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OSTEOBLASTS BEHAVIOR ON DIFFERENT PEEK IMPLANT SURFACES – *A PILOT STUDY*

carlotaidm@gmail.com

C. D. Mendonça[1], M. B. Cruz[1], J. Marques[1], G. Juanito[2], F. Silva[3], G. Miranda[3], R. Magini[2], J. Caramês[1], A. Mata[1]

1- Oral Biology and Biochemistry Research Group, LIBPhys, 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

Titanium is considered the *gold standard* material for implant surfaces, however due to aesthetics and mechanical properties, new materials have been proposed as alternatives[1].

Polyetheretherketone (PEEK), a synthetic thermoplastic polymer[1], has an excellent cell biocompatibility, strength and favorable Young's Modulus, similar to the mechanical properties of cortical bone[2,3]. However, it is biologically inert and this fact may compromise osseointegration[3]. Therefore, calcium phosphate-based coatings, such as hydroxyapatite (HA) and beta tricalcium phosphate (ßTCP), on a PEEK substrate have been proposed as a compatible scaffold for osseointegration[1,2,3]. Nevertheless, coatings are susceptible to delamination during implant placement[7,8,9]. A new approach to produce these materials was developed by our group based on combined pressing and sintering techniques to produce a hybrid material with bioactives incorporated in a PEEK scaffold.

The aim of this study was to compare the *in vitro* response of human fetal osteoblasts (hFOB1.19) in contact with new PEEK-based implant surfaces: pure PEEK (control), PEEK with 5% HA or PEEK with 5% ßTCP.

MATERIALS AND METHODS

8 sample discs (diameter of 8mm, height of 3mm and equivalent roughness) for each study group (PEEK, PEEK-HA and PEEK- ßTCP) were produced by a combination of uniaxial pressing (200MPa) and sintering techniques[4]. Human Fetal Osteoblasts hFOB 1.19 (ATCC; American Culture Collection, Manassas, VA, USA) were used and conditioned as recommended by the supplier[10,11,12]. All experiments were conducted at 37ºC at a density of 10^4 cells/well using cells from the 4th passage. Cell viability were evaluated using a Resazurin-based method - Cell-Titer Blue® reagent (Promega, Madison, WI, USA) at 1, 3, 7, and 14 days culture on a spectrofluorometer (LS50B-Perkin-Elmer®,EUA) using excitation/emission wavelenghts of 530/590nm. Morphology was determined 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. Alkaline phosphatase was evaluated at 7 and 14 days using Alkaline Phosphatase Assay Kit (Fluorometric, ab83371, Abcam®). Results were presented as mean and standard deviation of fluorescence intensity values (expressed in arbitrary units – A. U.) and for ALP as the amount of enzyme causing the hydrolysis of 1 µmol of non-fluorescent substrate 4-Methylumbelliferyl phosphate disodium salt (MUP) per minute at pH 10.0 and 25°C (glycine buffer) (mU/mL).

Differences between groups were tested using one-way ANOVA with Tukey's *post-hoc*. Values of $p < 0.05$ were considered significant.

