# Enhanced Osteoblastic Differentiation and Maturation by Titanium and Titanium-Zirconium Nanostructured and Hydrophilic Surface Modifications

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**Statement of Purpose:** Surface modifications are often used to alter the topography and hydrophilicity of titanium dental implants to improve retention rates. Recent studies have highlighted the importance of a nanostructured surface topography that yields a surface more similar to that of natural bone.[1] Additionally, surface modifications enhancing the hydrophilicity of the implant facilitate bone formation by promoting closer interaction between the implant surface and its surrounding environment.[2] Although titanium is commonly used for dental implants, some leading dental implant industries are developing titanium alloyed implants because of their improved material properties, particularly mechanical strength. However, the effects of some of these modifications on alloyed surfaces have yet to be determined on osteoblastic differentiation and maturation. In the present study, we compared the osteoblastic differentiation and maturation of two different cell types in response to grit-blasted, acid-etched and hydrophilic titanium (Ti) and titanium-zirconium (TiZr) implants.

**Methods:** Institute Straumann AG provided grade 4 Ti and TiZr (13-17wt% Zr) discs. Discs were modified by large grit sandblasting (250 - 500μm) followed by acid etching in a boiling mixture of HCl and $H_2SO_4$ with immediate storage in 0.9% NaCl solution. The subsequent Ti modSLA and TiZr modSLA surface parameters were evaluated with SEM, confocal laser microscopy, XPS, and contact angle measurements. Mesenchymal stem cells (MSCs, Lonza) and normal human osteoblasts (NHOsts, Lonza) were plated on tissue culture polystyrene (TCPS), Ti modSLA, or TiZr modSLA at a density of 10,000 cells/cm$^2$. Osteoblastic cell differentiation and inflammatory response were assessed by measuring the production of osteocalcin, osteoprotegerin BMP2, VEGF, IL6, and IL10 by sandwich ELISA and normalized to DNA content. Cell lysate was analyzed for alkaline phosphatase specific activity and normalized to total protein content. mRNA levels for integrin subunits (ITGA1, ITGA2, ITGA3, ITGA5, ITGA6, ITGAV, ITGB1, ITGB3), bone morphogenetic proteins (BMP4, BMP7), BMP receptors (BMPR1A, BMPR1B, BMPR2), and BMP inhibitors (NOG, BMPER, GREM1) were measured and normalized to GAPDH.

**Results:** Evaluation of the surface parameters revealed that the fabrication method was capable of inducing a micro/nanostructured and hydrophilic surface on both the Ti and TiZr discs. Contact angle measurements demonstrated equal hydrophilicities on both surfaces [Ti modSLA = TiZr modSLA = 0(°)]. Differences in microroughness (Ti modSLA [$S_A$ = 1.74μm], TiZr modSLA [$S_A$ = 1.45μm] were detected using confocal microscopy. Figure 1 shows the SEM images of the Ti modSLA (left) and TiZr modSLA (right) surfaces which reveal the small and dense nanostructures associated with Ti modSLA and large low density nanostructures for TiZr modSLA. Using XPS, the chemical composition showed the presence of zirconium in TiZr modSLA while Ti modSLA had no measureable percentage.

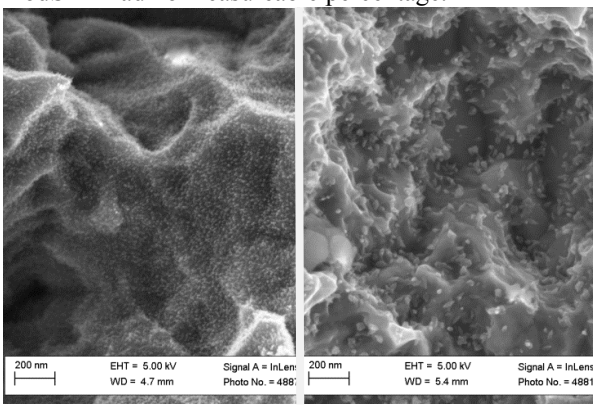


**Figure 1**: SEM images of the Ti modSLA (left) and TiZr modSLA (right) surfaces at 100Kx magnification.

Compared to TCPS, MSCs and NHOsts DNA content, ITGA5, and IL6 decreased. Alkaline phosphatase specific activity, osteoprotegerin, osteocalcin, VEGF, IL10, ITGA1, ITGA2, ITGB1, ITGA6, ITGAV, BMPS, BMP receptors, and BMP inhibitors increased compared to TCPS. No differences were detected in MSC ITGB3 expression and NHOst ITGA3expression. MSC DNA, alkaline phosphatase specific activity, BMP4, BMPER, and GREM1 levels were lower while ITGA3 and IL10 levels were higher on Ti modSLA compared to TiZr modSLA. No differences were detected between Ti modSLA and TiZr modSLA for any markers of osteoblastic differentiation and maturation.

**Conclusions:** Clinical grade titanium and titanium-zirconium alloy were successfully modified to enhance their hydrophilicity, create microroughness, and induce nanostructure formation. Ti modSLA and TiZr modSLA exhibit similar surface topographies and chemistries although minor differences can be seen due to the differences in bulk material. After assessment of the biological activity of the two metallic surfaces, our results suggest that osteoblastic differentiation and maturation were similarly enhanced on Ti and TiZr modSLA.

**References:**

1. Olivares-Navarrete R. Biomater. 2010;31;2728-2735.
2. Park JH. Biomater. 2012;33;7386-7393.