was also successfully applied to assemble other nanofibers into 3D well-aligned nanofibrous networks. Nanofibrous materials with well aligned and densely packed nanofibers were developed using a dipeptide, i.e. Phe-Phe (data not shown). By contrast, randomly distributed nanofibers were formed using the conventional drop-casting approach. Phe-based nanofibrous composites were fabricated by introducing polymers like PEI and PVA into Phe nanofibrous networks (**Fig. 3**). By labeling PEI with RhoB, we confirmed that PEI was uniformly distributed within the Phe-PEI nanofibers using confocal laser scanning microscopy. The secondary structures of Phe-PEI and Phe-PVA composites were found to be similar to that of Phe nanofibers (**Fig. 3f**).

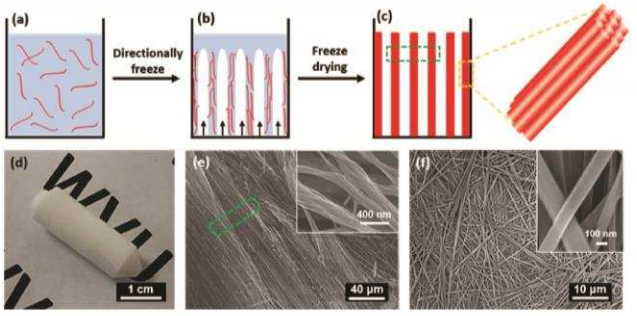

Fig. 1. Formation mechanism and morphology of aligned 3D Phe nanofibrous materials. (**a-c**) Scheme showing the formation of aligned nanofibers using directional freeze-drying. The Phe nanofibers in the solution are excluded from the freezing front and concentrate and aligned between the orientated solvent crystals. Aligned nanofibrous materials are obtained upon subsequent removal of solvent during the freeze-drying process. (**d**) Photo image of a freeze-dried 3D Phe nanofibrous monolith. (**e**) Typical FE-SEM image of Phe nanofibers obtained using directional freeze-drying. Inset is a high magnification image. (**f**) Typical FE-SEM image of Phe nanofibers prepared using conventional drop-casting.

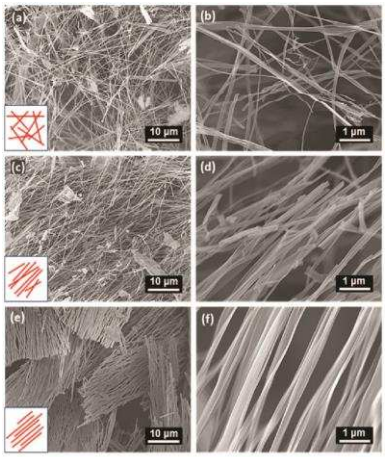

Fig. 2. Microstructures of 3D Phe nanofibrous materials prepared at (**a,b**) –20, (**c,d**) –80, and (**e,f**) –196 °C (liquid nitrogen). Concentration of Phe was 100 mM.

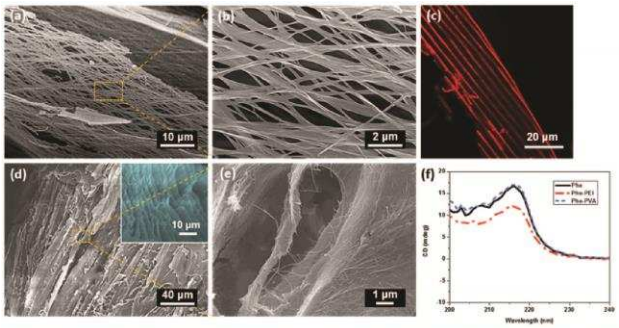

Fig. 3. Morphology of aligned Phe-based composite nanostructures prepared by directional freeze-drying using liquid nitrogen freezing. (**a,b**) SEM images of Phe-PEI nanofibrous materials. (**c**) Confocal microscopy image of freeze-dried Phe-PEI nanofibers. PEI was Rhodamine B (RhoB) labeled. (**d,e**) SEM images of Phe-PVA aligned porous materials. Inset of (d) shows the cross-section perpendicular to the direction of alignment. (**f**) CD spectra of Phe, Phe-PEI, and Phe-PVA solutions at a concentration of 100 μM.

Conclusions: 3D free-standing Phe nanofibrous monoliths have been successfully prepared using directional freeze-drying, and have presented a unique hierarchical structure with well-aligned nanofibers at the nanometer scale and an ordered compartmental architecture at the micrometer scale. We have found that the physical properties (e.g. nanofiber density and alignment) of the nanofibrous materials could be tuned by controlling the concentration and pH of the Phe solution and the freezing temperature. Moreover, the same strategy (i.e. directional freeze-drying) has been successfully applied to assemble peptide nanofibrous materials using a dipeptide (i.e. diphenylalanine), and to assemble Phe-based nanofibrous composites using polyethylenimine and poly(vinyl alcohol). The tunability of the nanofibrous structures together with the biocompatibility of Phe may make these 3D nanofibrous materials suitable for a variety of applications, including biosensor templates, tissue scaffolds, filtration membranes, and absorbents.

References. [1] Reches M. & Gazit E. Nat Nanotechnol **1**, 195 (2006). [2] Zhong ZH, et al. Science **302**, 1377 (2003). [3] Hung AM & Stupp SI. Nano Lett **7**, 1165 (2007).