Human Vascular Smooth Muscle Cell Calcification on Poly-lactic Acid 2D Films

Beili Zhu[1], Steven R. Bailey[2], and C. Mauli Agrawal[1].
[1]The University of Texas at San Antonio, [2]The University of Texas Health Science Center at San Antonio.

Statement of Purpose: Vascular Smooth Muscle Cell (VSMC) calcification is a phenomenon closely linked to atherosclerotic calcification, and atherosclerosis is the leading cause of death in western countries. Bone Morphogenetic Protein 2 (BMP-2), known as a potent inducer of osteoblastic calcification as well as a growth factor secreted by VSMCs themselves, hasn't been applied in the investigation of human VSMC calcification, yet. The purpose of this study is to evaluate the calcification ability of human VSMCs as a function of rhBMP-2, and ß-glycerophosphate (ß-GP) treatments. The cells were cultured on poly-lactic acid (PLA) 2D films. PLA has been widely used in tissue engineering to support and facilitate cell growth.

Methods: Commercially available Human Aortic Smooth Muscle Cells (HASMCs) and Human Embroynic Palatal Mesenchymal Cells (HEPMCs) were cultured on control bare wells and solution cast DL-PLA (IV=0.55-0.75dl/g; Mw= 75K-117K Daltons) films. Initial cell seeding density of each specimen is $4x10^4$. After 80% confluence, they were subjected to calcification treatments. For rhBMP-2 treatment, HASMCs were cultured with 150ng/ml rhBMP-2 in 10% FBS supplemented DMEM for 28 days. For ß-GP treatment, both HASMCs and HEPMCs were cultured with 10mM ß-GP, 50µg/ml L-ascorbic acid, 10nM dexamethasone, and 7% FBS in DMEM for 20 days. At above mentioned study end-points, calcification was assayed using Von Kossa Staining. The BMP-2 produced by HASMCs at 24 hours, 7, 14, 21, and 28 days was measured using an ELISA kit when cells were cultured in DMEM containing 10% FBS. pH changes were measured at 1, 7, 14, 21, and 28 days on both bare wells and PLA films in ddH_2O in the absence of cells. Samples were placed in a humidified atmosphere with 5% CO_2 at 37°C. For all experiments, N=6 were used for each study group at every pre-designed study endpoint.

Results: The typical high dosages of BMP-2 treatment (150ng/ml) used for osteoblastic calcification did not show any mineralization on human VSMCs (Fig.1).

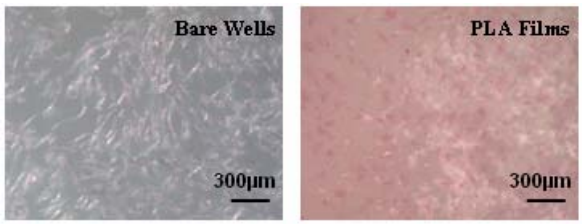

Fig.1. HASMCs with 150ng/ml rhBMP-2 treatment for 28 days and stained with Von Kossa. Calcium stains as black.

For the same ß-GP dosage treatment, PLA films had less calcium deposition than bare wells, and HASMCs had poorer mineralization than HEPMCs (Fig.2).

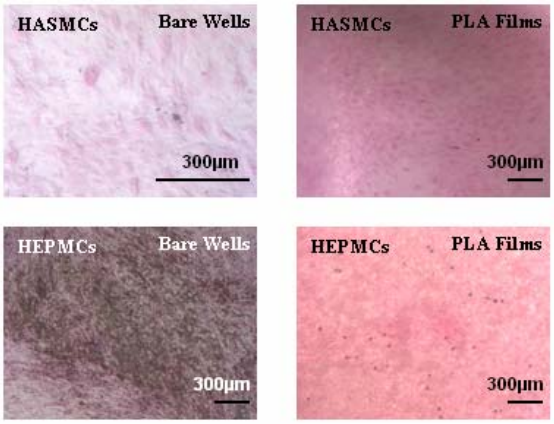

Fig. 2. HASMCs and HEPMCs with ß-GP treatment for 20 days and stained with Von Kossa. Calcium stains as black.

PLA films did not significantly alter BMP-2 production of HASMCs (Fig.3). The local low pH environment caused by PLA acidic by-products may inhibit calcium deposition on the surface (Fig.4).

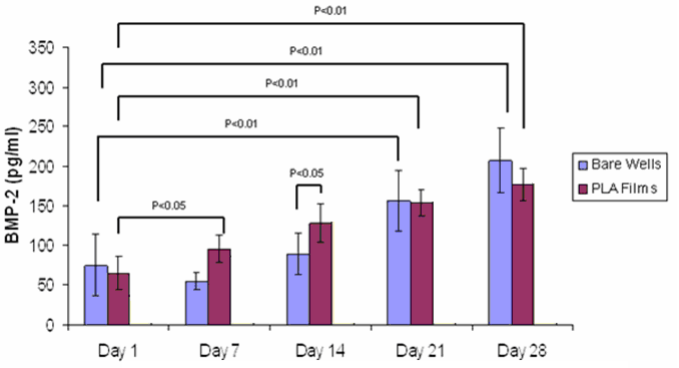

Fig.3. BMP-2 Production of HASMCs

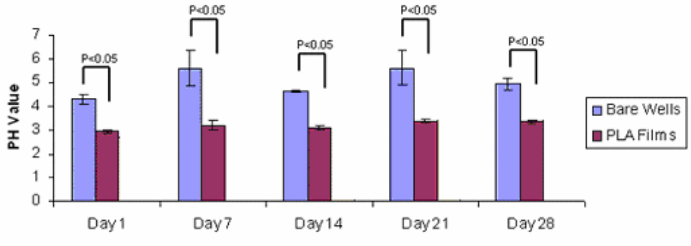

Fig.4. pH Measurement of Culture Substrates in ddH_2O

Conclusions: Human VSMCs are capable of calcification, but to a lower degree compared to bone-related cell types. PLA appears to deter cells from depositing calcium.