Protein Adsorption and Crystal Structure of Poly(L-lactic acid) Nano Fibers

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Statement of Purpose: Developing polymeric nanofibers for tissue engineering purposes has caught much attention in recent years[1-2]. A novel phase separation process has been developed in our laboratory to fabricate nano-fibrous poly(L-lactic acid) (PLLA) scaffolds. Previous experimental results show that nano-fibrous PLLA scaffolds selectively adsorb proteins and provide superior environment for the growth of osteoblastic cells[3]. Nano-fibrous materials have large surface areas, which should advantageously enhance the capacity of protein adsorption. However, the large surface area itself cannot explain the protein adsorption selectivity. This work focuses on the crystal structure of the nano-fibrous PLLA material and its effect on protein adsorption.

Methods: PLLA used in this study were purchased from Boehringer Ingelheim (Ingelheim, Germany). PLLA nanofibrous films were prepared using the method described in previously published articles[1]. Smooth PLLA films were prepared using solvent casting method. The crystal structures and the degree of crystallinity of films were determined using an X-ray Diffractometer (Rigaku D/Max-IIB) and Differential Scanning Calorimeter (DSC) (Perkin-Elmer DSC-7) respectively. Films were heated under vacuum to vary the degree of crystallinity. Bovine serum albumin (BSA) was used as a model protein to test the effects of polymer crystallinity on protein adsorption. Films were soaked inside 2% BSA phosphate-buffered saline (PBS) (0.1M, pH=7) solution at 37 °C overnight. The amount of protein adsorption was determined by using BCA™ protein assay kit (Piece).

Results / Discussion: Thermal annealing is widely used to vary the degree of crystallinity and/or crystal structure of polymeric materials. Here thermal annealing is employed to vary the degree of crystallinity of nano-fibrous and smooth PLLA films. Results show that the degree of crystallinity increases steadily with annealing time for smooth PLLA films (Figure 1). On the contrary, the degree of crystallinity of nano-fibrous PLLA films essentially does not change under the same thermal annealing conditions. The difference may result from the geometry restriction of the nano-fibrous structure. Further studies are needed to understand the exact mechanisms. DSC measurements at slow heating rate also show similar disparity. The spectrum of smooth films shows two melting peaks at very slow heating rates. The higher-temperature melting peak results from the reorganization of the polymer molecules to form larger crystal size during the slow heating process. It is interesting that the reorganization behavior is not observed in nano-fibrous samples. This phenomenon is consistent with that of thermal annealing monitored using X-ray diffraction. Both thermal annealing and DSC scanning results indicate that the structure of nano-fibrous PLLA films is more stable than that of the smooth PLLA films under heat treatment.

Smooth PLLA films with different degrees of crystallinity are used as substrates for the protein adsorption experiment. The results indicate that the degree of crystallinity not only affects polymer's mechanical properties and degradation rate, but also influences protein adsorption. The amount of BSA adsorption decreases with increasing degree of crystallinity (Figure 2). Since protein adsorption is critical to cell attachment and other cellular behaviors, the finding provides us with useful information to rationally design optimal scaffolds for tissue engineering.

The difference in polymer crystal structure is another possible factor that affects the amount or/and conformation of adsorbed proteins, which in turn affect cellular behaviors at surface. The most evident peaks on the XRD spectra of both the nanofibrous film and smooth film locate at essentially the same positions (2θ = 16.6 and 19 degrees), which are characteristics of a 10_3 helix crystal structure. The smaller peaks located at 12, 20.7 and 24.6 degrees on the spectrum of smooth film suggest that a fraction of polymer has a 3_1 helix structure. Further studies are underway to understand effect of different helical structures on protein adsorption.

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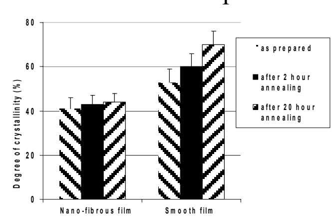

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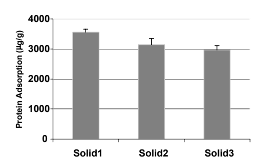

Figure 1: the effects of thermal annealing on degree of polymer crystallinity.

Figure 2: BSA adsorption on solid-wall PLLA with different degrees of crystallinity (solid 1: 53%, solid 2: 60%, and solid 3: 70%).

Conclusions: Experimental results show that the degree of crystallinity of PLLA affects the capacity of protein adsorption. Differences in polymer crystallization and crystal structure have been observed in nanofibrous and smooth PLLA films. The study may lead to a better understanding on how proteins and cells interact differently with nanofibrous PLLA surface from smooth PLLA surface.

References:
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2. Zhang SG NATURE BIOTECHNOLOGY 21 (10): 1171-1178 2003.
3. Woo KM, Chen VJ, Ma PX JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART A 67A (2): 531-537 2003.