The effectiveness of UV-irradiation for osteoblast-like cell adhesion to poly-ether-ether-ketone surfaces

[+1]Okada, Y; [2]Abe, N; [1]Yoshida, A; [1]Furumatsu, T; [1]Miyazawa, S; [1]Yokoyama, Y; [1]Takata, N; [2]Tanaka, R; [3]Takahashi, H; [3]Fujiwara, K; [3]Kimura, H; [1]Ozaki, T
[+1]Dept. of Orthopaedic Surgery, Okayama University Graduate School, Okayama
[2]Dept. of Intelligent Orthopaedic System, Okayama University Graduate School, Okayama
[3]Nakashima Medical Co., Ltd., Okayama
Senior author: yukimasaokada@gmail.com

INTRODUCTION

Poly-ether-ether-ketone (PEEK) has suitable biomechanical and stable chemical properties without cytotoxic effects. This biomaterial is now broadly accepted as a radiolucent alternative to metallic biomaterials in the spine community[1)]. We are considering using this biomaterial in the new method of the joint reconstruction, but few studies had been reported about the adhesion of mesenchymal cells to the PEEK surface[2)]. The UV-irradiation were sometimes used to improve the biocompatibility of biomaterials. In this study we investigate the effectiveness of UV-irradiation for the adhesion of osteoblast-like cells through the effect of the changed surface properties and this possibility for its adaptation to the minimally invasive joint replacement.

MATERIALS AND METHODS

Materials: PEEK substrates (Victrex PEEK 450G, Yasojima Proceed Co.,Ltd., Japan) were polished with 1.2~1.8 μm cerium oxide particles "SHOROX grade A-10" (Showa Denko K.K., Japan). We used the polished PEEK (the surface roughness Ra ≒ 20nm) plates manufactured 1mm in thick and 10 mm in diameter.

UV-irradiation: The UV treatment was performed by excimer irradiation unit with a 10 mW/cm^2 Xe_2 excimer lamp at 172 nm wavelength in the N_2 gas. The samples were fixed at a distance of 10 mm and irradiated for 1 h and 6h. Then, they were washed in order to remove the low molecular weight products, dried at room temperature and then characterized. Each PEEK plates groups were prepared as no UV-irradiated PEEK (UV0), UV-irradiated for an hour PEEK (UV1) and UV-irradiated for six hours PEEK (UV6). To evaluate the hydrophilic effect, the surface was analyzed by contact angles immediately following UV-irradiation and at seven days after treatment. Contact angles H_2O measured under room temperature using a DM-501 contact angle meter (Kyowa Interface Science Co., Ltd., Japan).

Cell cultutre: The mouse osteoblast-like cells (MC3T3-E1) were cultured on each PEEK plates at four days after UV-irradiation treatment. Cells were plated at a 100,000 cells/well in DMEM with L-glutamine and phenol red supplemented with penicillin–streptomycin and 10% fetal bovine serum. We collected each PEEK plates at six, twelve and twenty-four hours after culturing cells to analysis about cell adhesion described below.

Cell adhesion and proliferation assay: We analyzed the adhered cells on the PEEK plates by crystal violet staining assays (CVS). After each PEEK plates were washed with PBS, the cells attached on the plates were fixed and stained with 0.05% crystal violet solution for 30 min. After the plates was washed with distillated water and dried, the absorbance at 540 nm was measured by the microplate reader.

Scanning electron microscopy (SEM): For SEM analysis, at the end of the culture period as described above, cells were washed with serum-free medium and then fixed with 2.0% glutaraldehyde and 2.0% formaldehyde in PBS for eight hours, then washed with PBS for one hour. The cells were dehydrated through an increasing ethanol gradient to absolute ethanol, then substituted and dried with tert-butyl alcohol. The cells were mounted on specimen stubs and sputter coated with osmium. Samples were examined using an electron microscope (HITACHI Scanning Electron Microscope Model S-4800, Japan) with 15-kV accelerating voltage.

