Tethered Epidermal Growth Factor Increases Multipotent Stromal Cell Proliferation and Differentiation on Ligand-Coated Scaffolds

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Statement of Purpose: Bone marrow-derived multipotent stromal cells (MSCs) are adult stem cells capable of differentiating into osteoblasts and are useful in bone tissue engineering applications. Our ultimate goal is to develop a clinically used scaffold coated with ligands, such as epidermal growth factor (EGF) and extracellular matrix proteins, to promote attachment, survival, proliferation, and differentiation into osteoblasts to heal bone wounds and defects. We have previously shown that tethering EGF (tEGF) to a Poly(ethylene oxide) (PEO) – poly(methyl methacrylate) (PMMA) comb polymer improves cell spreading and survival through an ERK-mediated pathway[1]. Here, we hypothesized that tEGF on this polymer would improve MSC osteogenic differentiation and proliferation and that analysis of the associated signaling mechanisms would provide insight into key points driving this response.

Methods: Poly(ethylene oxide) (PEO) – poly(methyl methacrylate) (PMMA) comb polymers were synthesized which incorporated 22 wt.% or 33% wt % hydroxy poly(oxyethylene methacrylate) similarly to those previously described[2]. 33% wt polymer was activated with 4-nitrophenyl chloroformate (NPC) to react with primary amines on the EGF molecule. Thin films of this polymer blend were spin coated onto the cleaned and silanized glass coverslips and dried overnight under vacuum before murine EGF was covalently tethered. Mock activated substrates were produced following the same reaction steps but without EGF in the phosphate buffer coupling step. Primary human multipotent stromal cells (MSCs) were obtained from Tulane University and cultured according to their protocols[3]. Cells were seeded onto mock or tEGF surfaces and over defined time periods from 5 minutes to 7 days, cell lysates were collected for dynamic measurement of EGFR and ERK using the Bioplex phosphoprotein measurement setup and Western blots. Concurrent samples of cells were collected for alkaline phosphatase activity assays as determinants of osteogenic differentiation. Cell numbers after defined periods of time were assayed using Cyquant fluorescent dyes (Invitrogen, Carlsbad, CA).

Results: Tethered EGF significantly increased osteogenic differentiation of MSCs compared to the mock control according to the alkaline phosphatase activity assay after seven days of culture with osteogenic supplemented media ($n=3$, $p<.05$). Further, to show that this increase in differentiation was through an EGFR-mediated pathway, we incubated the MSCs with 1 μM AG1478, an EGFR kinase inhibitor which significantly reduced osteogenic differentiation in the MSCs cultured on tEGF (See Figure 1). We analyzed total cell number on the polymer surfaces after 4 hours to determine initial attachment, 24 hours, 4 days, and 7 days to measure the effect of tEGF on cell proliferation. Tethered EGF increased MSC initial attachment (4 and 24 hours) and proliferation (4 day and 7 day) compared to mock coupled surfaces in both the culture expansion medium that maintains multipotency and in the medium with osteogenic supplements that drives differentiation.

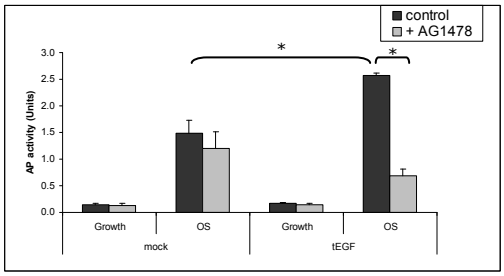

Figure 1. Tethered EGF increase osteogenic differentiation by an EGFR-mediated mechanism.

Dynamic measurement of EGFR expression over the time frame of the osteogenic differentiation revealed that freshly isolated MSCs initially have low levels of EGFR, but increase its expression in culture. MSCs cultured on tEGF surfaces, however, have lower levels of total EGFR but a higher amount of phosphorylated EGFR suggesting that there may be downregulation of the receptor as it is activated by the tethered ligand in the osteogenic differentiation process. Higher phosphorylation of ERK 1/2 correlate with the increased EGFR activation in the MSCs on the tEGF surfaces during the investigated time points during the MSC differentiation providing a downstream mechanism of propagation of this signal to elicit a cellular response.

Conclusions: Bone marrow derived MSCs showed improved proliferation and differentiation responses when cultured on tethered EGF polymer surfaces suggesting that this approach could be extremely useful in healing bone wound defects. Since it is known that EGFR and integrins have crosstalk, our future work is to not only investigate the intracellular signaling network driving these effects through ERK and probably Runx2, but to determine the additive benefits of adsorbing extracellular matrix proteins such as collagen, fibronectin, and laminin with tEGF to fully optimize MSC osteogenic differentiation on our polymer.

References: 1) Fan VH. Stem Cells. 2007; 5:1241-51. 2) Irvine DJ. Biomacromolecules. 2001; 2(1):85-94. 3) Colter DC. PNAS. 2001; 98(14): 7841-7845