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IMPLANT SURFACES MANUFACTURED WITH A NOVEL TECHNIQUE – *IN VITRO* STUDY

M.B. Cruz[1], G. Juanito[2], J. Marques[1], F. Silva[3], M. Costa[3], J. Souza[2], D. N. Marques[1], A. Mata[1], J. Caramês[1]

1- Oral Biology and Biochemistry Research Group, LIBPhys-FCT UID/FIS/04559/2013 , Universidade de Lisboa , Faculty of Dental Medicine, Lisboa, Portugal
2- Centre for Research on Dental Implants, School of Dentistry, Federal University of Santa Catarina, Florianópolis, Brazil
3- Centre for Microelectromechanical Engineering, University of Minho, Guimarães, Portugal

INTRODUCTION AND OBJECTIVES

Although titanium has been regarded as the material of choice for dental implants, it is associated with adverse events such as hypersensitivity reactions, esthetics and corrosion [1]. Due to these limitations, alternative materials have been the subject of intensive research. Zirconia and biocompatible polymers, such as Polyetheretherketone (PEEK) have been proposed as options [2,3] based on their biocompatibility, esthetics and favorable mechanical properties [4-6].

A new advanced technology of multimaterial manufacturing resulting in materials structured in a hierarchical way to produce a favorable concentration gradient – functionally-graded materials - was developed under a joint research consortium between the School of Mechanical Engeneering at the Universidade do Minho and the Faculty of Dental Medicine at the Universidade de Lisboa. The present study was performed to validate the biological properties of these new materials.

The aim of this study is to characterize the behavior of human fetal osteoblasts (hFOB1.19) in contact with PEEK or Zirconia-based dental implant biomaterials produced using a new manufacturing procedure comparing to Titanium as a gold standard material.

MATERIALS AND METHODS

8 sample discs for each study group (PEEK, Zr and Ti) were produced by a combination of uniaxial pressing (200MPa) and sintering at 1500°C for 2 hours (Zirkonofen 700 furnace) with heating and cooling rates of 8°C/min [7]. Final samples had a diameter of 8mm and height of 3mm. All samples were sandblasted with alumina particles (250 mm) under the same conditions to achieve a equivalent surface roughness between samples. Commercially pure grade IV Ti discs were used as control. Human Fetal Osteoblasts hFOB 1.19 (ATCC; American Culture Collection, Manassas, VA, USA) were used. Cells were cultured at 37°C in an atmosphere of 5% CO_2 and 100% humidity in proper culture cell medium as per manufactures instructions. Cell viability and proliferation was evaluated using a rezasurin-based viability assay at 1, 3, 7, and 14 days culture on a spectrofluorometer (LS50B-Perkin-Elmer®,EUA) and expressed in fluorescence intensity values arbitrary units (A.U.). Alkaline phosphatase (ALP) activity was measured using a fluorimetric enzymatic assay at 7 and 14 days and values were converted to mU/ml of enzyme (ALP) based on the standard regression equation. Results were presented as mean and standard deviation. Differences between groups were tested using one-way ANOVA with Tukey's post-hoc. Values of $p< 0.05$ were considered significant. In a subsequent culture, cells were fixed and stained with OsteoImage™ Mineralization Assay (Lonza®,Switzerland) after 7 and 14 days in culture, and observed under a fluorescence confocal microscope Bio-Rad MRC600 (Leica©,Germany). In a third round of cell culture, samples were fixed, dehydrated and metalized after 1 of culture and observed under a Ultra-high resolution Field Emission Gun Scanning Electron Microscopy (FEG-SEM), NOVA 200 Nano SEM, FEI, Oregon, USA. Images were analyzed by two calibrated observers.

RESULTS

CELL VIABILITY AND METABOLIC ACTIVITY

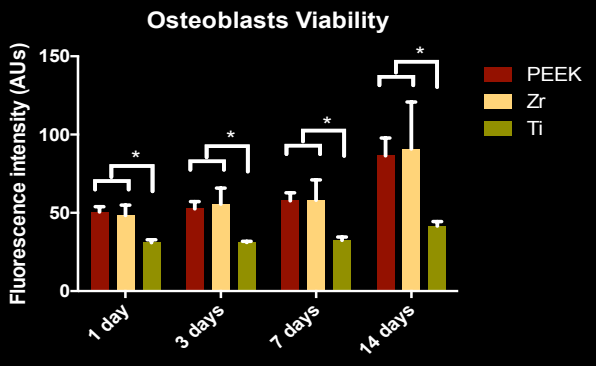


Figure 1 – Barchart representing cell viability outcomes expressed as resorufin formation measured by fluorescence expressed in arbitrary units (A.U.) as mean +/- standard deviation of human osteoblast hFOB1.19 cell culture at 1, 3, 7, and 14 days on PEEK, Zr and Ti surfaces. The results refer to 8 replicates of representative experiments.

