In vitro apatitic formation on the surface of poly(methyl methacrylate)

Sun-Mi Choi, Ju-Yeon Han, Jung-A Lee, Su-Youn Kang and Woo-Kul Lee †

Laboratory of Biomaterials and Tissue Engineering, Department of Chemical Engineering, Institute of Tissue Regeneration Engineering, College of Engineering, Dankook University, Jukjeon-dong, Yongin-si, Gyeonggi-do, Korea

Statement of Purpose: Poly(methyl methacrylate) (PMMA) is a biopolymer widely used as bone cement [Kenny, 2003]. But PMMA is bioinert and it is a typically hydrophobic polymer. Therefore, biological apatite is not readily formed on PMMA in vivo. The purpose of the current study was surface modification through base hydrolysis to improve apatitic film formation. Although several studies have attempted to modify the surface properties of PMMA for apatite formation, it has not been so successful yet [Varma, 2003]. In order to examine the effect of surface modification on in vitro apatitic film formation, PMMA was treated with NaOH. Surface hydrophobicity of the NaOH-treated PMMA was determined by measuring contact angle and XPS. Apatitic film on treated PMMA samples was analyzed by FE-SEM, FTIR and XPS. To examine the cytotoxicity of PMMA, we performed adhesion and proliferation assays of osteoblast-like MG63 cells.

Methods: Intact PMMA(P1) was purchased from LG MMA(HP202, Korea) and cut into a desired shape. PMMA samples were treated with NaOH solution at room temperature (P2). Hydrophobicity of P1 and P2 samples was measured using the goniometer (Phoenix-300, SEO, Korea). The chemical state of the film was examined with XPS (ESCALAB 250 XPS Spectrometer, VG Scientifics, England) and was identified through peak analysis. P1 and P2 put into 4-well dish and put into calcium and phosphorus ionic solution. An apatitic thin film was formed. The surface morphology of apatite-coated PMMA (P1CP) and apatite-coated PMMA after NaOH treatment (P2CP) was observed using field emission scanning electron microscopy (S-4300, HITACHI, Japan) and a chemical bond of apatite layers was examined using ATR-FTIR (Perlkin Elmer, USA). To evaluate the biocompatibility and cytocompatibility of P1 and P2, human osteoblast-like MG63 cells were cultured on PMMA samples. The adhesion and proliferation of MG63 cells were assayed using crystal violet assay kit (Sigma, C-6258) and cell counting kit (CCK-8, Sigma), respectively.

Results: After PMMA surfaces were treated through base hydrolysis method, contact angle measurements were decreased with treatment time, indicating that PMMA surface became hydrophilic. Surface analysis on XPS spectrum revealed that the oxygen-containing carbon was increased, implying that the PMMA surface became more hydrophilic. At the same time, SEM micrographs showed that apatitic film was sparsely formed on P1CP while the film was fully grown film on P2CP [Fig. 1].

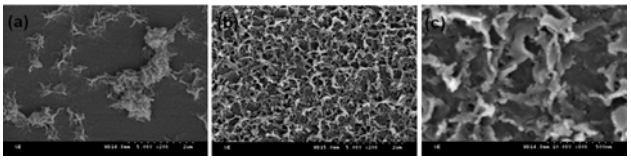

Fig. 1. SEM micrographs of apatitic film formed on PMMA: (a) P1CP(x20000), (b) P2CP(x20000), and (c) higher magnification of (b) (x80000).

IR spectral band of P2CP showed a peak at 1025 cm^{-1} which can be assigned to the ν_3 vibration of PO_4^{-3}. This indicates that apatitic film was formed successfully [Sailaja, 2006]. The value of Ca/P ratio for P2CP was 1.3 indicating that the apatitic film was similar to OCP. To determine the biocompatibility of the apatitic film, in vitro cellular assay using osteoblast-like MG63 cells displayed that cell adhesion ability was increased and no cytotoxic effects were detected. Fig. 2 shows adherent cell morphology with well-developed processes.

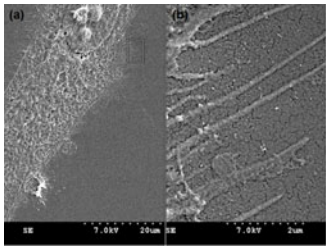

Fig. 2. Cell morphology: (a) adherent cell morphology on P1 (2000x), (b) higher magnification of the part of (a) (x20000).

Conclusions: This study demonstrated that the surface modification of PMMA mediated by base hydrolysis appeared to be effective to promote the in vitro apatitic film formation. When PMMA was treated with NaOH, the hydrophilicity of PMMA was increased. Time required for apatitic film formation was greatly shortened. We confirmed that apatitic film was well formed using FT-IR and XPS. The characteristics of the apatitic film resemble OCP. The prepared apatitic film was confirmed to be cytocompatible via cellular assays. The information obtained in this study will be useful for the understanding of in vivo bone formation

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