RESULTS

CELL VIABILITY AND DIFFERENTIATION

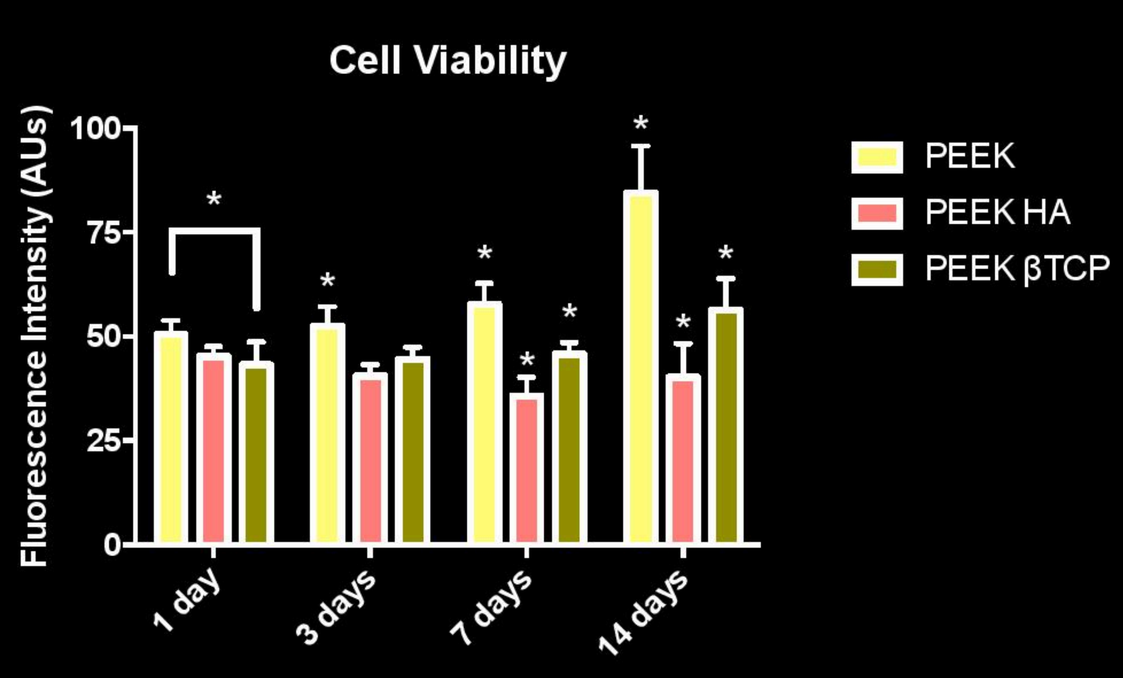


Figure 1 – Barchart representing mean cell viability expressed as resorufin formation measured by fluorescence expressed in arbitrary units (A.U.) of human osteoblast hFOB1.19 cell culture at 1, 3, 7, and 14 days on PEEK, PEEk-HA and PEEK- ßTCP surface. Error bars represent standard deviation

