Probing the Osteoinductivity of Siloxane Containing Shape Memory Polymers

Robert A. Culibrk[1], Ahmad S. Arabiyat[1], Daniel J. Yeisley[1], Felipe O. Beltran[2], Melissa A. Grunlan[2], Mariah S. Hahn[1]
[1]*Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180*. [2]*Department of Materials Science and Engineering, Texas A&M University, College Station, Texas 77843-2120.*

Statement of Purpose: Autografts – the current "gold standard" treatment for irregularly shaped bone defects – are inherently limited in their ability to promote comprehensive and long-term bone tissue regeneration [1]. The complicated geometries of defects, such as those obtained from craniomaxillofacial injury, preclude adequate graft-tissue interfacing, resulting in undesired immune responses, fibrosis, and premature degradation of the treatment construct [2]. Toward addressing this issue, we previously demonstrated the capacity of polydopamine-coated PCLDA shape memory polymers to intrinsically stimulate osteogenic differentiation of human bone marrow derived mesenchymal stem cells (hBMSCs) in the absence of standard osteogenic supplements [3]. Herein, we endeavor to maximize the osteogenic capacity of PCLDA foams by incorporating siloxane groups via PDMA-DMA and PMHS-DMA, affording enhanced mineralization and scaffold degradation rates. These novel copolymer architectures may provide a suitable platform for superior bone tissue regenerative therapies.

Methods: Scaffolds of varying molar siloxane content were fabricated as previously described [4]. For *in vitro* studies, pooled hBMSCs from three donors were seeded onto scaffolds at a density of 3.5 x 10^6 cells/mL in experimental medium (DMEM-HG + 10% heat-inactivated, MSC-qualified FBS + 1x GlutaMAX + 1% P/S). hBMSCs seeded on PCLDA hydrogels in experimental medium in the presence or absence of osteogenic supplements served as growth medium (GM) and osteogenic controls (OM), respectively. Full experimental medium changes were performed every 2 days. Following 14 days of culture, whole cell lysate protein expression of osteogenic (RUNX2, BMP-4, COL1A1, OPN, SPARC, VEGF, RANKL) and off-target differentiation (COL2A1, CEBP-a, AFABP, SOX9) markers was assessed via MAGPIX multiplex immunoassay and western blot. Alizarin Red S staining was utilized to assess calcium deposition as described previously [5]. Experimental group means were compared using one-way ANOVAs and post-hoc Dunnett's tests ($\alpha = 0.05$).

Results: hBMSCs cultured in PCL-DA/PMHS-DMA 60:40 scaffolds expressed significantly lower COL1A1 (~2.9-fold; $p = 0.0002$) and SPARC (~2-fold; $p = 0.0002$) relative to the PCL-DA GM control. Contrarily, hBMSCs cultured in PCL-DA/*linear*-PDMS-DMA 60:40L scaffolds express greater VEGF (~1.4-fold; $p = 0.047$) and OPN (~2-fold; $p = 0.023$) relative to the PCL-DA GM control. Both PCL-DA/*linear*-PDMS-DMA 75:25L and 60:40L groups expressed significantly greater BMP-4 (~1.5-fold; $p = 0.02$, ~1.45-fold; $p = 0.039$, respectively) than the PCL-DA GM control. All scaffold formulations evaluated demonstrated a trend toward greater RANKL expression relative to both the PCL-DA OM and GM controls. PDMS 60:40 groups demonstrated significantly decreased expression of adipogenic marker AFABP relative to PCLDA GM controls (~4.0-fold, $p = 0.009$). Quantification of Alizarin Red S staining revealed a trend toward decreasing calcium deposition with increasing PMHS content, with PMHS 60:40 groups demonstrating a significant reduction in staining relative to the PCL-DA GM controls (~3.3-fold, $p = 0.0023$).

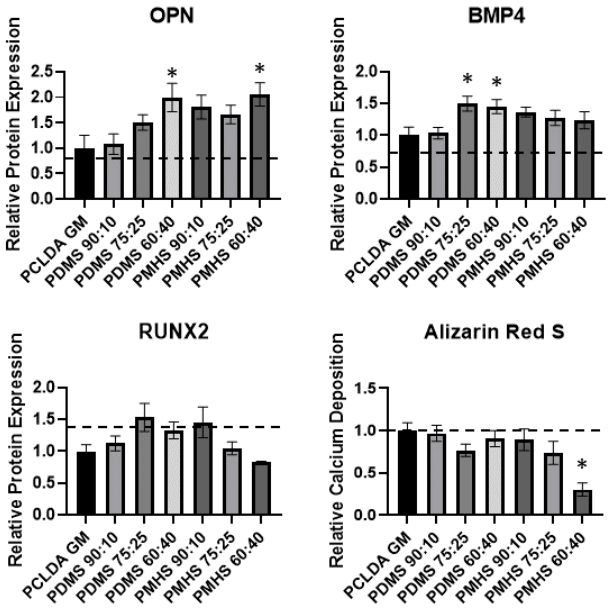

Figure 1. Relative protein expression of select osteogenic markers and Alizarin Red S staining following 14-day hBMSC culture. Dashed lines represent the PCLDA OM control. * denotes significant differences from the PCLDA GM control ($p < 0.05$).

Conclusions: Select scaffold formulations, particularly those containing greater siloxane content (e.g., PDMS 60:40) exhibited elevated expression of markers associated with hBMSC osteogenesis, including BMP-4 and OPN, and comparable calcium deposition to a known osteoinductive platform (PCLDA). Decreased Alizarin Red S staining in PMHS 60:40 groups, despite the significant elevation in OPN, a regulator and indicator of bone mineralization, may indicate a delayed mineralization response. Future studies should incorporate longer experimental timepoints (>21 days) to accommodate adequate calcium deposition and a larger osteogenic marker panel. Altogether, the results disclosed herein may indicate the intrinsic osteoinductive capacities of the evaluated siloxane-containing shape memory polymers.

References: [1] (Alsberg, E. Crit Rev Oral Biol Med. 2001;12:64-75.) [2] (Neovius, E. JPRAS. 2010;63:1615-1623.) [3] (Erndt-Marino, J. ACS Biomater Sci Eng. 2015;1:1220-1230.) [4] (Bailey, B. Act Biomater. 2012;8:4324-4333.) [5] (Frassica, M. Biomacromolecules. 2020;21:5189-5199.)