Proliferation of Aortic Adventitial Fibroblasts on Three Novel Polyisobutylene (PIB)-based Thermoplastic Elastomers (TPES)

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Statement of Purpose: Coronary stents revolutionized interventional cardiology to treat narrowing of the coronary arteries caused by the build-up of atherosclerotic plaque (heart disease), the leading cause of death for both women and men in the United States. Drug Eluting Stents (DES) reduced the incidence of restenosis (renarrowing of the artery).[1] The *Taxus* DES is coated with the Translute® polymer, (poly(styrene-*b*-isobutylene-*b*-styrene) SIBS for short, invented at the University of Akron[2]. SIBS was shown to have excellent biocompatibility.[3]This paper will discuss cell proliferation studies on new generations of SIBS-type polymers with branched (dendritic) PIB core[4], targeted for stent-coating and other applications.

Materials and Methods: SIBS (M_n = 67,000 g/mol, M_w/M_n = 1.2, end block 31 wt% PS), D_IBS23 (119,000 g/mol, 2.3, 23wt% PS), D_IBMS31 (300,000 g/mol, 2.6, 31 wt% PpMeSt (PMS)) were dissolved in methylcyclohexane with a ratio of 1.25g of sample/70 mL solvent. The solution was then centrifuged at 3200 RPM (IEC CENTRA-7R Refrigerated Centrifuge, International Equipment Company). 0.1mm thick films were cast with a Dr. Blade Instrument (Richard E Mistler, Inc., Yardly, PA). The films were then allowed to dry for 48 hours and were then placed into a drying oven 110°C for two weeks. The films were then cut and secured over the opening of polyethylene PE microwells and sterilized by ethylene oxide. Silicone (NuSil) and PVC (Teknor Apex) were used as controls.The samples were submerged in SCBM (stromal cell basal medium- Lonza Inc.) for 24 hrs. at 37 °C in a 5% CO_2 atmosphere in a Forma Scientific Infrared CO_2 incubator to remove the residuals of the ethylene oxide. The prepared medium (500mL of SCBM , 0.5mL-rhFGF-β (Human Fibroblast Growth Factor-_β, 0.5mL-Insulin, 25mL – FBS (Fetal Bovine Serum) and 0.5mL-GA-1000 (Gentamian Sulfate Amphotericin- β)_was stored at 4°C. Human Aortic Adventitial Fibroblasts (AoAF) cells were stored at 37°C in a 5% CO_2 atmosphere in a Forma Scientific Infrared CO_2 incubator. The AoAF cells were used on the 4th through the 10th passages. The PE microwells were seeded with 32,000 cells/cm^2. Then 4 replicates of microwell dishes were prepared (per sample) in a 24 well tissue culture dish with the following: 0.1 ml of the cells and 0.3 ml of prepared medium. The samples were cultured for 11 days. The cell counts were obtained using Trypan blue stain (GIBCO™, Invitrogen Corporation) 0.4% and a hemocytometer. To compare the cellular growth of the AoAF cells on the 3 different polymers and PVC and silicone controls the independent Student *t* test was used as a statistical method.

Results: Figure 1 shows that the silicone control and the test polymers had similar cell growth to the blank culture dish ($p > 0.05$), except for D_IBMS31. For the first 5 days this polymer had similar cell growth to the materials above but after that the cells started to show a decrease in growth. The PVC control showed a decrease and a negative interaction with the cells after Day 1.

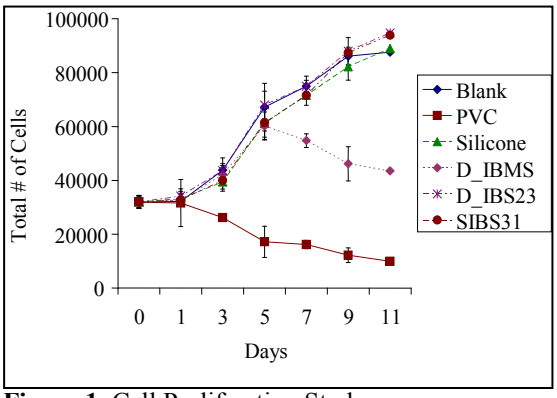

Figure 1. Cell Proliferation Study.

The D_IBMS31 film was not transparent at the beginning of the cell proliferation studies, due to solvent entrapment by the very high molecular weight polymer. The entrapped solvent was able to diffuse from the interior of this sample only very slowly, affecting the cells only after 5 days. To verify that this was responsible for the abnormal cell behavior, SCBM with the D_IBMS31 leachates were added into the cell culture. Cell proliferation with 9-day leachates from the polymers showed a similar negative result for D_IBMS31. Within 48 hours the total number of cells attached to the flask started to decrease.

Conclusions: The cell culture method effectively compared the test polymers, showing that these materials are non-toxic to vascular wall cells. The results also point out the necessity of longer leachate tests on SIBS-type polymers than the recommended 24 hrs, due to the very low permeability of the PIB segment.

Acknowledgements: This material is based upon work supported by the National Science Foundation under Grant DMR #0509687 and #0804878.

References:

(1) (Muni, NI. N. Engl. J. Med. 2004; 351:1593-1595.)
(2) (Kennedy, JP. New thermoplastic elastomers. U.S. 4,946,899, 1990.)
(3) (Pinchuk, L. *Biomaterials.* 2008;29:448-460.)
(4) (Puskas, J.E. Novel Highly Branched Thermoplastic Elastomers. US Patent 6,747,098, 2004.)