The Ca^{2+}-Dependent but Not the Canonical Wnt Pathway Enhances Osteoblast Maturation on Titanium Microstructured Surfaces

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Statement of Purpose: Titanium implants have been used successfully in dental and orthopedic treatments. Osseointegration or biological fixation of the implant to the bone is a requisite for the success of the treatment and long-term implant stability. *In vitro* studies have shown that surface microtopography can regulate and influence osteoblast maturation and terminal differentiation, increasing alkaline phosphatase specific activity and osteocalcin (OCN), osteoprotegerin (OPG), TGF-β1, and PGE2 levels, as well as increasing angiogenic factors such as VEGF and FGF. Wnt family genes encode a large a number of proteins that regulate patterning, development, proliferation, and differentiation in a large variety of organs and tissues. Wnt proteins function in both an autocrine and paracrine fashion. They bind to specific G protein-coupled receptors of the Frizzled family (Fzd) and trigger one of several signaling pathways according to the ligand-receptor combination. The role of Wnts in bone regeneration and osseointegration are not understood. The aim of this study was to elucidate if Wnt pathways are involved in osteoblast differentiation on titanium microstructured surfaces and the possible contribution of the Wnt canonical and Ca^{2+}-dependent pathways in this process.

Methods: Human osteoblast-like MG63 cells (ATCC) were grown until confluence on TCPS or Ti surfaces (PT [Ra<0.2μm], SLA [Ra=3.4μm], modSLA [hydrophilic-SLA]). RNA extraction and real-time PCR were performed to analyze several Wnt activators, receptors and co-receptors, and inhibitors. In a second experiment, cells were seeded on TCPS or microstructured Ti substrates and treated with exogenous Wnt3a or Wnt5a or blocking antibodies against Wnt3a or Wnt5a. After confluence, cell number was determined and alkaline phosphatase (ALP) specific activity and OCN used as measures of osteoblast differentiation and levels of OPG, BMP2, BMP4, and VEGF were used as an indicator of soluble factor production. Data were calculated as means±SEM for n=6 independent cultures for each variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

Results: Expression of WNT3a was not detectable in cells grown on any of the Ti surfaces. WNT5a expression increased with increasing roughness. Wnt pathway receptors FZD5, FZD6, FZD7, ROR2, LRP5, LRP6, and KREM1 expression increased with increasing surface roughness. Inhibitors DKK1, DKK2, and WIF1 were increased on SLA and modSLA surfaces. FZD4 and β-catenin were downregulated on SLA and modSLA surfaces as compared to TCPS. Cell number decreased as surface roughness increased. This effect was enhanced with Wnt5a treatment, which had the lowest cell number in comparison to control treatment. Wnt3a did not affect cell number on any of the tested surfaces. ALP activity increased when cells were treated with Wnt5a and the effect was synergistic with surface roughness, with the highest activity on modSLA surfaces. Treatment with an antibody against Wnt5a decreased ALP (Fig 1A) activity on all surfaces in comparison to control. Wnt3a or antibody against Wnt3a had no effect on ALP activity. Wnt5a strongly increased levels of OCN (Fig 1B), OPG, VEGF, and latent and active TGF-β1 in comparison to untreated cells. This effect was more robust on SLA and modSLA surfaces. Wnt5a blocking antibody decreased levels of OCN, OPG, and active and latent TGF-β1 in all surfaces. Addition of Wnt3a or Wnt3a antibody did not affect any of the soluble factors measured in this study.

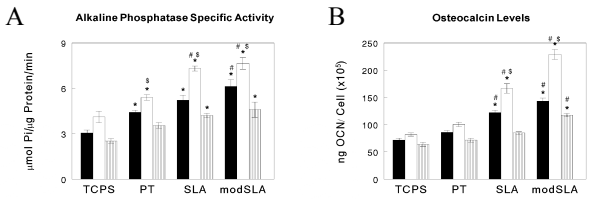

Figure 1: Black, control; White, Wnt5a treated; Hatched, Wnt5a Ab treated. The effect of surface on alkaline phosphatase specific activity (A) and the synergistic effect when treated with Wnt5a and levels of osteocalcin in the conditioned media (B). *p<0.05, Ti surface vs. TCPS; #p<0.05, SLA or modSLA vs. PT; $p<0.05, Treated vs. untreated.

Conclusions: The present study shows for the first time the contribution of the Ca^{2+}-dependent Wnt signaling pathway on osteoblast maturation on titanium microstructured surfaces. The results suggest Wnt5a plays a major role increasing osteoblast maturation and the effect can be enhanced with surface microstructure. Antibodies blocking this pathway showed that cells grown on titanium microstructured surfaces produce Wnt5a and when blocked, osteoblast maturation is compromised, indicating that it acts as an autocrine mediator. In contrast, Wnt3a, an activator of the canonical Wnt pathway, had no effect on osteoblast maturation, suggesting it has an important role for progenitor cell commitment but not in cells already in the osteoblastic linage.

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