The influence of milled vs. laser-grooved Zirconia implant surfaces on human osteoblast behavior

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Abstract

Introduction: Zirconia is a potential alternative to titanium for dental implants, however, there is a need for the improvement of surface treatment protocols to enhance its bioactivity. Several strategies have been described to achieve this aim, including the manufacture of surface modifications that increase roughness using laser, micro-machining or surface treatments as sandblasting and acid etching (SB-AE). The benefits of laser graving versus milling are not yet known.

Objective: *In vitro* comparison of osteoblast response to Zirconia with SB-AE surfaces and additional groove-textures produced by micro-machining or laser, regarding cell attachment, cell proliferation and differentiation.

Materials and Methods: Milled (M-YTZP) and laser (L-YTZP) groove textured zirconia discs with 10, 100, and 100 µm features of depth, width and spacing, respectively, were produced using press and sintering. Untextured (S-YTZP) discs were used as controls. SB-AE protocol was applied to all samples to achieve a mean roughness ranging between $2.49 \pm 0.3\mu\text{m}$ and $2.03 \pm 0.48\mu\text{m}$ ($p>0.05$). Human osteoblasts were cultured on discs for 14 days. Morphology and cellular adhesion were observed using scanning electron microscopy (SEM) after 1 day. Cell viability and proliferation were evaluated at 1,3,7 and 14 days using a resazurin-based method. Alkaline phosphatase (ALP) activity was evaluated at 7 and 14 days and Collagen type I, Osteopontin and IL-1β were evaluated at 3 and 7 days using ELISA. All results were presented as mean $\pm$ standard deviation (SD). Group comparisons were tested using Anova (Tukey's post-hoc) using appropriate statistical software and significance was set at $p<0.05$.

Results: Cell viability and proliferation increased over time in all study groups. M-YTZP and L-YTZP viability was significantly higher than in S-YTZP group at 7 and 14 days ($p<0.05$). Collagen secretion was higher in M-YTZP and L-YTZP when compared to S-YTZP at 7 days ($p<0.05$). Cell viability or collagen secretion were not statistically different between M-YTZP and L-YTZP ($p>0.05$). However, Osteopontin secretion and ALP activity were significantly increased at 7 and 14 days, respectively, in M-YTZP surfaces comparing to L-YTZP and S-YTZP ($p<0.05$). IL-1b levels were not significantly different between groups.

Conclusion: In SB-AE Zirconia surfaces, micro-texture grooves manufactured by milling induced enhanced osteoblast differentiation comparing to laser-textured surfaces. Although cell viability and inflammatory marker production were not affected by the micropatterning technique, our results suggest that conventional milling combined with SB-AE may lead to a higher ability of bone cells to produce and mineralize bone matrix when compared to laser-textured or untextured surfaces with similar surface treatment.

Results

Figure 2 – Bar chart showing osteoblast viability as mean and standard deviation using fluorescence intensity expressed in arbitrary units (AU). Statistical significance: * $P<0.05$.

Figure 1 – SEM micrographs of surfaces at baseline and after 1 day of culture with 100x and 200x magnifications, respectively.

Figure 3 – Mean and standard deviation of collagen type 1 (A) and osteopontin (B) concentration obtained in pg/ml in the osteoblastic culture supernatant in all study groups at 3 and 7 days. One-way ANOVA with Tukey's post-hoc were used to test for differences between study groups. The error bars represent standard deviation. Statistical significance: * $P<0.05$.

Figure 5 – Alkaline phosphatase mean activity expressed in mU of hydrolyzed substrate per µl solution in the osteoblastic culture for all study groups at 7 and 14 days, respectively. One-way ANOVA with the Tukey's post-hoc test were used to test for differences between study groups. The error bars represent the standard deviation. Statistical significance: * $P<0.05$.

Figure 6 – Mean interleukin 1β concentration (mU/µl) in the osteoblastic culture supernatant at 1st and 3rd day in culture. One-way ANOVA with the Tukey's post-hoc test were used to test for differences between study groups. The error bars represent standard deviation. Statistical significance: * $P<0.05$.

Background and Aim

Zirconia is a potential alternative to Titanium for dental implants,^{1,2} however, there is a need for the improvement of surface treatment protocols to enhance its bioactivity.^{3,4} Several strategies have been described to achieve it, including the manufacture of surface modifications that increase roughness using laser, micro-machining or surface treatments as sandblasting and acid etching (SB-AE).⁴ The benefits of laser graving versus milling are not yet known.^{5,6}

In vitro comparison of osteoblast response to Zirconia with SB-AE surfaces and additional groove-textures produced by micro-machining or laser, regarding cell attachment, cell proliferation and differentiation.

Conclusion

In SB-AE Zirconia surfaces, micro-texture grooves manufactured by milling induced enhanced osteoblast differentiation comparing to laser-textured surfaces. Within these dimensions, conventional milling combined with SB-AE may lead to a higher ability of bone cells to produce and mineralize bone matrix when compared to laser-textured or untextured surfaces with similar surface treatment.

Methods and Materials

Milled (M-YTZP) and laser (L-YTZP) groove textured Zirconia discs with 10, 100, and 100µm features of depth, width and spacing, respectively, were produced using press and sintering techniques. Untextured (S-YTZP) discs were used as controls. SB-AE protocol was applied to all samples to achieve a mean roughness ranging between $2.49 \pm 0.3\mu\text{m}$ and $2.03 \pm 0.48\mu\text{m}$ ($p>0.05$). Fetal human osteoblasts were cultured on discs for 14 days, as described in previous studies⁷. Morphology and cellular adhesion were observed using SEM after 1 day. Cell viability and proliferation were evaluated at 1,3,7 and 14 days using a resazurin-based method. ALP activity was evaluated at 7 and 14 days and collagen type I, osteopontin and IL-1b were evaluated at 3 and 7 days using ELISA. All results were presented as mean $\pm$ standard deviation (SD). Group comparisons were tested using Anova (Tukey's post-hoc) using appropriate statistical software and significance set at $p<0.05$.

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