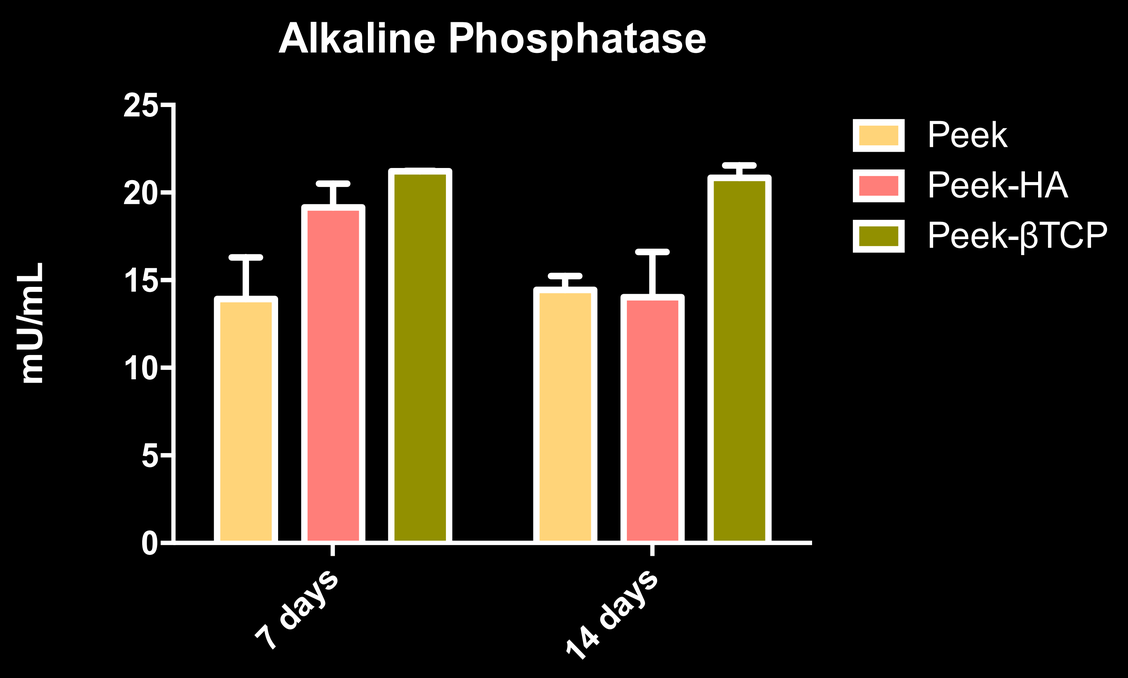
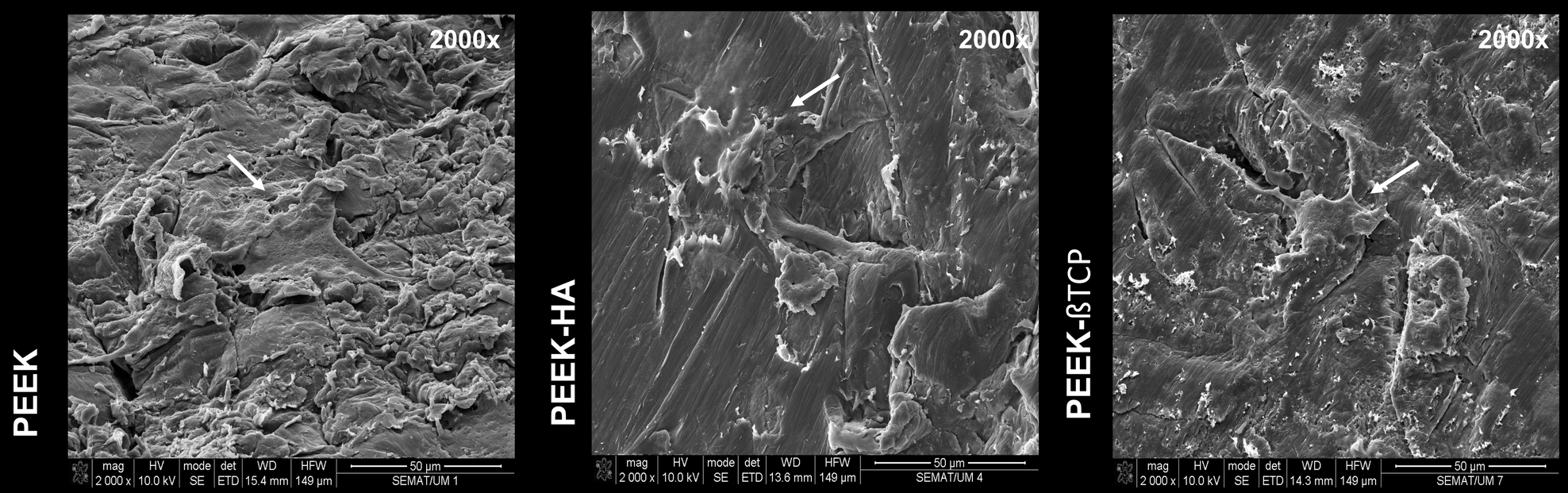


Figure 2 – Barchart representing mean alkaline phosphatase activity measured in human osteoblast hFOB1.19 cell culture at 7 and 14 days on PEEK, PEEK-HA and PEEK- ßTCP surfaces. Error bars represent standard deviation.

CELL ATTACHMENT AND MORPHOLOGY



Figures 4, 5, and 6– Scanning electron micrographs of hFOB1.19 osteoblasts cultured on PEEK, PEEK-HA and PEEK-ßTCP samples after 1 day. 2 000x magnification is shown.

DISCUSSION

- Cell viability increased in PEEK group over time, showing significant difference for PEEK-βTCP at 1 and 3 days ($P<0.05$). At 7 and 14 days, that difference was significant for all groups.
- PEEK-βTCP displayed the highest ALP values in both time points, compatible with increased differentiation. However, differences between groups at the same time-points were not significant.
- A perceived higher number of cells and fillopodia is apparent in PEEK samples compared to other groups after 1 day in culture, compatible with early cell attachment and proliferation in this group.

CONCLUSIONS

Pure PEEK presented higher viability comparing to PEEK-HA and PEEK- ßTCP. Adding bioactive materials to a PEEK scaffold, under the tested conditions, did not result in any benefit in osteoblast response.

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