Effects of Polymer Degradation on Smooth Muscle Cell Phenotype: Implications for Stent Design

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Statement of Purpose: Intravascular metal stents are currently being used to restore patency in atherosclerotic coronary arteries. A variety of different stent designs have been proposed and it has been found that different stents have different in-stent restenosis rates.[1] The incidence rate of complications due to stenting has stimulated researchers to pursue synthetic polymer stents, to be employed alone or as drug delivery vehicles to decrease stent thrombogenicity and/or neointimal hyperplasia. A landmark study by van der Giessen et al. in 1996 demonstrated an acute inflammatory and neointimal response to several biodegradable polymer-loaded stents following deployment in the porcine coronary artery via balloon angioplasty.[2] Since, there have only been a handful of studies that attempt to explain the results of van der Giessen et al., and none have examined the phenotypic profiles of smooth muscle cells. To this end, we investigated the effects of biodegradation products of several polymers on the phenotype and function of human coronary artery smooth muscle cells (hCASMCs) using real-time RT-PCR and proliferation assays, respectively. We hypothesize that polymers that create an acidic microenvironment for hCASMCs stimulate an inflammatory reaction, thereby increasing proliferation and de-differentiating the cells from a mature/contractile state to a synthetic/proliferative phenotype, indicative of increased probability of restenosis and neointiaml hyperplasia.

Methods: Human coronary artery smooth muscle cells were purchased from ATCC and cultured in Lonza M199 supplemented with 50ml FBS, 5ml pen/strep, and 5ml L-glutamine. Cells were plated at 10k cells/cm^2 in growth medium and allowed to attach for 24 hours, upon which medium was switched to growth arrest the cells for 48 hours (M199 with 5ml pen/strep, 5 ml L-glutamine, 5ml ascorbic acid, 3.125ug sodium selenite, and 2.5mg/ml apo-transferrin). Cells were used up to passage 10 for experiments. Following growth arrest, cells were stimulated for 24 hours with polymer-conditioned medium and then harvested for PCR or proliferation assays. Several polymers were used: Methyl ester-capped 50:50 poly(lactide-co-glycolide) (PLAGA), 71 kDa (slow-degrading); acid-capped 50:50 PLAGA, 13.5 kDa (fast-degrading); polycaprolactone (PCL); and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). Additionally, glycolic acid and lactic acid, degradation products of PLAGA, were used as controls (10mM) to determine if results were caused by changes in pH or the specific acidic byproducts themselves. The following genes were investigated: smooth muscle alpha-actin (SMA), indicative of proliferative phenotype, and myocardin (MyoC), suggestive of a contractile phenotype. 18sRNA was used as a housekeeping gene. Proliferation was quantified using the Invitrogen Quant-iT PicoGreen dsDNA assay.

Results: PLAGA degrades into acidic byproducts, namely lactic acid and glycolic acid. Incubation of fast-degrading and slow-degrading PLAGA in PBS shows a sharp drop in solution pH in the first 24 hours. Lowering medium pH from 7.94 to 6.43 does not significantly change hCASMC proliferation (Figure 1). This suggests that pH change alone is not causing the phenotypic switching of SMCs from a contractile to proliferative phenotype.

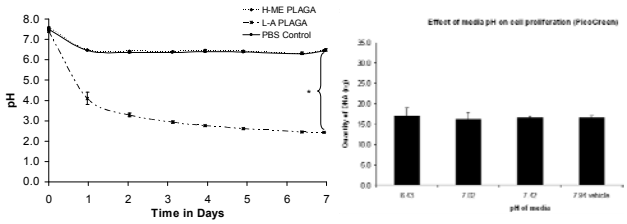

Figure 1. (left): Incubation with acid-capped PLAGA significantly lowers pH of PBS after 7 days. (right): Drop in pH of M199 medium (addition of HCl) does not affect cell proliferation after 24 hours of stimulation.

Incubation of hCASMCs with both PLAGAs, PCL, PHBV, or 10mM glycolic or lactic acid did not significantly alter proliferation compared to growth medium alone; all samples were significantly greater in terms of DNA quantity compared to cells in growth arrest medium (positive control) (data not shown). RT-PCR results show an average increase in SMA expression and average decrease in MyoC for PLAGA-stimulated cells (acidic degradation) (Figure 2).

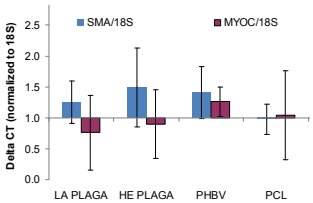

Figure 2. Changes in SMA and MyoC expression after 24-hr stimulation with various polymers (n=4).

Conclusions: Results show that hCASMC proliferation is not altered by a drop in pH or incubation with polymer degradation products for 24 hours. There was large variability in biological replicates for PCR data, which was most likely contributed to the size of degradation products in the media. Results are being repeated with cells cultured directly on the polymers themselves. Results have important implications in stent design, as we try to create polymer-coated stents that decrease the probability of thrombogenic events.

References:

1. McClean, R., Reviews in Cardiovascular Medicine 3, S16–S22.
2. van der Giessan WJ, et al. Circulation. 1996;94:1690-1697.