Attachment of Osteoblasts to New Surface-Modified Substrates - A Preliminary Report

P.L. Tate [a], M.A. Hucks[a], S. Nagatomi[a], M.A. Vaughn[a], M. Shalaby[b], and S.W. Shalaby[a]
[a]Poly-Med, Inc., Anderson, SC
[b]Lehigh Valley Hospital, Allentown, PA

Statement of Purpose: Results of earlier studies conducted in this laboratory on surface phosphonylated endosteal dental implants of carbon fiber reinforced polyether ether ketone (CFR-PEEK) have indicated that these implants do encourage bone growth onto these active surfaces resulting in osseointegration with surrounding tissues [1,2]. This led to the postulate that surfaces capable of immobilizing and chelating calcium ions, such as those activated by phosphonylation or c-succinylation, provide a preferred active substrate for the attachment and proliferation of osteoblasts. This study is designed to test the viability of such postulate using c-succinylated surfaces.

Methods: Cells: Human fetal osteoblasts (ATCC, Manassas, VA) were maintained under recommended culture conditions in a 33.5 ºC, humidified, 5% CO_2 / 95% air environment in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's Nutrient Mixture F12 supplemented with 10% fetal bovine serum (ATCC), 15 mM HEPES, and 0.3 mg/mL G418 (Invitrogen, Carlsbad, CA). Films: Polypropylene and CFR-PEEK films of less than 1 mm thickness were prepared using a heated, automatic hydraulic press (Carver, Wabash, Indiana) and then surface c-succinylated to introduce succinic acid side groups as described earlier[3] or left untreated. The individual films were cut into 1 cm^2 pieces and sterilized by ultraviolet irradiation for 20 minutes. Prior to cell seeding, films were fixed to the bottom of tissue-culture wells with a small amount of sterile silicone grease and soaked in media for 2 hours. Experiments: Osteoblasts were seeded onto films at a density of 1.3 x 10^4 cells/cm^2. After 7 days of culture, films were transferred to a new tissue-culture plate and visualized with propidium iodide or alizarin red staining.

Results / Discussion: Direct microscopic examination of alizarin red-stained specimens are illustrated in Figures 1 and 2. Compared to untreated polypropylene controls (Figure 1), there was enhanced osteoblast attachment and proliferation on c-succinylated polypropylene films (Figure 2). Specimens stained with propidium iodide and viewed with fluorescence microscopy revealed that there was osteoblast adhesion on both control and c-succinylated CFR-PEEK films (Figures 3 and 4), but adhesion was hardly enhanced on c-succinylated films.

Conclusions: Available results of this preliminary study indicate that c-succinylated surfaces, at least for polypropylene, encourage the attachment and proliferation of osteoblasts.

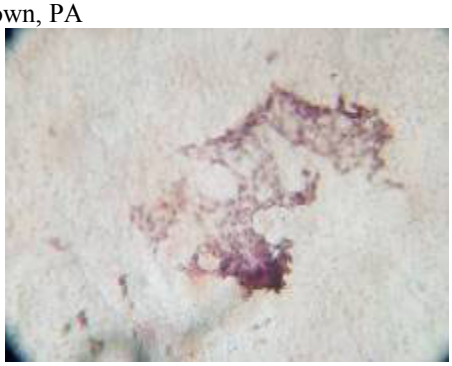

Figure 1. Control polypropylene

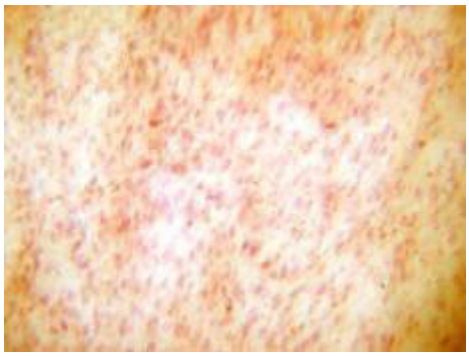

Figure 2. c-succinylated polypropylene

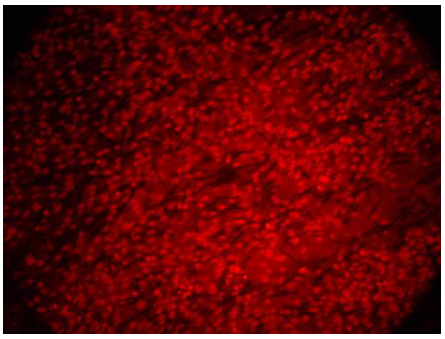

Figure 3. Control CFR-PEEK

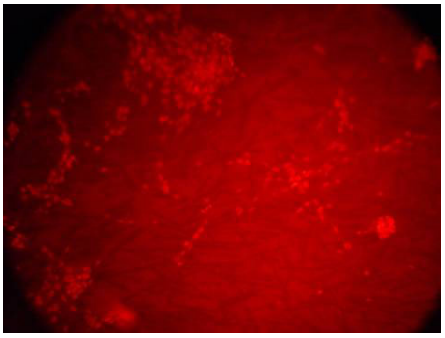

Figure 4. c-succinylated CFR-PEEK

References:

1. Anneaux BL et *al*. Trans Soc Biomater. 2005;28:129.
2. Anneaux BL et *al*. Trans Soc Biomater. 2005;28:431.
3. Shalaby SW. U.S. Patent Appl. 2005;#60/662:908.