Osteogenic Differentiation of Encapsulated Mesenchymal Stem Cells in Dexamethasone-Functionalized Semi-IPN Hydrogels

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Statement of Purpose: Point-of-care stem cell therapy has been widely applied to multiple types of orthopaedic tissue regeneration. The development of scaffolds incorporating osteoinductive stimuli to direct cell differentiation *in-situ* is critical for such applications. Bone morphogenetic proteins (BMPs) are the most well-established and potent growth factors in engineered bone regeneration. However, the clinical use of BMPs is associated with significant side effects and exorbitant costs. Dexamethasone (DX) is a synthetic glucocorticoid that stimulates osteogenic differentiation of MSCs by initiating and regulating the expression of osteoblast-specific transcription factors such as runt-related transcription factor 2 (RUNX2), and osterix (SP7) [1,2]. While a number of systems have been developed that provide chemically-controlled release of DX, there are few release systems designed to be cell-responsive. Previously, we have described semi-IPN networks composed of hydrolytically degradable PEG-diacrylates and hyaluronic acid (HA) that support hyaluronidase-dependent cellular remodeling [3,4]. The objective of this study was to synthesize DX-conjugated HA for incorporation into these semi-IPNs and evaluate its ability to support MSC differentiation.

Methods: Dexamethasone (DX) was reacted with succinic anhydride to obtain DX-monosuccinate (DXM) and then the carboxylic acid group of DXM was activated with 1,1'-Carbonyldiimidazole (CDI) in anhydrous DMSO. The mixture was then added to a solution of HA (1500 kDa, 50 mg) dissolved in 20 ml anhydrous DMSO and the reaction was stirred for 48 hours in the dark. Human mesenchymal stem cells (hMSCs, Lonza, 12.5 × 10^6 cells/ml) were encapsulated in PEG-based hydrogel (6% PEG-bis-(2-chloropropanoate) [PEG-bis-AP] with 0.36% DX-conjugated hyaluronate (HA-DXM), 0.1% I2959 photoinitiator, and 1 mM acrylate-PEG-GRGDS. Photopolymerized semi-IPNs with HA-DXM (n=4) were cultured in 12 well plates in low glucose DMEM supplemented with 10% FBS, 1% pen/strep, 50 uM ascorbic acid-2-phosphate, and 10 mM beta-glycerophosphate. Semi-IPNs containing native HA cultured in the same media were used as negative controls, while those cultured in medium with conventional soluble DX were used as positive controls. The osteogenic effect of HA-DXM was analyzed by RT-PCR and alkaline phosphatase activity, and matrix mineralization was evaluated by atomic absorption spectroscopy and histology (von Kossa and Alizarin red S staining).

Results: Hyaluronate-Dexamethasone monosuccinate (HA-DXM) was successfully synthesized and characterized by thin layer chromatography (TLC) and ATR-FTIR. HA-DXM was stable during 7 days incubation in PBS, while free DX was released in the presence of hyaluronidase and esterase enzymes (data not shown). Calcium deposition was detected in histological sections from HA-DXM containing semi-IPNs by von Kossa and alizarin red S staining and consistent with the positive control receiving soluble DX (Fig. 1). At 14 days of incubation, mRNA expression of osteogenic markers (RUNX2, Osterix/SP7, and OCN) was significantly upregulated in semi-IPNs containing HA-DXM compared to the negative control and similar to the positive control (Fig. 2). Elevated calcium contents and ALP activity in semi-IPNs containing HA-DXM demonstrated that released dexamethasone from HA-DXM macromer enhanced the differentiation of hMSCs to the osteoblastic lineage (data not shown).

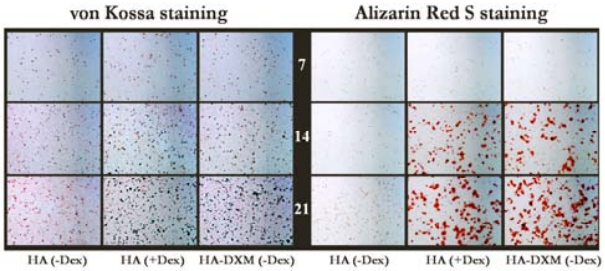

Figure 1. Histological analysis of the hydrogel constructs with von Kossa and Alizarin Red S staining over 21 days.

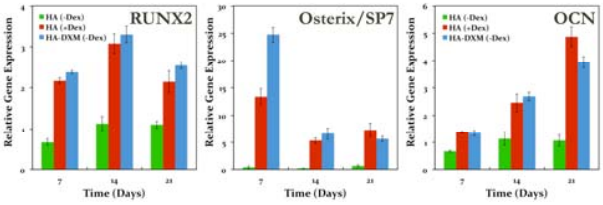

Figure 2. Gene expression profiles of osteoblast-specific markers over 21 days: RUNX2, Osterix/SP7, and OCN.

Conclusions: Semi-IPNs containing HA-DXM supported osteogenic differentiation of hMSCs similar to positive controls provided soluble DX supplementation and significantly greater than negative controls cultured in the absence of DX. These results suggest covalent immobilization of DX within semi-IPNs is an efficient system for local delivery of osteogenic molecules empowering point of care applications.

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