Calcification of Human Aortic Smooth Muscle Cell Cultures on Gas Plasma Treated Polylactic Acid Films

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Statement of Purpose: Calcification by Vascular Smooth Muscle Cells (VSMCs) is closely linked to vascular calcification, and vascular calcium accumulation is a hallmark in the senior stage of atherosclerosis. The purpose of this study is to evaluate the capability of calcium induction in human VSMC cultures as a function of Calcium/Phosphate (Ca/Pi) and ß-glycerophosphate (ß-GP) treatments. The biomaterial utilized here is poly-lactic acid (PLA). The long term goal of this project is to reproduce vascular calcification in an animal artery.

Methods: Commercially available Human Aortic Smooth Muscle Cells (HASMCs) were cultured on control bare wells, solution cast DL-PLA (IV=0.55-0.75dl/g; Mw= 75K-117K Daltons) films, and gas plasma (GP) treated PLA films. Films were subjected to GP treatment in a glow discharge system in a 100% oxygen environment at a pressure of 0.2 torr for 3 minutes with a 100W power setting. Initial cell seeding density of each specimen is $4x10^4$. Eighty percent confluent HASMCs were subjected to ß-GP and Ca/Pi calcification treatments. (1) For ß-GP treatment, cells were cultured with 10mM ß-GP, 50µg/ml L-ascorbic acid, 10nM dexamethasone, and 7% FBS in DMEM for 20 days. (2) For Ca/Pi treatment, $CaCl_2$ and NaH_2PO_4 were used for calcium and phosphate supplements. HASMCs were cultured with Ca/Pi at concentrations of 3.6mM/2.9mM for 10 days in DMEM containing 20% FBS. At above mentioned study end-points, calcification was assayed using Alizarin Red Staining. The BMP-2 produced by HASMCs on bare wells, PLA films, and GP films was measured using an ELISA kit at 24 hours, 7, 14, 21, and 28 days. The culture condition was DMEM containing 10% FBS. Number of specimens used was 6 for each group at every pre-designed study endpoint.

Results: HASMCs had little mineralization on three culture substrates (bare wells, PLA films, and GP films) after 20 days exposure to ß-GP (Fig. 1).

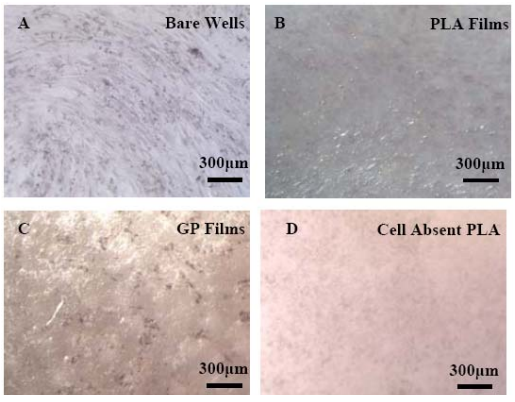

Fig. 1. HASMCs with ß-GP treatment for 20 days and stained with Alizarin Red. Calcium stains as red.

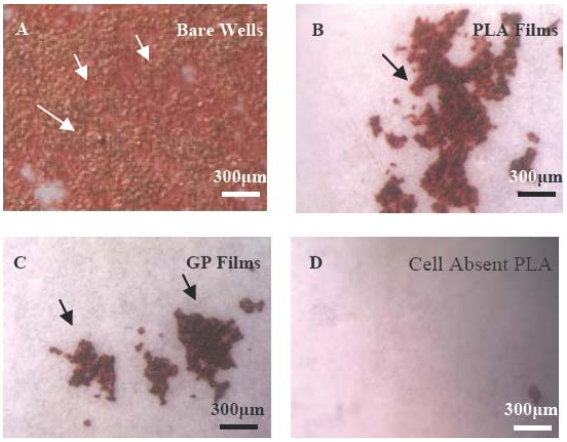

Fig. 2. HASMCs with Ca/Pi treatment for 10 days and stained with Alizarin Red. Calcium stains as red. Arrows point to calcium.

After 10 days of treatment with Ca/Pi, Calcium deposits were present on bare wells, PLA films and GP films (Fig. 2). Across three study groups, HASMCs on bare wells deposited the most calcium.

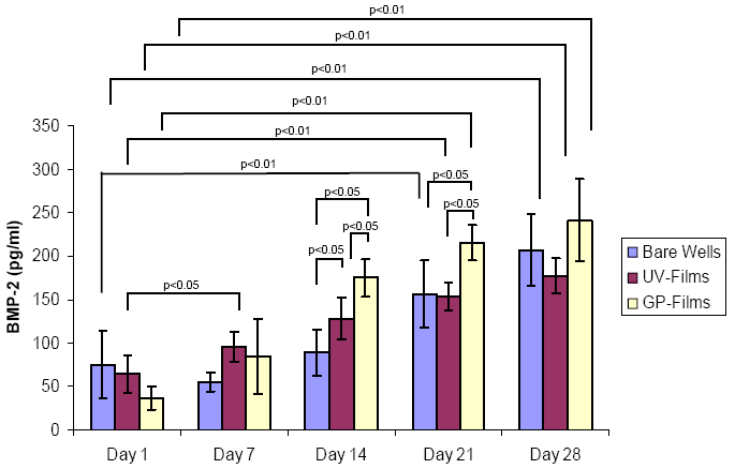

Fig. 3. BMP-2 Production of HASMCs

BMP-2 is a growth factor produced by HASMCs. BMP-2 production on GP films started to accelerate and exceed other substrate groups at Day 14 and Day 21 ($p<0.05$) (Fig. 3). The amount measured at both Day 21 and Day 28 was significantly enhanced for each substrate groups compared to its respective level at Day 1 ($p<0.01$).

Conclusions: HASMC cultures are capable of depositing calcium through Ca/Pi stimulation, but not through ß-GP treatment. Gas plasma treatment enhanced cell secreted BMP-2 on PLA films at Day 14 and Day 21. Gas plasma treatment on PLA had no effect on HASMC culture calcification.

Reference: Zhu B *et al*. Transactions of the 33rd SFB 2008, 31, Abstract # 192