

PEG-PDMS-BMP-2 Hydrogels for Directed Osteogenic Differentiation of MSCs from Alternative Sources

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Statement of Purpose: Mesenchymal stem cell (MSC)-based tissue engineering is a promising alternative to autograft- or allograft-based treatments of craniomaxiofacial (CMF) bone defects. Critical to the success of MSC-based CMF tissue engineering approaches is a biomaterial scaffold which simultaneously promotes: (1) integration/bonding with surrounding bone tissue and (2) osteoinductivity. Many scaffolds have therefore been designed to include bioactive glass or hydroxyapatite (HAp). However, these additives generally render the scaffold susceptible to brittle failure. Also critical is an MSC source which is both readily available and which has strong osteogenic potential. Although adipose-derived MSCs (AMSCs) can be extracted at high levels with minimally invasive procedures, AMSCs are generally considered to have significantly reduced osteogenic potential relative to bone marrow-derived MSCs (BMSCs). Unfortunately, BMSC collection requires painful and invasive cell harvesting. Synovial fluid-derived MSCs (Sy-MSCs) are more accessible than BMSCs². However, the strength of the osteogenic potential of Sy-MSCs has not been evaluated.

To address these cell source and scaffold challenges, we propose scaffolds based on the incorporation of inorganic, elastomeric methacrylated star poly(dimethylsiloxane) (PDMS_{star}-MA) into hydrophilic, elastomeric poly(ethylene glycol) diacrylate (PEG-DA) scaffolds. In contrast to pure PEG hydrogels, these PEG-PDMS scaffolds have been demonstrated to intrinsically support HAp deposition when in simulated body fluid¹. Thus, these hybrid scaffolds allow for the surface mineralization necessary for osseointegration while maintaining a highly elastomeric, non-brittle scaffold. While PEG-PDMS scaffolds have also been demonstrated to support MSC osteogenesis even in the absence of osteogenic supplements¹, we propose to enhance this intrinsic osteoinductivity by tethering bone morphogenetic protein-2 (BMP-2) to the PEG-PDMS gels. The conjugation of BMP-2 to the scaffold maintains its localization and prevents its removal by cell internalization³. It is thus anticipated that lower concentrations of the tethered growth factor will be needed than would be necessary for non-covalently bound BMP-2. This is significant as the high BMP-2 levels frequently employed in bone scaffolds have been associated with ectopic bone formation. Finally, we propose to evaluate the osteogenic potential of Sy-MSCs relative to MSCs derived from the iliac crest bone marrow (IC-MSCs) toward identifying an alternate MSC source.

Methods: *Synthesis of Macromers.* PEG-DA (3.4 kDa) and PDMS_{star}-MA (2 kDa) were prepared as previously described¹. Acrylated BMP-2 and acrylated cell adhesion peptide RGDS were synthesized per standard NHS-

chemistry. *Fabrication of constructs:* Three distinct aqueous hydrogel precursor solutions (each containing 1mM acrylated RGDS and photoinitiator) were prepared: (i) 10 wt% PEGDA, (ii) 10 wt% PEGDA + 2 wt% PDMS, (iii) and 10 wt% PEGDA + 2 wt% PDMS + 100 ng/ml acrylated BMP-2. Canine IC-MSCs and canine Sy-MSCs were encapsulated within these 3 hydrogel formulations by 6 min exposure to 365 nm UV light. *Construct Culture and Cell Characterization:* Following 21 days of culture in media lacking osteogenic supplements (DMEM plus 10% FBS), the differentiation status of MSCs encapsulated within each gel formulation was analyzed relative to day 0 using semi-quantitative immunostaining and von Kossa staining.

Results and Discussion: For both IC-MSCs and Sy-MSCs inclusion of PDMS in the PEG scaffold structure appeared to significantly increase the day 21 levels of osteopontin (OPN) [Figure 1A]. In contrast, PDMS inclusion resulted in enhanced mineralization at day 21 only in scaffolds containing Sy-MSCs [Figure 1B]. Since day 21 calcium deposition represents the combined results of intrinsic scaffold mineralization and cell-mediated mineralization, the PDMS appeared to have an osteoinductive effect on the Sy-MSCs beyond its intrinsic ability to support HAp formation. As anticipated, inclusion of BMP-2 in the PEG-PDMS hydrogels stimulated further increases in calcium deposition by both IC-MSCs and Sy-MSCs. Similar results were observed for OPN following inclusion of BMP-2.

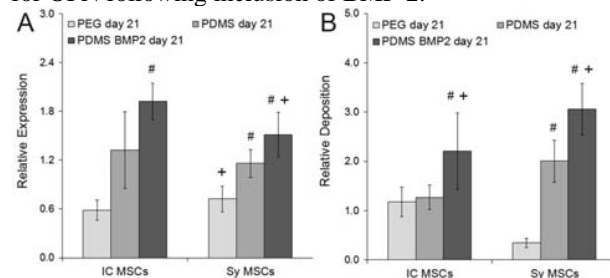


Figure 1: Relative levels of (A) osteopontin (OPN) and (B) calcium deposition. '#' significantly different from PEG day 21, '+' significantly different from PDMS day 21, $p < 0.05$

Conclusions: The results from the present study indicate that PEG-PDMS-BMP2 scaffolds showed significant potential for directed osteogenic differentiation of MSCs. Furthermore, the current data indicates that Sy-MSCs have similar osteogenic potential as bone marrow-derived IC-MSCs and therefore may be a promising cell source for bone tissue engineering applications.

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