RESULTS

The contact angle H_2O (figure 1) of the UV0, UV1 and UV6 were 87.1°(A), 38.5°(B), 35.9°(C) immediately following UV-irradiation, and seven days later from UV-irradiation the contact angle H_2O of each PEEK were 87.1°(D), 73.2°(E), 71.9°(F). The contact angle H_2O was reduced with the UV-irradiation time. However, these effects were not continued and thus contact angle H_2O were increased back at seven days after UV-irradiation. The absorbance of the CVS was higher in UV1 and UV6 compared with UV0, but there was no significant differences among them (figure 2). At the SEM analysis (figure 3), larger amount and more flattened cells were shown on the UV1 (D, E, F) and UV6 (G, H, I).

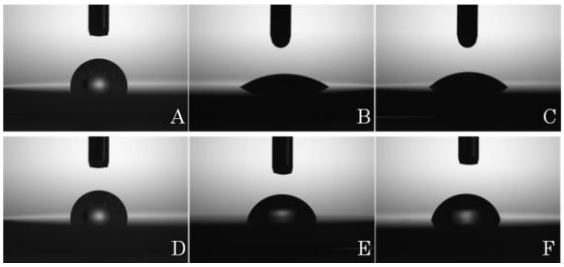

Figure 1. The contact angle H_2O, immediately after UV-irradiation of the UV0 (A), UV1 (B), UV6 (C), at seven days after UV-irradiation of UV0 (D), UV1 (E), UV6 (F).

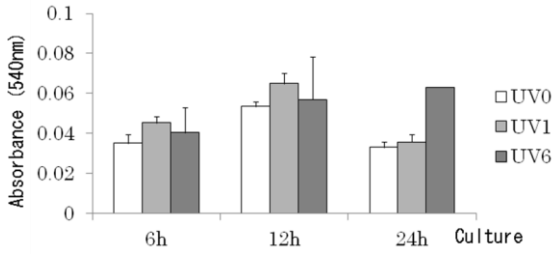

Figure 2. The absorbance of the CVS were analyzed with UV0, UV1, UV6 at six, twelve and twenty-four hours after culturing.

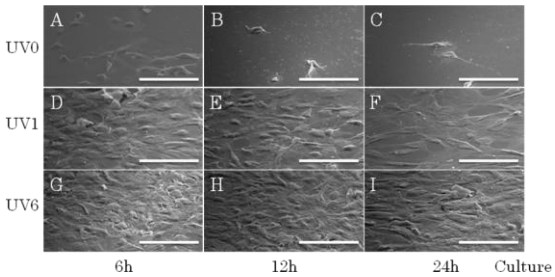

Figure 3. SEM analysis of the cultured cells on the PEEK surface: (A) UV0, 6h (B) UV0, 12h (C) UV0, 24h (D) UV1, 6h (E) UV1, 12h (F) UV1, 24h (G) UV6, 6h (H) UV6, 12h (I) UV6, 24h. Bars, 100μm.

DISCUSSION

The UV-irradiation is one of the surface treatment methods which improve the surface hydrophilicity of metals or plastic materials. There are two mechanisms improving surface hydrophilicity, which are the UV sterilization effect to degrade the organic contaminants on the surface and the UV surface modification to produce the hydrophilic functional groups on the surface[3)]. In this study, the contact angle H_2O was decreased by the UV-irradiation, but this effect was not continued. We assume that only the effect of UV surface modification remained when we cultured the cells at four days after UV-irradiation. And thus our results showed 1 hour duration of UV-irradiation could be proper for cell adhesion and proliferation. The UV surface modification might be able to improve the biocompatibility of the PEEK surface.

SIGNIFICANCE

Our results indicated that the biocompatibility of the PEEK was increased by UV-irradiation and this technique might be applied in the minimally invasive joint reconstruction.

REFERENCES

1) Steven M Kurtz, John N Devine. Biomaterials 2007;28:4845-69.
2) Karen B Sagomonyants, Marcus L Jarman-Smith, et al. Biomaterials 2008;29:1563-72.
3) Iva Pashkuleva, Alexandra P. Marques, et al. J Mater Sci Mater Med 2010;21:21-32.