Enhancement of cell adhesion and proliferation on polyetheretherketone by using plasma surface modification

[1]Yeung K W K; [1]Lui S C; [2]Chu P K; [1]Luk K D K; +[1]Cheung K M C;
+[1]The University of Hong Kong, Hong Kong, China; [2]City University of Hong Kong, Hong Kong, China

Statement of Purpose: Polyetheretherketone (PEEK) has been widely used as bone substitute in orthopaedic surgery, since its mechnical properties are similar to that of human bone. Its bioinertness, however, associates with the unsatisfactory bone-implant integration. To increase its bioactivity, incorporating additional bioactive substance into PEEK matrix has been recently studied. However, alternation of its original mechanical properties is concerned. Alternatively, surface modification using plasma immersion ion implantation (PIII) has been developed in order to incorporate new biofunctional groups onto PEEK surface. The present study aims at investigating the feasibility of ammonia and water plasma treatment in enhancing the surface bioactivity of PEEK.

Methods: PEEK samples measured 5mm in diameter and 3 mm thick were prepared and polished to mirror finish. Water and Ammonia PIII treatments were applied at implantation energy of 10kV, 20kV and 30kV, with radio frequency at 50Hz and pulse width at 30μs for 2 hours. Surface bioactivity assessments using SaOs-2 osteogenic cells were conducted. In cell adhesion assay, ten thousands cells were cultured on all sample surfaces for 4 hours and the total number of adhered cells was estimated according to the image-sample surface area ratio. Cell proliferation was measured by MTT assay at day 2, 4 and 7 of culturing. Alkaline phosphatase (ALP) expression was assessed by using ALP kit after culturing for 4, 7 and 14 days with supplement of 100 ug/ml ascorbic acid and 10mM β-glycerol phosphate. Mineralization assay was applied at day 14 and the mineralized area was quantified by image analyzer. The minerals formed on the surfaces were qualified by energy-dispersive X-ray spectroscopy (EDX).

Results: The result of cell adhesion testing revealed that more cells attached to the ammonia plasma treated samples (20kV and 30kV) as compared with the untreated PEEK ($p<0.05$) (Fig. 1). In general, all treated samples except NH_3 10kV were better than the untreated one. The ALP expression of all plasma samples treated with 30kV was higher than the untreated at Day 7 ($p<0.05$) (Fig. 2). At Day 14 cell culturing, the ALP expression and mineralization of the plasma treated samples (H_2O 30kV and NH_3 30kV) were not significantly different from the untreated (Fig. 3). Apatite-like structure was found on the ammonia plasma treated surface. The EDX analysis confirmed the minerals were Ca and P rich substances.

Discussion: Our biological testing results suggested that the bioactivity of PEEK could be enhanced by water and ammonia plasma treatments in particular to the initial cell attachment and proliferation. Based on the results of cell adhesion, high implantation energy can produce better biological response as compared with low energy implantation. We suspect that new biofunctional groups cannot be established by low energy implantation. Additionally, ALP expression at day 4 and 7 suggested that osteoblast activity was significantly increased in the treated samples (H2O 30kV and NH3 30kV). The evidences suggested that the current plasma treatments could stimulate osteoblast activity at the early stage of cell-material interaction. The effect comes to plateau after 14 days of culturing. All these bioactivity enhancements may be attributed to the newly formed nitrogen- or oxygen-containing functional groups on PEEK surfaces, which have been characterized in our previous studies.

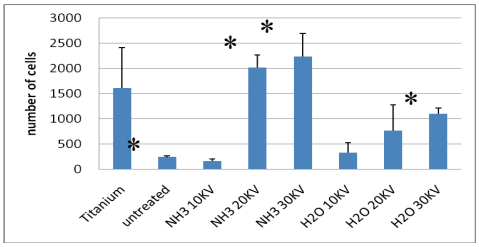

Fig.1 Result of cell adhesion testing of plasma treated, untreated PEEK and medical grade titanium alloy serving as control

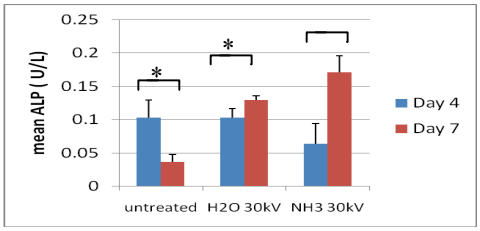

Fig. 2 ALP assay results at day 4 and day 7

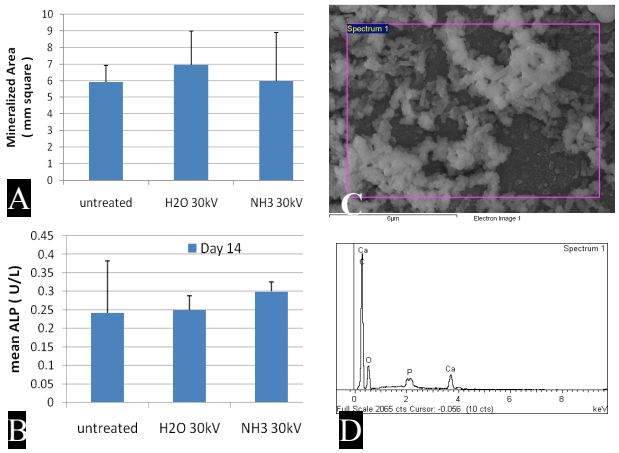

Fig. 3 (A) Mineralized area on the sample surfaces after 14 days culturing; (B) The corresponding ALP expression at day 14; (C) SEM micrograph of surface morphology of the ammonia plasma treated surface (NH_3 30kV); and (D) EDX analysis of the minerals formed on the plasma treated surface

Conclusions: Water and ammonia plasma immersion ion implantation treatments can help promote osteoblast adhesion and proliferation on PEEK surface. High implantation energy can result in better biological response.

Acknowledgement: This study was financially supported by HKU Seed Funding for Basic Research.