

Improving Human Primary Osteoblast Cytocompatibility to PEEK by Plasma Surface Modification

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Introduction: Metal devices can obscure soft and hard tissue integration to implants during evaluation by X-ray or MRI. Polyetheretherketone (PEEK) has been used as a replacement for metals in devices such as spine cages and patient specific craniomaxillofacial (CMF) implants, due to its radiolucency. PEEK has come to the forefront in the field of biomaterials due to its high strength and good wear properties compared to polymers such as UHMWPE¹. However, many polymers including PEEK have an intrinsic low surface energy which can restrict the cellular attachment, and this can lead to implant loosening, as a result of fibrous encapsulation. Surfaces with high energy have been shown to promote rapid cellular adhesion and spreading^{2,3}. To improve cellular attachment the surface energy can be increased by plasma surface treatment. The present study aims to investigate the effect of oxygen plasma treatment of PEEK on the adhesion and functionality of primary human osteoblast-like cells (HOB).

Materials and Methods: Injection moulded PEEK Optima™ discs (Invivo) with a 13mm diameter were modified by plasma treatment, and these were compared to Thermanox (THX) and standard medical grade micro-rough titanium (cpTi ISO 5832/2) (Synthes). Using an EMITECH RF plasma treater, the samples were exposed to varying treatment times. Surface chemical compositions of treated and untreated surfaces were characterised by XPS, wettability by contact angle, topographic changes by AFM and SEM. HOB cells isolated from human femoral heads removed during total joint replacement operations were grown to 70-80% confluence in DMEM (10% FCS in 5% CO₂ at 37°C), and plated at 10000 cells/cm². Alpha-MEM (0.1µM dexamethasone and 10mM beta-glycerophosphate) was used as mineralisation media over the 28 day experiments. Cell functionality was assessed by alkaline phosphatase expression (ALP), phenotypic gene expression by qPCR, mineralisation by Alizarin red S (ARS) staining of calcium, cell attachment by SEM and cell density through the alamarBlue™ assay, sampling was performed at 1, 7, 14, 21 and 28days.

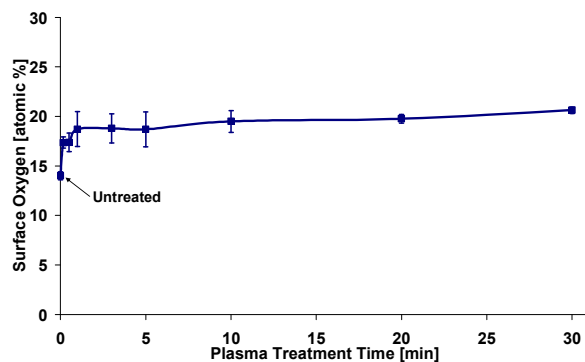


Figure 1: Surface oxygen concentration of PEEK surfaces with increasing plasma treatment time.

Results: XPS analysis of the untreated PEEK discs showed 14 atomic% surface oxygen, as expected, indicating that these surfaces are relatively hydrophobic in character⁴. Analysis of the plasma treated PEEK surfaces showed that the surface oxygen concentration increased with increasing treatment time up to ~20 atomic% after 30min treatment (Fig 1). High resolution C1s spectra showed a greater increase in C-OR type functional groups than C=O and O-C=O with increasing treatment time. Shelf life experiments have to date shown the surface treatment to be stable for up to 8 months. The surface roughness was not found to be affected by the surface treatment at the early treatment times, but after 30min marked changes in the surface micro- and nano- structure were observed (Fig 2). To study the effects of the surface treatment on cell attachment and functionality, the cells were examined after plating on the treated and untreated PEEK, THX and cpTi surfaces. Cell adhesion was observed by SEM. The HOB cells were found to attach more readily to the treated surfaces with higher concentrations of C-OR functional groups than the untreated PEEK surfaces, and higher densities were measured within 72hrs. By day 21 the treated PEEK surfaces were shown to have similar cell densities to cpTi.

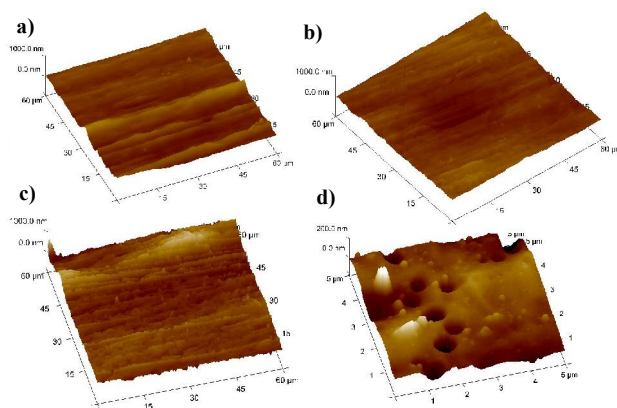


Figure 2: AFM images of a) the untreated PEEK as a relatively smooth surface, b) the surface after 10min treatment showing no significant change in the surface roughness, c) the surface after 30min treatment, where surface modification has started to alter the surface topography, and d) a 5µm scan of the same region showing the pits created.

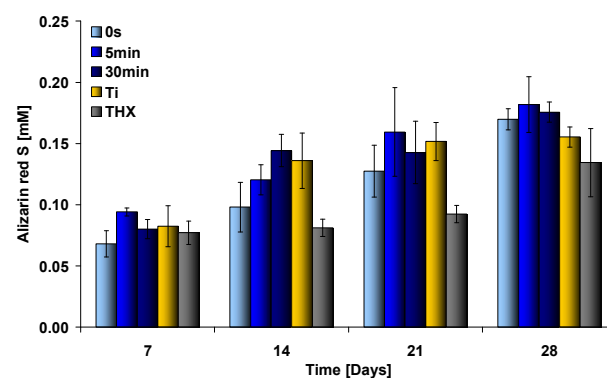


Figure 3: In vitro mineralisation determined by Alizarin red S staining of calcium depositions produced by the HOB cells.

ALP expression was observed to be more characteristic towards osteoblast phenotype on the treated PEEK surfaces, than untreated PEEK over the 28 day experiments. Initial findings indicate that collagen type I and osteocalcin gene expression were upregulated on the treated PEEK surfaces compared to untreated PEEK surfaces by day 7. Nodule formation quantified by dissolving the ARS stain was found to be greater on the PEEK surfaces than on the THX surfaces, and similar to the levels on the cpTi surfaces (Fig 3). The level of mineralisation was higher on the treated PEEK surfaces from 7 days onward, indicating that the cells had started to mineralise earlier on these treated surfaces, and showed similar levels to cpTi throughout the 28 day experiments.

Discussion/Conclusions: Surface modification of PEEK by oxygen plasma treatment can be used to increase the surface energy and thereby aid the adhesion of HOB cells. This surface modification has led to more characteristic osteoblast behaviour of the HOB cells, indicating that these treated surfaces are likely to improve bony integration to implants.

References: ¹Kurtz, S.M. and Devine, J.N. *Biom.*, 28, 4845, 2007. ²Lopez, G.P., Ratner, B.D., et al. *J Biom. Res.* 26, 415, 1992. ³Kasemo, B. *Surf. Sci.* 500, 656, 2002. ⁴Comyn, J., Mascia, L., and Xiao, G. *Int J Adhesion & Adhesives*, 16, 97, 1996.

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