Stable Oxygen Plasma Surface Modification of PEEK to Improve Osteoblast Cytocompatibility

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Statement of Purpose: Polyetheretherketone (PEEK) has bulk properties such as radiolucency, high strength and good wear resistance which have been shown to be advantageous in medical devices[1]. However, an intrinsic problem for many polymers, including PEEK, is a low surface energy, which can limit cellular adhesion. This in turn can lead to implant loosening, as a result of fibrous encapsulation. Surfaces with higher energy are known to promote rapid cellular adhesion and spreading, in contrast to surfaces with lower energy[2,3]. The aim of this study is to investigate the effect of oxygen plasma treatment of PEEK to increase the surface energy and evaluate the effect of these modified surfaces on the adhesion and functionality of primary human osteoblast-like cells (HOB) compared to untreated PEEK, Thermanox (THX) and standard micro-rough medical grade titanium (Ti).

Methods: Discs (13mm diameter) of injection moulded PEEK-OPTIMA® discs (Invibio Biomaterial Solutions, UK), THX (Nunc, DK) and titanium (Ti, ISO 5832/2) (Synthes, CH) were used in this study. PEEK discs were exposed to oxygen plasma for varying treatment times using an EMITECH RF K1050X plasma reactor (Quorum, UK). Surface chemical compositions were characterized by X-ray photoelectron spectroscopy (XPS), wettability by water contact angles (WCA) and changes in topography by atomic force microscopy (AFM) and scanning electron microscopy (SEM). HOB cells isolated from human femoral heads (Graubunden Ethical Commission, 18/02) removed during total joint replacement surgeries were grown to 70-80% confluence in DMEM (10% FCS in 5% CO_2 at 37°C), and plated at 10^3 cells/cm^2. Alpha-MEM (0.1µM dexamethasone and 10mM β-glycerophosphate) was used as mineralization media over the 28d experiments. Cell functionality was assessed by alkaline phosphatase activity (ALP), phenotypic gene expression by qPCR, mineralization by Alizarin red S (ARS) staining of calcium deposits, cell attachment by SEM and cell density through the alamarBlue™ assay. Sampling was performed at 1, 3, 7, 14, 21 and 28d. Statistical analysis was performed using SPSS v.16.0. ($P<0.05$). General linear model ANOVA with Tukey simultaneous *post-hoc* tests.

Results: Analysis of the surface chemistry by XPS showed the unmodified PEEK to have ~12 atom% surface oxygen. The plasma modified PEEK demonstrated the surface oxygen to increase with increasing treatment time up to ~17 atom% after 2400s exposure. High resolution C1s spectra showed the oxygen incorporation to be a result of increasing C=O and O-C=O functional groups. Examination of the surface topography by AFM showed no significant change in the surface topography of the modified PEEK. However, the modified surfaces did appear to be etched after treatment times longer than 1200s, at which point some pitting was observed, this could potentially provide anchorage points for HOB cells.

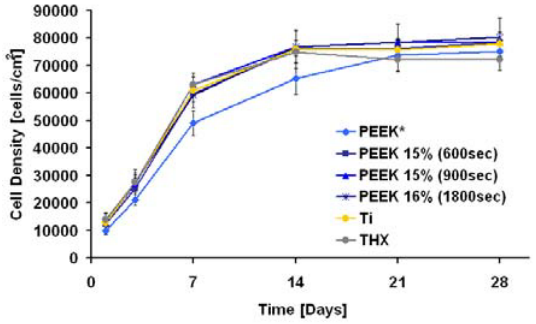

Figure 1. *HOB cell adhesion to 600s, 900s and 1800s modified PEEK, Ti and THX was significantly higher than to unmodified PEEK over the 28d experiments (± std. dev, n=4).*

WCA of the treated surfaces were shown to decrease, confirming the increase in surface energy. Cell density was determined by the alamarBlue™ assay (Fig 1). Within 24h the 600, 900 and 1800s treated surfaces, Ti and THX were shown to have statistically significantly higher cell densities than the untreated PEEK surfaces. The cells on the 600, 900 and 1800s treated surfaces, Ti and THX surfaces reached confluence between 7d and 14d, while the cells on the untreated PEEK only reached confluence after 14d. These findings indicate that modified PEEK surfaces with higher C=O and O-C=O functional group concentrations promote higher initial HOB cell attachment and this trend continues to 21d. As a result of confluence being reached earlier on the modified surfaces the ALP expression was observed to be more characteristic, leveling off with mineralization, while on the untreated PEEK the production of ALP by the HOB cells continued to increase to day 28. A significantly higher up-regulation of osteonectin, was observed from day 14 to 21 on the 1800s modified PEEK and Ti surfaces compared to untreated PEEK. Mineralization was confirmed by ARS staining of nodule formation and was found to be more abundant on all the modified PEEK and Ti surfaces from day 14 onwards.

Conclusions: Increasing the surface energy of the PEEK by oxygen plasma modification has been found to aid the adhesion, proliferation and mineralization of HOB cells *in vitro*. Ongoing experiments have shown that this surface treatment continues to be stable for more than two years. These findings indicate that this method of oxygen plasma surface modification is likely to improve bony integration to PEEK implants.

References: [1]Kurtz, S.M., Biom., 28, 4845, 2007. [2]Lopez, G.P., J Biom. Res, 26, 415, 1992. [3]Kasemo, B. Surf. Sci. 500, 656, 2002.

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