Extracellular matrix proteins mediate osteogenic differentiation of human mesenchymal stem cells on phosphate functionalized gels through integrin mediated focal adhesion kinase signaling

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Introduction: Differentiation of human mesenchymal stem cells (hMSCs) has been shown to be dependent on various biochemical and biophysical cues. Benoit *et al* (1) demonstrated that small functional groups when incorporated into poly(ethylene glycol) (PEG) gels can induce differentiation of hMSCs. The phosphate (PO_4) functionality has been shown to induce osteogenic differentiation in hMSCs in the absence of osteogenic supplements (OS). The aim of the present study was to investigate the possible mechanisms involved in how the PO_4 functionality alone can induce osteogenic differentiation in hMSCs. We found that incorporation of PO_4 functional groups induces extracellular matrix (ECM) protein adsorption from serum and mediates cell attachment via integrins. Our studies also show that focal adhesion kinase (FAK) is involved during osteogenic differentiation of hMSCs on PO_4-PEG gels.

Methods: Ethylene glycol methacrylate phosphate (EGMP) was added at a concentration of 50mM to a monomer solution, containing poly(ethylene glycol) dimethacrylate (M_n~550 Da) and 0.5wt% photo-initiator (I651, Ciba), rendering PO_4 functionalized PEG gels. For all cell culture studies, 10mm diameter disks were cut from gels formed by polymerizing monomer solution between two glass slides and sterilized before use. For all experiments, cells were cultured on PO_4-PEG gels in normal growth media.

Integrin blocking studies: PO_4-PEG gels were incubated in serum media for overnight to pre-coat the gels with serum proteins. Cells were incubated with either β1, β3, β1+β3 or isotype control antibodies (Abcam) for 30mins and seeded onto serum precoated PO_4-PEG gels. Cells were allowed to attach overnight (12hrs), washed and stained with calceinAM (Invitrogen). Attached cells were imaged and counted manually using ImageJ software.

FAK inhibition studies: The FAK inhibitor, PD573228 (Tocris Biosciences), was added to the media at a concentration of 3μM to inhibit phosphorylation of FAK. DMSO was used as a control for FAK inhibition studies. ALP activity of cells was measured by adding photometric substrate to cell lysate and measuring absorbance at 401nm.

Results: β1 and β3 integrins mediate attachment of hMSCs to PO_4-PEG gels: Results (not shown) indicate that ECM proteins from serum adsorb onto the PO_4-PEG gels at low concentrations (~500 ng/cm^2) and mediate hMSC attachment. Blocking β1, β3 integrins with antibodies before cell seeding reduced attachment on the PO_4-PEG gels compared to those cells blocked with isotype control (Figure.1A). This results shows that hMSCs interact with the adsorbed serum protein layer via β1 and β3 integrins. Immunostaining for vinculin revealed the presence of well-developed focal adhesions (Figure 1B). Taken together these findings point us to the hypothesis that osteogenic differentiation of hMSCs on PO_4-PEG gels is mediated by integrin signaling.

To test the above hypothesis, we monitored the osteogenic differentiation of hMSCs by measuring ALP activity when cultured in the presence or absence of chemical inhibitor to FAK. Figure.2 shows the effect of FAK inhibition on ALP activity of cells cultured on PO_4-PEG for 7 or 14 days normalized to day 1. ALP activity of cells cultured on tissue culture polystyrene (TCPS) in OS media in the presence and absence of FAK inhibitor is also included for comparison. ALP activity of cells on PO_4-PEG gels increased by day 7 and decreased slightly by day14 similar to cells cultured in OS media. Inhibition of FAK phosphorylation significantly reduced the ALP activity of cells on PO_4-PEG gels and on TCPS in OS media suggesting the involvement of FAK during osteogenic differentiation of hMSCs on PO_4-PEG gels.

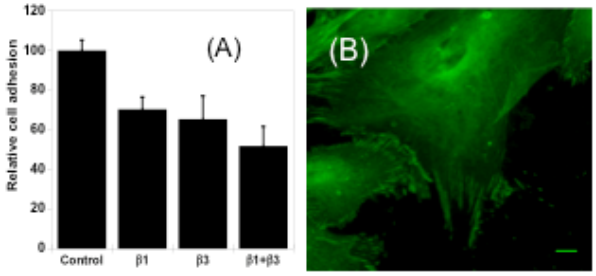

Figure.1. hMSC attachment on PO_4-PEG gels is mediated by integrins. (A) Cell attachment is decreased when integrins present on the cell surface are blocked with antibodies before seeding. (B) Immunostaining for vinculin (green) shows well-developed focal adhesions (scale bar=100μm).

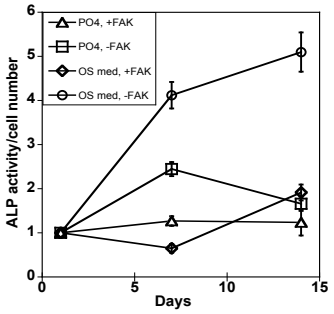

Figure2. Osteogenic differentiation of hMSCs on PO_4-PEG (shown as PO4) and TCPS+OS media (shown as OS med) with and without FAK inhibitor (shown as +/- FAK)

Conclusions: Results show that ECM proteins present in serum mediate hMSC attachment to PO4-PEG gels via integrins. Results demonstrate a possible putative mechanism of how osteogenic differentiation of hMSCs is mediated on PO4-PEG gels.

References: 1. Benoit DSW. Nat.Mat. 2008;7:816-823.

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