Focal Adhesions of Osteoblasts on Poly(d,l-lactide)/Poly(vinyl alcohol) Blends by Confocal Fluorescence Microscopy

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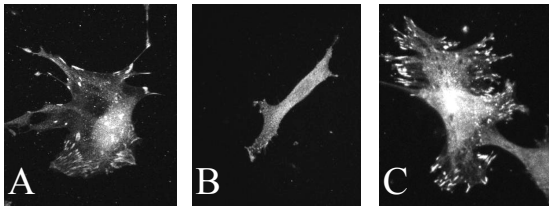
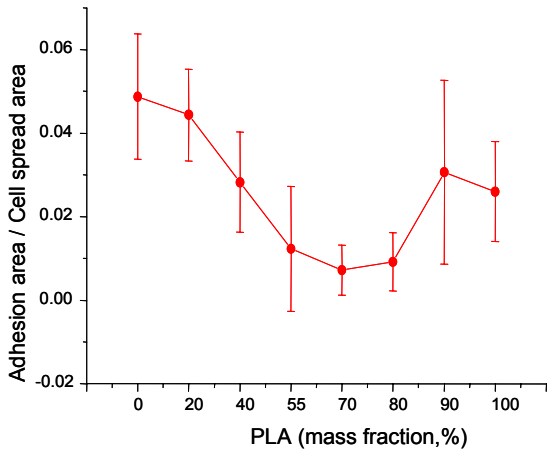
Introduction: Blends of poly(lactic acid) [PLA] and poly(vinyl alcohol) [PVOH] have been considered as biomaterials for tissue engineering scaffolds and for encapsulation of drugs. For example, Sun et al. used PLA /PVOH membranes for microencapsulation of cartenoids[1] and Mooney et al. investigated PLA/PVOH sponges for hepatocyte transplantation.[2] However, to our knowledge, there has been no attempt to use a combinatorial approach to characterize the biocompatibility of PLA/PVOH blends. Therefore, the purpose of the project described here was to measure focal adhesions, cell spread area, and cell number as indicators of biocompatibility for PLA/PVOH blends of various compositions.

Experimental[#]: PVOH (100 % hydrolyzed, molecular mass of 15,000 g/mol; Aldrich Chemical Company), poly (d,l-lactic acid) [PLA, inherent viscosity of 0.17 dL/g; Birmingham Polymers, Inc], and hexafluoro-isopropanol (HFIP; TCI America) were used without further purification. Solutions of different compositions were prepared by mixing appropriate amounts of a solution of PVOH (mass fraction of 1 %) in HFIP and a solution of PLA (mass fraction of 1 %) in HFIP. Films of PLA, PVOH and PLA/PVOH blends [PLA mass fractions of (20, 40, 55, 70, 80, and 90) %] were prepared by casting these solutions on cover slips 12 mm in diameter. These films were allowed to dry in the ambient air for 1 d, and then at room temperature for 2 d under high vacuum. The films were quenched to room temperature after they were heated at 200 °C for 3 min. Finally, the films were further dried under high vacuum for 3 d at room temperature.

MC3T3-E1 osteoblast-like cells obtained from Riken (Ibaraki, Japan) were cultured in alpha Minimal Essential Medium (MEM; BioWhittaker, MD), supplemented with 10 % mass fraction fetal calf serum (FCS, Gibco, Grand Island, NY) and 60 µg/mL of kanamycin sulphate (KS; Sigma, St. Louis, MO). The cells were kept at 37 °C in a fully humidified atmosphere of air containing 5 % volume fraction of CO_2. The cells were fed every (3 or 4) d. Before the cells became confluent, the cells in a 25-cm^2 flask were incubated with 5 mL of 0.25 % mass fraction trypsin in 1 mmol/L EDTA-4Na (Gibco, Grand Island, NY) for 5 min at 37 °C. The cells were resuspended in MEM (supplemented with 10 % mass fraction FCS and 60 µg/mL of KS) and plated onto films of PLA/PVOH blends. After 24 h of incubation, cells were prepared for immunofluorescence staining of vinculin, a focal-adhesion protein, as described by van Kooten and coworkers[3]. A confocal fluorescence microscope was used to acquire the images of the stained cells.

Results: In the micrographs shown herre, the bright spikes indicate the locations of vinculin molecules in the focal adhesions, which were made visible through an immuno-fluorescence technique. It is seen that while the cells seeded on a PVOH film (A) and on a PLA film (C) exhibited prominent focal adhesions, the cell seeded on a blend film (B, 70 % mass fraction of PLA) exhibited few focal adhesions. Similarly, the cell spread area and the cell number for the cells that were seeded on the blend films of intermediate compositions [mass fraction of (55, 70, and 80) % PLA] were less than those for the cells seeded on the PLA or PVOH films.





The plot above gives the focal adhesion area (normalized by the cell spread area) as a function of the blend composition. Each error bar, an estimate of the standard uncertainty, is the standard deviation of the mean value derived from twenty measurements. It is worth noting that the cells seeded on the blend films of intermediate compositions [mass fractions of (55, 70, and 80) % PLA] exhibited diminished focal adhesions. The mechanisms of the diminished focal adhesions at these intermediate compositions are under investigation.

References:

1. Sun ZM, Poncelet D, Conway J, and Neufeld RJ, J Microencapsulation, **12**, 495-504 (1995).
2. Mooney DJ, Park S, Kaufmann, PM, Sano K, McNamara K, Vacanti JP, and Langer R, J Biomed Mater Res, **29**, 959-965 (1995).
3. van Kooten TG, Klein CL, Wagner M, Kirkpatrick CJ, J Biomed Mater Res, **46**, 33-43 (1999).

[#] **Disclaimer.** Certain commercial materials and equipment are identified in this work for adequate definition of the experimental procedures. In no instances does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or that the material and the equipment identified is necessarily the best available for the purpose.