In Vitro Biocompatibility of Hydrolytically Degraded Poly(d,l-lactic acid)

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Introduction: Poly(d,l-lactic acid) [P(d,l-LA)] has a history of safety and efficacy as well as the FDA approval. Therefore, P(d,l-LA) has often been considered as a candidate biomaterial for applications in tissue-engineering therapy. Knowledge of the adhesive interactions between osteoblasts and biodegradable polymers is needed for the evaluation of these polymers, because the adhesion of cells to biomaterials is an important indicator of cell-biomaterial interactions. Therefore, we measured the adhesion of MC3T3-E1 osteoblasts to hydrolytically degraded P(d,l-LA) disks in order to evaluate how the degradation affected the interactions between the osteoblasts and P(d,l-LA).

Experimental[#]: Racemic P(d,l-LA) powder with an average molecular mass of 17,000 g/mol, was obtained from Birmingham Polymers (Birmingham, AL). P(d,l-LA) disks (10 mm in diameter and 1.6 mm in thickness) were prepared by compression moulding. The disks were sterilized with 70 % mass fraction ethanol for 15 min, kept in serum-free alpha Minimal Essential Medium (MEM; BioWhittaker, MD) for one hour, and then washed with fresh serum-free MEM to remove ethanol.

MC3T3-E1 osteoblast-like cells (from Riken, Ibaraki, Japan) were cultured in MEM, supplemented with 10 % mass fraction fetal calf serum (FCS, Gibco, Grand Island, NY) and 60 μg/mL of kanamycin sulphate (KS; Sigma). The cells were maintained 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 P(d,l-LA) disks.

Cell Area MC3T3E-1 cells from fifth-passage cultures were plated onto the P(d,l-LA) disks that had been degraded for (0 to 4) weeks by immersion in serum-free MEM (supplemented with 60 μg/mL of KS) [hereafter referred to as the hydrolyzing medium], as well as empty wells of tissue culture-polystyrene plates (as controls), at a density of 8 x 10^3 cells/well in MEM containing 10 % mass fraction FCS and 60 μg/mL of KS. After 24 h of incubation, the cells were fixed with 3.7 % mass fraction formaldehyde, and rinsed three times with phosphate-buffered saline (PBS; without calcium and magnesium chloride). To stain the plasma membranes of the cells, the cells were incubated in PBS containing 5 μmol/L of a fluorescent probe DiIC(16) [Molecular Probes], at room temperature for 10 min. After the staining, the cells were washed three times in PBS and then mounted with a mounting medium [1:1 mass ratio of glycerol and PBS; with 0.02 % mass fraction sodium azide and 100 mg/mL Dabco (Aldrich; 1,4-diazabicyclo[2.2.2]octane)]. Stained cells were examined by fluorescence microscopy (Nikon Eclipse TE300/TE200, Tokyo) with a 10x objective and a standard rhodamine filter. Five separate viewing fields (x 100; center, north, west, south, east) on each of four independent disk surfaces were digitized. For each degradation period, cell areas of at least 112 cells on each of four independent disk surfaces were measured. Cell areas were calculated from each digitized image using the NIH Image (Version 1.61).

Focal Adhesions Cells from fifth-passage cultures were seeded onto P(d,l-LA) disks in six-well plates at a density of 1.0 x 10^4 cells/well. After 24 h of incubation in MEM containing 10 % mass fraction FCS and 60 μg/mL of KS, cells were prepared for immunofluorescence staining of vinculin, a focal-adhesion protein, as described by van Kooten and coworkers.[1]

Live Cell Number Cells from the fifth-passage cultures were seeded on P(d,l-LA) disks that had been degraded by immersion in the hydrolyzing medium for (0 to 6) weeks in six-well plates at a density of 1.5 x 10^4 cells/well. After 16 h of incubation in MEM containing 10 % mass fraction FCS and 60 μg/mL of KS, disks were rinsed twice with serum-free MEM and then stained for at least 15 min at 37 °C with 2 μmol/L calcein-AM (acetoxy methyl ester, Molecular Probes Inc.) in serum-free MEM. The cells were then photographed by fluorescence microscopy with a 10x objective using a standard FITC filter.

Results: The cell-spread area of the cells on P(d,l-LA) disks that were not degraded did not differ significantly from that of the cells on tissue-culture polystyrene, but the degradation of P(d,l-LA) disks affected cell spreading. The cell-spread area decreased linearly with the degradation time of the disks at a rate of (-741 ± 307) μm^2/week (all uncertainties quoted are expanded uncertainties at the 95% confidence level). Compared with the cells on non-degraded P(d,l-LA) disks, the cells on P(d,l-LA) disks that had been degraded for 4 weeks also showed irregular morphologies. Focal adhesions began to disappear for the cells on P(d,l-LA) disks degraded for one week. The number of the live cells [up to (2.099 ± 0.268) cells/mm^2 in $\log_{10}$ units, depending on the measurement location within the samples] on P(d,l-LA) disks also decreased linearly with the degradation time of the disks at a rate of up to (-0.175 ±0.064) (cells/mm^2)/week in $\log_{10}$ units, again depending on the measurement location within the samples. These results indicate that degraded P(d,l-LA) is less biocompatible than non-degraded P(d,l-LA), and focal adhesions are more sensitive indicators of the biocompatibility of degraded P(d,l-LA) than the cell-spread area.

References:

1. van Kooten TG, Klein CL, Wagner M, Kirkpatrick CJ, J Biomed Mater Res, **46**, 33-43 (1999)

[#]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.