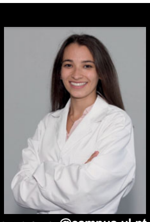

beatriz-ferreira@campus.ul.pt

Inflammatory response of peri-implant cells to piezoelectric-ceramic composite implant surfaces – *in vitro study*

Fernandes, B.[1], Tiainen, L.[2], Gasik, M.[3], Carvalho, O.[2], Cruz, M.[1], Mata, A.[4,5]

1 Grupo de Investigação em Biologia e Bioquímica Orais (GIBBO-UICOB), Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal;
2 Center for Microelectromechanical Systems (CMEMS), Departamento de Engenharia Mecânica, Universidade do Minho, Guimarães, Portugal
3 Department of Chemical and Metallurgical Engineering, Aalto University, Espoo, Finlândia
4 Grupo de Investigação em Biologia e Bioquímica Orais – LIBPhys FCT UID/FIS/04559/2013, Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal
5 CEMDBE – Cochrane Portugal, Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal

BACKGROUND AND OBJETIVE

Osseointegration is the major success factor for dental implants.[1] In order to optimize the biological response, several strategies have been investigated.[2] Due to its piezoelectric properties similar to bone electric potentials generated in loading function, barium titanate ($BaTiO_3$) piezoelectric ceramic is a potential approach for promoting osteogenic proliferation and differentiation and, consequently, accelerating osseointegration.[3, 4] In this sense, a new technique was developed to stimulate the cells in the implant bed through the use of piezoelectric ceramics as a source of electrical stimulation. However, the potential inflammatory effects in peri-implant cells and tissues are not defined.

The aim of this in vitro study was to evaluate the inflammatory response of gingival fibroblasts and human fetal osteoblasts in contact with barium titanate functionalized zirconia implant surfaces with piezoelectric properties.

MATERIALS AND METHODS

Composite discs with 5 wt.% $BaTiO_3$ in Yyttria- stabilized zirconia (YSZ) were prepared through press-and-sintering technique (n=15). Contact poling was carried out in silicon oil bath under DC 2 kV/mm electric field at 130 °C for 30 min followed with field cooling.[6] Reference samples of YSZ were processed parallel to the composites. Human gingival fibroblasts (hTERT) and fetal osteoblasts (hFOB1.19) were cultured on discs for 14 and 7 days, respectively. Cell viability was evaluated at 1, 3, 7 and 14 days using a commercial resazurin-based method. IL-1β and IL-6 were evaluated at 1 and 3 days in each fibroblast and osteoblast culture and osteopontin was measured in osteoblast cell culture at 3- and 7-days using ELISA (pg/mL). Osteoblasts alkaline phosphatase (ALP) activity was measured using an enzymatic colorimetric assay at 7 and 14 days (µmol/min/mL). All results were presented as mean ± confidence interval (CI). Group comparisons were based on one-way ANOVA repeated measures or Kruskal-Wallis and Tukey's post-hoc using appropriate statistical software (IBM® SPSS® for Mac version 27.0.1.0) and significance was set at $p<0.05$.

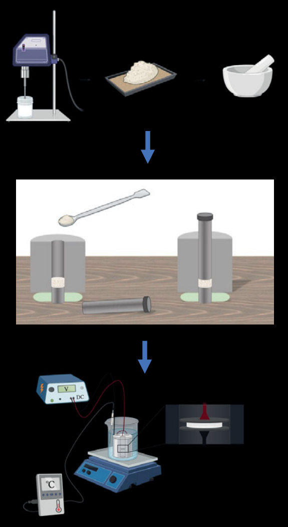

Figure 1 – Illustrative images of sample preparation. Image created with BioRender ©.

RESULTS

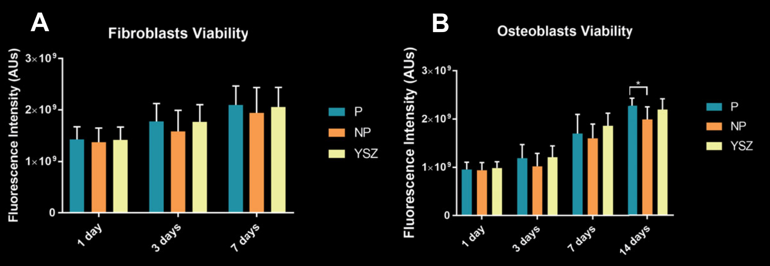

Figure 1 – Bar chart with mean and standard deviation of fibroblasts (A) and osteoblasts viability (B) using fluorescence intensity expressed in arbitrary units – AU. Statistical significance: *p<0.05.

