Inhibition of Noggin Increases Osteoblast Maturation on Microstructured Titanium Surfaces

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Statement of Purpose: Implants made with titanium (Ti) and Ti alloys that have microstructured features have been shown *in vivo* to increase bone-to-implant contact and decrease healing time. *In vitro* it has been demonstrated that physical properties such as micron- and submicron-scale topographies and chemical properties like high surface energy decrease osteoblast proliferation, increase osteoblast differentiation and local factor production, and increase cell response to exogenous regulatory factors. While the resulting increase in differentiation of osteoblasts grown on rough surfaces is known, the surface dependent factors that contribute to this phenotype have not been well studied. Bone morphogenetic proteins (BMPs) are associated with bone development, but whether surface topography regulates expression of BMPs is unclear. BMP2 and BMP4 induce osteoblast differentiation of mesenchymal stem cells and osteoprogenitor cells. BMP2 has been used clinically to enhance bone formation. Osteoblasts regulate BMP2/4 through production of the inhibitor Noggin, which blocks the binding of BMPs to the receptor. In this study, we examined whether the BMP pathway regulated osteoblast differentiation on microstructured Ti surfaces and whether autocrine or paracrine factors produced by cells on these surfaces can be modulated to enhance osteoblast differentiation.

Methods: MG63 cells were grown to confluence on tissue culture polystyrene (TCPS) or microstructured Ti surfaces: pretreated titanium (PT, Ra<0.4μm); sand-blasted and acid-etched (SLA, Ra=3.2μm); and hydrophilic SLA (modSLA). Changes in mRNA expression of BMP2, BMP4, BMP7, NOG, GREM1, CER1, FST, and RUNX2 were measured by real-time PCR. Protein levels of BMP2, BMP4, BMP7, and Noggin were quantified by ELISA. MG63 cells were grown on TCPS or Ti surfaces and treated with exogenous BMP2, Noggin protein, or antibody against Noggin (Noggin Ab). In another experiment, MG63 cells were transduced with lentivirus particles containing the NOG-shRNA template. MG63 cells and selected NOG-silenced (shNOG) cells were cultured on the test surfaces ± exogenous BMP2 protein. For both studies, total cell number and alkaline phosphatase specific activity (ALP) were measured as well as levels of osteocalcin (OCN), osteoprotegerin (OPG), TGF-β1, BMP2 and BMP4 protein levels in the conditioned media. For each experiment, there were n=6 independent cultures per variable. All experiments were repeated to validate the results. Data presented are from one representative experiment. Statistical significance was determined by ANOVA followed by Bonferroni's modification of Student's t-test.

Results: The rough SLA and modSLA surfaces increased expression of BMP2, BMP4, BMP7, NOG, GREM1, CER1, FST, but decreased RUNX2 expression. ALP and BMP2 (Fig 1A), BMP4, BMP7, and Noggin (Fig 1A) protein levels were greater on SLA and modSLA substrates than on PT. BMP2 treatment increased BMP2, ALP, OCN, OPG (Fig 1B) and TGF-β1 in cells on SLA and modSLA surfaces. However, treatment with Noggin Ab caused a more robust response than BMP2 treatment. Exogenous Noggin increased cell number and reduced all factors examined when compared to control. Noggin was reduced 75% in shNOG MG63 cells from wild type as measured by ELISA and Real-time PCR. shNOG cells produced higher levels of ALP, OCN, OPG, TGF-β1, BMP2, and BMP4 than wild type cells on all Ti substrates, with the greatest effect on the rougher surfaces, particularly modSLA.

Conclusions: These results show that osteoblasts produce BMPs (BMP2, BMP4, BMP7) and BMP inhibitors (Noggin, Gremlin, Cerberus) when cultured on Ti surfaces and that production is regulated by surface microstructure and energy. Inhibiting Noggin using Noggin Ab was more effective in osteoblast differentiation than treatment with BMP2 and this maturation was enhanced by surface roughness. These results suggest that osteoblasts on microstructured titanium surfaces produce enough BMP to create an adequate osteogenic environment and that additional BMP2 has a minimal effect on osteoblast maturation. However, the potency of cell-produced BMPs may be increased through regulation of endogenous levels of BMP inhibitors. Noggin silenced cells increased the osteogenic markers examined in a roughness dependent manner, confirming the Noggin Ab effect. Here, we demonstrate that BMP signaling pathway molecules regulate osteoblast maturation on microstructured titanium surfaces and that treatment with BMP2 to cells on these surfaces does not increase osteoblast maturation. Regulation of Noggin may be a more successful approach to enhance osteoblast differentiation and peri-implant bone formation.

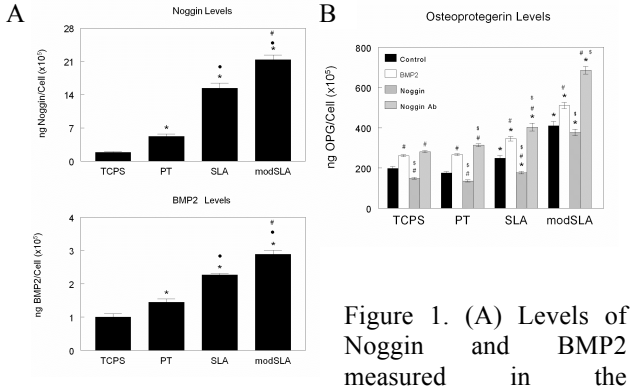

Figure 1. (A) Levels of Noggin and BMP2 measured in the conditioned media in MG63 cells grown on Ti surfaces. (B) Levels of OPG in MG63 cells cultured on Ti surfaces and treated with BMP2, Noggin, or Noggin Ab. P<0.05, * v. TCPS; # v. Control; $ v. BMP2.

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