Human osteoblast behavior on Zirconia implant surfaces treated with Nd:YAG laser – In vitro study

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Abstract

Background and Aim: Yttria-stabilized tetragonal Zirconia polycrystal (Y-TZP) has emerged in dentistry as a promising material for various applications, such as dental implant surfaces. Nd:YAG laser surface treatments has been considered an innovative texturing technique to improve roughness and early osteointegration. However, the optimal parameters and surface texture characteristics to be applied with Nd:YAG laser in zirconia are yet to be determined. The aim of this study was to evaluate the behavior of human fetal osteoblasts in contact with laser-machined micro pillar Zirconia implant surfaces. Secondary aim was to compare the effect of different distances between micro-pillars in cell response.

Materials and Methods: Zirconia discs were produced using press-and-sintering techniques. All surfaces were treated with Nd:YAG laser and each group had different distance between micro pillar array (A= 25µm, B=30µm and C=35µm). Untextured Zirconia (UT) discs were used as controls. Sandblasting and acid etching (SB-AE) protocol was applied to all samples. Human osteoblasts were cultured for 14 days by previously described studies. Morphology and cellular adhesion were observed using scanning electron microscopy (SEM). Cell viability and proliferation were evaluated at 1,3,7 and 14 days using a commercial resazurin-based method. Collagen type I was evaluated at 3 days using enzyme-linked immunosorbent assay. All results were presented as mean ± standard deviation (SD). Alkaline phosphatase (ALP) activity was evaluated at 7 days using a colorimetric enzymatic technique. Group comparisons were tested using Anova (Tukey's post-hoc) using statistical software and significance was set at p<0.05.

Results: Cell viability and proliferation increased over time for all groups with statistically higher values for textured groups when compared UT at 3, 7 and 14 days of culture (p<0.05). Collagen type I levels were about 10-fold increased in laser treated groups when compared to UT (p<0.05). However, no statistical differences were detected for collagen secretion between the textured groups (A, B and C) (p>0.05). Group C presented higher ALP activity levels at 7 days (p<0.05) when compared with remaining laser textured groups.

Conclusion: The results suggest that laser-produced micro pillar array texture in Zirconia implant surfaces may benefit biological osteoblast response however micro pillar spacing at the range of 25-35µm does not seem to be a relevant variable. Subsequent studies should focus on alternative strategies of laser micro-texturing protocols to optimize cell behavior in Zirconia implant surfaces.

Results

Figure 2 – Bar charts showing collagen levels (A) and ALP activity (B) as mean and standard deviation using enzymatic assay expressed in pg/mL and umol/min/mL, respectively. Statistical significance: *p<0.05.

Figure 3 – SEM micrographs of surfaces at baseline and after osteoblast culture at 1 day with 120x and 500x, respectively.

Background and Aim

Yttria-stabilized tetragonal Zirconia polycrystal (Y-TZP) has emerged in dentistry as promising material for various applications, such as dental implant surfaces.^[1,2] Nd:YAG laser surface treatments has been considered an innovative texturing technique to improve roughness and early osteointegration.^[2, 3, 4] However, the optimal parameters and surface texture characteristics to be applied with Nd:YAG laser in zirconia are yet to be determined.^[4] **The aim of this *in vitro* study was to evaluate the behaviour of human fetal osteoblasts in contact with laser-machined micro-pillar Zirconia surfaces. Secondary aim was to compare the effect of different distances between micro-pillars in cell response, considering cell attachment, viability, proliferation and differentiation.**

Conclusion

Laser-produced micro pillar array texture in Zirconia implant surfaces may benefit biological osteoblast response, however, micro pillar spacing at the range of 25-35µm does not seem to be a relevant variable. Subsequent studies should focus on alternative strategies of laser micro-texturing protocols to optimize cell behavior in zirconia implant surfaces.

Methods and Materials

Zirconia discs were produced using press-and-sintering techniques. All surfaces were treated with Nd:YAG laser and different distance between micro pillar array in each group (A= 25µm, B=30µm and C=35µm). Untextured zirconia (UT) discs were used as controls. Sandblasting and acid etching (SB-AE) protocol was applied to all samples. Human osteoblasts were cultured for 14 days by previously described studies.^[1, 5, 6] Morphology and cellular adhesion were observed using scanning electron microscopy (SEM). Cell viability and proliferation were evaluated at 1,3,7 and 14 days using a commercial resazurin-based method.^[1, 5, 6] Collagen type I was evaluated at 3 days using enzyme-linked immunosorbent assay. Alkaline phosphatase (ALP) activity was evaluated at 7 days using a colorimetric enzymatic technique. All results were presented as mean ± standard deviation (SD) and group comparisons were performed using Anova (Tukey's post-hoc) with appropriate statistical software and significance set at p<0.05.

References

- [1] Penarrieta-Juanito G., Cruz MB, Costa M, Miranda G, Marques J, Magini R, Mata A, Caramês J, Silva FS. A novel gradated zirconia implant material embedding bioactive ceramics: physicochemical assessment and human osteoblast behavior. *Materialia*. 2018
- [2] Delgado-Ruiz RA, Gomez Moreno G, Aguilar-Salvatierra A, Markovic A, Mate- Sanchez JE, Calvo-Guirado JL. Human fetal osteoblast behavior on zirconia dental implants and zirconia disks with microstructured surfaces. An experimental in vitro study. *Clin Oral Implants Res*. 2016;27(11):e144-e53.
- [3] Carvalho BMP, E. P.; Moraes, Falcón-Antenucci, R. M.; Júnior, J. S. F. Tratamentos de superfície nos implantes dentários. *Rev Cir Traumatol Buco-Maxilo-fac*. 2009;9(1):123-30.
- [4] Taniguchi Y, Kakura K, Yamamoto K, Kido H, Yamazaki J. Accelerated Osteogenic Differentiation and Bone Formation on Zirconia with Surface Grooves Created with Fiber Laser Irradiation. *Clin Implant Dent Relat Res*. 2016;18(5):883-94.
- [5] Kohal RJ, Bachle M, Att W, Chaar S, Altmann B, Renz A, et al. Osteoblast and bone tissue response to surface modified zirconia and titanium implant materials. *Dental Materials*. 2013;29(7):763-76.
- [6] Cruz MB, Marques JF, Penarrieta-Juanito GM, Costa M, Souza JC, Magini RS, et al. Hard and Soft Tissue Cell Behavior on Polyetheretherketone, Zirconia, and Titanium Implant Materials. *Int J Oral Maxillofac Implants*. 2019;34(1):39-46.

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