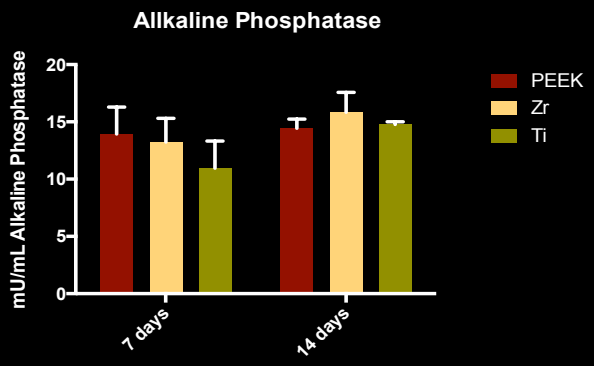


Figure 2 – Mean alkaline phosphatase activity measured in human osteoblast hFOB1.19 cell culture at 1, 3, 7, and 14 days on PEEK, Zr and Ti surfaces. Error bars represent standard deviation expressed in mU/ml of enzyme (ALP) as mean +/- standard deviation. The results refer to 8 replicates of representative experiments.

BONE MINERALIZATION

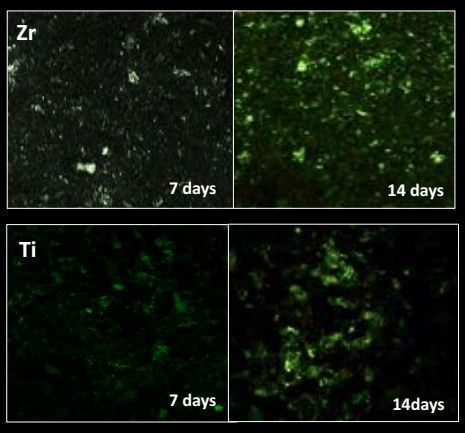
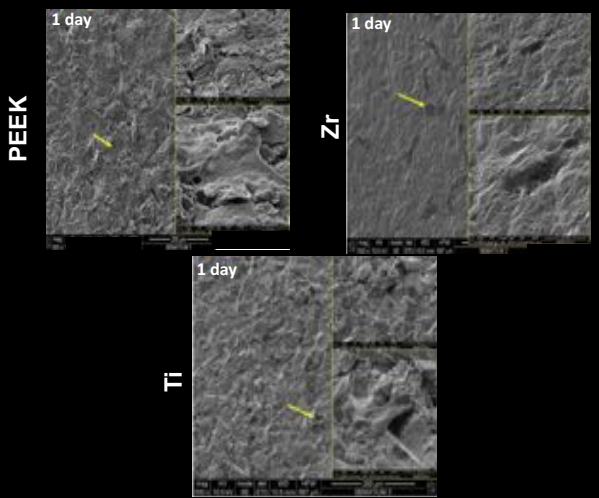


Figure 3 and 4– Confocal fluorescence micrographs show enhanced bone mineralization in Zr and Ti samples stained with OsteoImage™ at 7 and 14 days of culture . PEEK results not presented due to instrinsic autofluorescence.

CELL ATTACHMENT AND MORPHOLOGY



Figures 4, 5, and 6– Scanning electron micrographs of hFOB1.19 osteoblasts cultured on PEEK, Zr and Ti samples after 1 day. 500x and 5000x magnifications are shown.

DISCUSSION

- Zr and PEEK surfaces presented significant higher cell viability at 14 days culture when comparing with Ti ($p<0.05$).
- All groups presented increased ALP values over time, however, group comparisons at each time-point did not present significant differences.
- Improved mineralization was observed on Zr surfaces when compared to Ti after 14 days culture.
- An higher number of plasma membrane extensions was observed in Zr surfaces after 1 day culture which is compatible with a higher ability of this material to induce cell adhesion and proliferation when compared to the other study groups.
- Further comparative studies, including measurement of inflammatory markers, identification and measurement of specific bone and cell membrane proteins are needed to enable a comprehensive study of these novel implant surfaces.

CONCLUSIONS

The new production technique for Zirconia and PEEK-based materials presented improved in vitro cellular responses when compared to Titanium as reference material, with increased osteoblast viability, proliferation and matrix mineralization.

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