Plasma Surface Modification of PEEK – Effects on Bacterial Adhesion and Co-culture with Osteoblasts *in vitro*

[1,2]Rochford E.T.J;[3]Subbiahdoss G.;[1]Moriarty T.F.;[1]Poulsson A.H.C;[3]van der Mei H.C.;[3]Busscher H.J.;[1,2]Richards R.G.
[1]AO Research Institute,CH.[2]IBERS, Aberystwyth University,UK.[3]University Medical Center Groningen and University of Groningen,NL.

Introduction: Oxygen plasma treatment of polyetheretherketone (PEEK) has been shown to enhance human primary osteoblast cytocompatibility by increasing surface energy[1]. The oxygen plasma surface modification may also affect bacterial adhesion. This *in vitro* study compares the adhesion of clinically relevant bacteria to untreated PEEK versus oxygen plasma treated PEEK. Additionally, the effect of this surface modification was further investigated by co-culturing bacteria and U-2OS osteoblasts under flow conditions *in vitro*.

Methods: Injection moulded PEEK-OPTIMA® discs and PEEK films (Invibio Biomaterial Solutions, UK) were exposed to oxygen plasma for 900s or 1800s using an Emitech K1050X plasma cleaner (Quorum Tech., UK). Surfaces were characterised for wettability by water contact angle, topography by atomic force microscopy, and surface chemistry by X-ray photoelectron spectroscopy. Adhesion of 3 strains of *Staphylococcus aureus* and 3 strains of *Staphylococcus epidermidis* to the sample discs was evaluated by the addition of bacterial suspensions in PBS ($1x10^7$cfu ml^{-1}) into a custom made adhesion chamber, based on the CDC biofilm reactor (BioSurface Technologies Corp, USA) containing the sample discs. A method adapted from Miles and Misra[3] was used to obtain total viable counts of adherent bacteria after incubation for 2.5h (37°C, 125rpm)

Bacterial adhesion, osteoblast adhesion and co-culture experiments were performed on the PEEK films with a parallel plate flow chamber (PPFC)[2]. Briefly, $3x10^6$ bacteria ml^{-1} suspensions of *S. aureus* V8189-94 (clinical isolate) and *S. epidermidis* RP12 (ATCC 35983) in PBS were pumped through the PPFC for 2.5h (shear rate: $11s^{-1}$ at 37°C without fluid recycling). The density of adhered bacteria was determined visually using light microscopy. U-2OS osteosarcomal cell (ATCC HTB-96) adhesion was performed in the PPFC with a suspension of $6x10^6$ cells (passage 13-24) in 10ml of modified culture media (DMEM + 10%FBS + 2%HEPES + 2% tryptone soy broth + 0.1%AA2P). U-2OS adhesion under static conditions was conducted for 1.5h (37°C) before a flow of sterile modified culture media was engaged for 48h (shear rate: $0.14s^{-1}$, 37°C). The samples were then removed from the PPFC, fixed and stained to evaluate cell number and confluency. Co-culture experiments were conducted by preparing a bacterial suspension and a U-2OS suspension as described above. Approximately $1x10^4$ bacteria cm^{-2} were first adhered to the films, then a U-2OS suspension was added and initial static adhesion was followed by flow of modified culture media for 48h as above. Cell-bacterial interactions were evaluated by microscopy.

PASW statistics 18 (IBM SPSS) was used for data analysis and significance was identified when $P<0.05$.

Results: Plasma treatment of PEEK resulted in a decrease in water contact angle, an increase in atom% surface oxygen, corresponding to an increase in oxygen functional groups, and a slight increase in surface roughness after 1800s treatment due to surface etching.

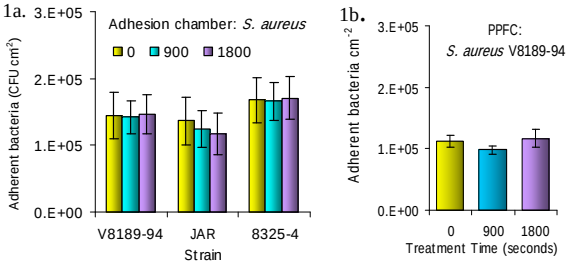

Fig.1. S. aureus adhesion to plasma treated PEEK in the adhesion chamber (1a) and PPFC (1b). n=3 ±s.e.

The quantity of adherent bacteria after 2.5h was shown to not increase on plasma treated surfaces for both *S. aureus* (Fig.1) and *S. epidermidis* using both methods.

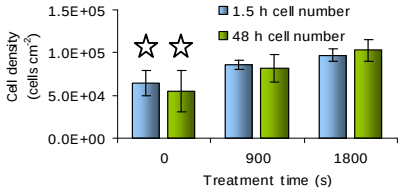

Fig.2. U-2OS cell density after the initial 1.5h adhesion and 48h growth periods in the presence of S. epidermidis RP12.n=3 ±s.e.

Plasma treatment did not result in a significant change in U-2OS cell adhesion density ($P=0.51$) or confluency ($P=0.80$) after 48h. Co-culture with *S. epidermidis* RP12 did not lead to U-2OS cell death on any of the surfaces. U-2OS cell density was significantly higher on the treated PEEK surfaces after the initial static adhesion period compared to the untreated surface in the presence of *S. epidermidis* RP12 (Fig.2). This trend was also reflected after 48h ($P=0.04$). The correlation between U-2OS cell density and confluence was strong for all surfaces in the co-culture experiments ($r^2=0.87$), suggesting that there was no difference in individual cell spreading on the different surfaces (Fig.2). The co-culture of *S. aureus* V8189-94 with U-2OS cells resulted in observed cell death after ~10h on all surfaces.

Conclusions: The incorporation of polar functional groups and increase in surface roughness caused by oxygen plasma treatment of PEEK was shown to not cause an increase in bacterial adhesion in PBS by two different methods. Therefore, plasma treatment of PEEK remains a promising technique for increasing osseointegration without increasing the risk of bacterial adhesion. In future work the role of proteins in bacterial adhesion to plasma treated PEEK and active antibacterial techniques will also be investigated.

References: [1]Poulsson AHC, Richards RG *Eur Cell Mater* 2008; [2]Subbiahdoss G, *et al. Acta Biomater* 2009; [3]Miles AA, Misra SS *J. Hygiene* 1938.