Sequestering proteoglycans from serum enhances mesenchymal stem cell proliferation and osteogenic differentiation

Gregory A. Hudalla, William L. Murphy
Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI

Statement of purpose: Cell-secreted proteoglycans (PGs) integrate into the extracellular matrix (ECM) via non-covalent interactions with core ECM constituents, such as proteins. In turn, PGs influence cell behavior by non-covalently localizing growth factor activity. We proposed that biomaterials presenting the peptide Lys-Arg-Thr-Gly-Gln-Tyr-Lys-Leu (HEPpep), which is derived from the heparin-binding domain of fibroblast growth factor-2, would sequester PGs. In turn, sequestered PGs would amplify the activity of heparin-binding growth factors, analogous to PGs within native ECMs. Our approach relied on self-assembled monolayer (SAM) substrates that present peptides in an otherwise bio-inert background. HEPpep SAMs sequestered serum-borne PGs via specific non-covalent interactions. Human mesenchymal stem cell (hMSC) proliferation on HEPpep/RGDSP SAMs was enhanced in an FGF-dependent manner during culture in serum-supplemented medium. hMSC osteogenic differentiation on the same substrates was enhanced in a BMP-dependent manner during culture in osteogenic induction medium. Together, these results demonstrate that sequestering PGs from serum amplifies the bioactivity of cell-secreted or serum-borne growth factors, as no recombinant growth factor supplements were required to enhance hMSC proliferation or osteogenic differentiation.

Methods: SAMs were formed by incubating clean gold slides in an ethanolic solution of HS-$(CH_3)_{11}$-triethylene glycol and carboxylate-terminated HS-$(CH_3)_{11}$-hexaethylene glycol overnight. Peptides were coupled to SAMs by activating carboxylic acid groups with NHS/EDC, followed by incubating the SAMs in a 1x PBS solution (pH 7.4) containing 1 mM peptide. hMSCs were allowed to attach to SAMs overnight in serum-supplemented medium. After overnight attachment, substrates were washed to remove loosely bound cells and maintained in medium supplemented with 10% serum or 10% serum plus 50 μg/mL 2-phosphate ascorbic acid, 10 mM β-glycerophosphate, and 100 nM dexamethasone (osteogenic induction medium, OM) for 3 or 7 days, respectively. Cell proliferation was assessed by manually counting cell number every 24 hours. Alkaline phosphatase (AP) activity was measured using the SensoLyte FDP alkaline phosphatase assay kit and was normalized by measuring total DNA in the sample. Quantitative polymerase chain reaction was used to quantify osteopontin (OPN) mRNA.

Results: hMSCs cultured on HEPpep/RGDSP SAMs proliferated faster than cells cultured on RGDSP SAMs in serum-supplemented medium. The rate of hMSC proliferation on HEPpep/RGDSP SAMs in medium supplemented with 10% serum was similar to the rate of hMSC proliferation on RGDSP SAMs in medium supplemented with 10% serum and 1 ng/mL recombinant FGF-2 (Fig. 1A). hMSCs cultured on HEPpep/RGDSP SAMs in medium supplemented with 10% serum and the FGF receptor inhibitor PD-173074 proliferated at the same rate as cells cultured on control SAMs (Fig. 1B).

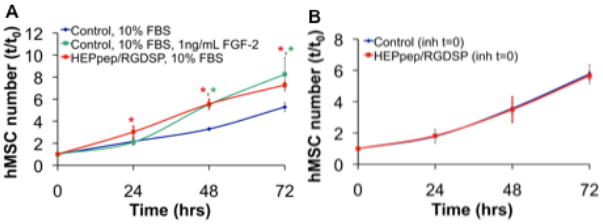

Figure 1: A) hMSC proliferation on HEPpep/RGDSP SAMs in serum-supplemented medium or RGDSP SAMs in medium supplemented with 10% serum +/- 1 ng/mL recombinant FGF-2. B) hMSCs cultured on HEPpep/RGDSP SAMs in medium supplemented with 10% serum and 200 nM PD-173074, an FGF receptor inhibitor. * represents significant difference compared to control ($P < 0.05$).

HMSC AP activity after culture on HEPpep/RGDSP SAMs in OM was significantly greater than that of cells cultured on RGDSP SAMs or polystyrene (PS) in OM (Fig. 2A). When the BMP receptor inhibitor LDN-193189 was added to osteogenic induction medium, AP activity in cells cultured on HEPpep/RGDSP SAMs was similar to that of cells cultured on PS. hMSC OPN mRNA expression was also significantly up-regulated after culture on HEPpep/RGDSP SAMs in OM when compared to cells cultured on PS (Fig. 2B). However, unlike AP activity, OPN mRNA expression was not inhibited by LDN-193189, suggesting that a mechanism other than BMP signaling is involved.

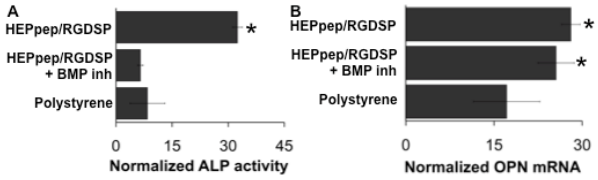

Figure 2: A) Alkaline phosphatase activity and B) OPN mRNA expression by cells cultured on HEPpep/RGDSP SAMs or PS in OM. * represents significant difference compared to polystyrene condition ($P < 0.05$)

Conclusion: Culture substrates that non-covalently sequester PGs enhance hMSC proliferation and osteogenic differentiation by amplifying growth factor activity. As no recombinant growth factor supplements were required to influence stem cell behavior, these data suggest that sequestered PGs amplify the activity of serum-borne or cell-secreted growth factors by concentrating them at the cell-material interface.