



Title: Phorbol Myristate Acetate-Differentiated THP-1 Cell Adhesion and Viability on Biomaterials

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Encapsulation of long-term implants confounds biomaterials design, inspiring the development of an assay of biocompatibility based on macrophage behavior. Preliminary work shows that the functionalization of polydimethylsiloxane (PDMS) with pendant poly(ethylene oxide) (PEO siloxanes) reduces deposition and activation of the human monocytic THP-1 cell line, increasing biocompatibility. THP-1 cells are also more viable on these biomaterials. Phorbol myristate acetate (PMA), which induces the differentiation of THP-1 cells into macrophages (THP-1s), activates them and arrests their proliferation, was introduced to THP-1 cells to further examine these results.

The adhesion, activation and viability of THP-1s to a variety of polymer biomaterials was appraised using inverted digital video microscopy for 14 d of incubation at 37 °C in 5% CO₂. Activation criteria include spread area/cell and periphery/cell. Polymers were spin-coated onto glass coverslips from 3% solutions of polymer in toluene to form a homogeneous reproducible film. Controls are the bare coverslips or those coated with commercial biomaterials, including the Cytonix precoating. Before spin-coating, the coverslips were made hydrophobic to be compatible with the PDMS base polymer of the siloxanes. This was accomplished by immersing the coverslips in Cytonix FluoroPel PFC 1601A, a fluoropolymer solution developed by the Cytonix Corporation.

Clean discs of each biomaterial, including controls, were placed in 24-well tissue culture plates and incubated with 10⁵ cells/well/ml of medium. Cells were examined using inverted digital video microscopy. The human monocytic THP-1 line (ATCC, Manassas, VA) was maintained in RPMI 1640 medium (Life Technologies, Frederick, MD) supplemented with 50 μM 2-mercaptoethanol, 10% fetal calf serum, Glutamax I, 50 U/ml penicillin and 50 μg/ml streptomycin and incubated in tissue culture flasks at 37 °C in 5% CO₂. Proper differentiation was achieved by 24 h exposure to 10 ng of PMA /ml of DMSO after the cells had been subcultured up to 14 d. The biomaterials appraised thus include tissue-culture polystyrene, glass, Cytonix, unfunctionalized polydimethylsiloxane, PDMS (molecular weight 56,000; UCT, Bristol, PA) pendant-functionalized with poly(ethylene oxide)-poly(propylene oxide) (PEOPPOPDMS), and PDMS (molecular weight 10,200; UCT) pendant-functionalized with poly(ethylene oxide) (PEOPDMS). Our results show greater viabilities than previous studies (1). We determined that the most biocompatible biomaterials investigated were the PEO siloxanes. Moreover the results for THP-1s suggest a convenient in vitro experimental model that might be useful for long-term predictions.

REFERENCES:

1. M Belanger, Y Marois, R Roy, Y Mehri, E Wagner, Z Zhang, MW King, M Yang, C Hahn, R Guidoin, *Artif. Org.* 24: 879 (2000).