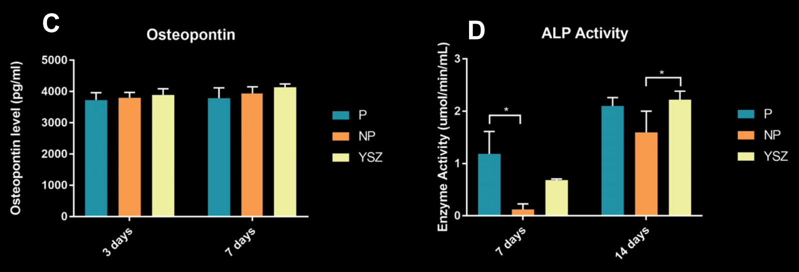

Figure 2 – Bar charts showing osteopontin levels (C) and ALP activity (D) as mean concentration in pg/mL and enzymatic activity in µmol/min/mL, respectively. Error bars represent standard deviation. One-way repeated measures ANOVA with post-hoc Tukey test were used for comparisons between study groups. Statistical significance: *p<0.05.

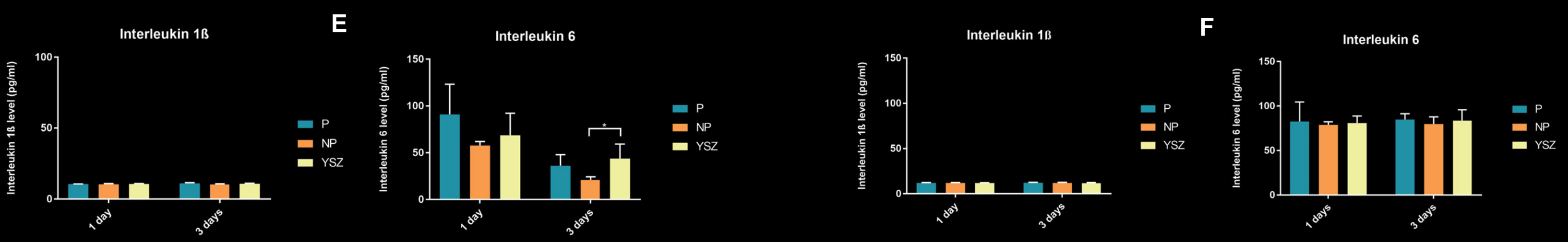

Figure 3 – Bar charts showing fibroblasts (E) and osteoblasts (F) interleukin 1β and interlekin 6 levels as mean concentration in pg/mL. Error bars represent standard deviation. One-way repeated measures ANOVA with post-hoc Tukey test were used for comparisons between study groups. Statistical significance: *p<0.05.

DISCUSSION

Poled zirconia surfaces with 5% $BaTiO_3$ demonstrated superior results of osteoblasts viability and initial differentiation compared to non-poled samples. These results agree with the literature.[3-5] Fibroblasts behavior does not seem to be influenced by polarization. Concerning osteoblasts inflammatory markers, IL-1β secretion remain constant and an increase in IL-6 was observed in poled group (p>0.05). Fibroblasts secretion of IL-1β was similar to osteoblasts, with constant values over time in line with several studies with other cell lines in literature.[7-8] However, IL-6 secretion decreased in all groups, with values significantly lower in non-poled group compared to YZS at 3 days (p>0.05), which suggests a possible anti-inflammatory role of non-poled $BaTiO_3$. The results of this study suggest that zirconia samples with 5% of $BaTiO_3$ are not cytotoxic to peri-implant tissue cells and, additionally, do not seem to affect their long-term inflammatory profile. In this sense, further studies should be carried out to assess the potential piezoelectric effect on bone tissue when load is applied and in a biological systems.

CONCLUSION

Zirconia composite surfaces with the addition of $BaTiO_3$ is not cytotoxic to peri-implant tissues cells. Additionally, samples with or without piezoelectric properties do not affect cellular differentiation and inflammatory profile. Nevertheless, the addition of non-poled $BaTiO_3$ to YSZ may have a potential reduction effect in IL-6 mediated —inflammatory activity in fibroblasts.

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