Osteogenic Medium Affects Wnt and BMP Signaling Molecules in MSCs

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Statement of Purpose: Osteogenic medium (OM) is the gold standard to induce progenitor or stem cells towards the osteogenic linage for tissue engineering and regenerative medicine applications. This media formulation contains dexamethasone (a synthetic glucocorticoid), β-glycerophosphate (βGP), and ascorbic acid. Alkaline phosphatase activity and mineralization nodules are classical assays to determine osteoinduction by OM. However, it is not clear how progenitor cells are affected by OM and if the media additives affect signaling pathways involved in osteoblastic differentiation. We have reported a method for spontaneous osteoblastic differentiation using topographical cues on the biomaterial surface. These modifications employ micro/nanostructures that regulate important molecules associated with osteoblastic differentiation including specific integrins (ITG) and growth factors. Cells grown on these modified substrates produce an osteogenic environment rich in bone morphogenetic proteins (BMP2, BMP4, BMP7), WNTs, and angiogenic factors, without the addition of exogenous molecules or OM. The aim of this study was to elucidate the effect of OM on BMP and Wnt signaling molecules on stem cells grown on micro-nanostructured modified surfaces.

Methods: Top down surface modifications of titanium (Ti) were achieved by sand-blasting and acid etching. Resulting disks were characterized to determine surface roughness, chemical surface composition, and surface energy. Human mesenchymal stem cells (MSCs) were grown on smooth or micro-nanostructured Ti or control tissue culture polystyrene (TCPS) in mesenchymal basal growth media (GM) or OM for 7 days. After incubation, RNA isolation and real time-PCR were performed. In a parallel experiment, conditioned media were harvested to quantify soluble proteins and cell layers were harvested to quantify growth factors and extracellular matrix components. Finally, MSCs were grown on the same substrates in GM or GM+dexamethasone, GM+βGP, GM+ascorbic acid, and mRNA levels measured after 7 days of incubation.

Results: On TCPS and smooth Ti, OM increased alkaline phosphatase specific activity and osteocalcin and decreased osteoprotegerin in comparison to GM. On rough surfaces, OM increased alkaline phosphatase and decreased osteocalcin and osteoprotegerin in comparison to GM. OM increased levels of BMP2 (Fig. 1A) and BMP4 on TCPS and PT but had no effect on rough surfaces. Wnt3a decreased in GM on rough surfaces, while OM increased levels of Wnt3a (Fig. 1B) independently of surface topography. In GM, Wnt5a increased on rough surfaces, but OM dramatically reduced Wnt5a levels on rough surfaces (Fig. 1C). Surface roughness increased ITGA1, ITGA2, and ITGB1 mRNA and decreased ITGA5 mRNA in GM. Culture in OM enhanced expression of ITGA1 and ITGA2 on TCPS and PT, and increased ITGA5 in all surfaces tested. OM did not alter expression of ITGB1. Addition of dexamethasone to GM had the strongest effect on TCPS and PT, increasing BMP2, BMP4, DKK1, and ITGA5 expression. However, it decreased expression of NOG, GREM1, WNT5A, ITGA1, and ITGA2 on rough surfaces. Similar results were obtained when cells were grown in GM+βGP. Addition of ascorbic acid to GM increased expression of ITGA1, ITGA2, and ITGB1 independently of surface topography.

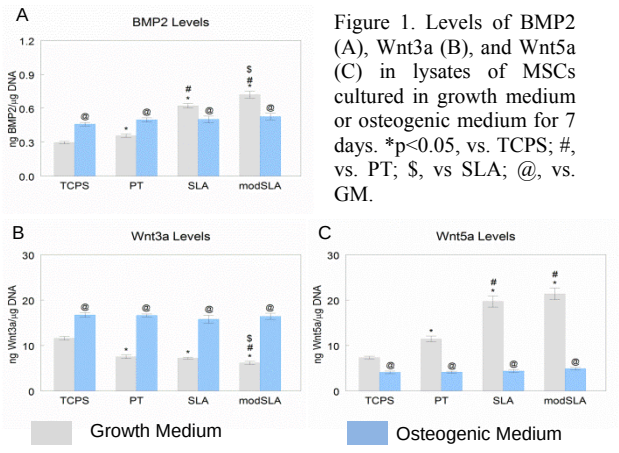

Figure 1. Levels of BMP2 (A), Wnt3a (B), and Wnt5a (C) in lysates of MSCs cultured in growth medium or osteogenic medium for 7 days. *p<0.05, vs. TCPS; #, vs. PT; $, vs SLA; @, vs. GM.

Conclusions: MSCs underwent osteoblastic differentiation in response to surface micro/nanostructure in GM without exogenous factors. OM had the strongest effect on smooth surfaces, but had no effect on nano-microstrutured surfaces. OM failed to enhance the osteogenic differentiation induced by complex structures surfaces and decreased later osteogenic markers. OM modified Wnt and BMP signaling in a roughness-dependent fashion. Taken together, the results suggest that osteogenic induction with synthetic factors can overshadow molecules that *in vivo* may be involved in osteoblastic differentiation and bone formation.