Title

Modification of the cell adhesion and hydrophilic characteristics of poly(ether-ether-ketone) by 172-nm Xenon excimer radiation

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Abstract

BACKGROUND: Poly-ether-ether-ketone (PEEK) has biomechanical and chemical properties that are excellent for biomedical applications; however, PEEK adhesion to bone or chondral tissue proceeds slowly due to poor hydrophilicity and other surface characteristics.

OBJECTIVE: We investigated the structural change, hydrophilicity, and cytocompatibility of a PEEK surface after 172-nm xenon excimer UV-irradiation.

METHODS: The surface characteristics before and after irradiation were evaluated by contact angle and ATR-FTIR measurements. Mouse osteoblast-like cells (MC3T3-E1) were cultured on PEEK plates and collected after 6, 12, and 24 h for cell adhesion analysis by crystal violet staining (CVS) and scanning electron microscopy (SEM).

RESULTS: UV irradiation improved PEEK surface hydrophilicity, as indicated by a significant drop in water contact angle ($p < 0.05$). Irradiated PEEK showed additional peaks around 3370 cm^{-1} and 1720 cm^{-1}, highlighting the generation of hydroxyl and carbonyl groups. CVS and SEM revealed improved adhesion to the PEEK surface after UV-irradiation.

CONCLUSION: Our results suggest that 172-nm UV-irradiated PEEK may be used in biomedical applications that require good cell adhesion.

1. Introduction

Poly-ether-ether-ketone (PEEK) has stable and desirable biomechanical and chemical properties and produces no cytotoxic side effects [1,2]. Metal alloys are typically used for joint replacement in orthopedic surgery; however, they are stiffer than human bone and can cause periprosthetic resorption of the bone through stress protection [3]. The good mechanical and chemical properties of PEEK avoid the disadvantages of metal and provide a suitable implant material [4]. PEEK elasticity is similar to that of human bone, so stress protection is not expected after implantation. It has a good combination of stiffness, tensile strength, distortion, abrasion, and fatigue resistance; it thus seems suitable for syndesmoplasty [4]. However, PEEK adhesion to the bone or chondral tissue proceeds slowly because of the roughness, wettability, and chemical composition of the PEEK surface [5,6].

Several reports have described PEEK surface modifications, including plasma treatment, to provide a better substrate for cell attachment and proliferation [4,7,8]. UV irradiation is another effective technique for modifying polymer surface characteristics [2,3]. UV irradiation can be performed rapidly, is low-cost, and no bulk materials are lost during treatment [9,10]. Changes in the chemical composition of the PEEK surface through excimer laser treatment has been reported [11-13], but to the best of our knowledge, no similar report has described the use of excimer lamps to improve cell adhesion to PEEK.

In this study, we investigated the structural change, hydrophilicity, and cytocompatibility of PEEK surfaces after UV irradiation with a 172-nm xenon excimer lamp. PEEK surfaces were irradiated for various periods and cell adhesion was monitored over time. The excimer lamp used in this study is suitable for irradiation of large complex shapes, such as orthopedic implants.

2. Materials and Methods

2.1 Materials

PEEK substrates (Victrex PEEK 450G, Yaojima Proceed Co., Ltd., Japan) were polished with 1.2~1.8 μm cerium oxide particles (SHOROX grade A-10, Showa Denko K.K., Japan). The polished PEEK plates (surface roughness Ra ≈ 20 nm) were 1 mm thick and 10 mm in diameter.

2.2 UV irradiation

All samples were washed with acetone and distillated water to remove low-molecular-weight products, and then dried at room temperature. UV treatment was performed with an excimer irradiation unit with a 10 mW/cm^2 Xe_2 excimer lamp at 172-nm in N_2 gas. The samples were fixed at a distance of 10 mm and irradiated for 1 and 6 h. PEEK plate groups were classified as non-UV-irradiated (UV0), 1-h irradiated (UV1), and 6-h irradiated (UV6).

2.3 Surface analysis

Surface hydrophilicity was evaluated by measuring water contact angles. Measurements were performed at room temperature using a DM-501 contact angle meter (Kyowa Interface Science Co., Ltd., Japan). Changes in surface functional groups were estimated by ATR-FTIR. Spectra were collected with an FTIR spectrometer (Spotlight100S, Perkin Elmer, Inc.) by a universal attenuated total reflection infrared method [14] with a resolution of 4 cm^{-1} and averaged over eight scans.

2.4 Cells and cell culture

Mouse osteoblast-like cells (MC3T3-E1) were cultured on the irradiated and non-irradiated PEEK plates. 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 the plates after 6, 12, and 24 h culture to analyze cell adhesion.

2.5 Cell adhesion assay

Adhered cells were analyzed by crystal violet staining (CVS) as described by Saotome et al. [15]. After washing with PBS, the adhered cells were fixed and stained with 0.05% crystal violet for 30 min. After washing with distillated water and drying, the dye was solubilized with methanol, transferred to 96-well plates, and absorbance was measured at 540 nm in a microplate reader.

2.6 Scanning electron microscopy

Adhered cells were observed by scanning electron microscopy (SEM) after being washed with serum-free medium, fixed with 2.0% glutaraldehyde and 2.0% formaldehyde in PBS for 8 h, and then washed with PBS for 1 h. 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.

2.7 Statistical analysis

Contact angle measurements were performed five times and CVS assays were repeated seven times. Results are reported as the mean ± standard error of the mean. Significant differences were evaluated by Student's *t*-test. P-values < 0.05 were considered statistically significant.

3. Results

3.1 Contact angle

UV irradiation improved PEEK surface hydrophilicity (Fig. 1). The average contact angle on the UV0, UV1, and UV6 PEEK surfaces was 87.1° ± 3.4°, 60.9° ± 4.1°, and 61.4° ± 5.1°, respectively (Fig. 1, A-D). The contact angles of UV1 and UV6 were significantly lower than that of UV0 ($P < 0.05$; Fig. 1D).

3.2 FTIR spectroscopy

A peak around 3370 cm^{-1} was also observed in irradiated samples and was attributed to the hydroxyl group (Fig. 2A). In the carbonyl region, spectral differences revealed the presence of a maximum around 1720 cm^{-1} (Fig. 2B) representing the C=O stretching frequency of aromatic esters and accounting for the photo-transformation of the benzophenone units.

3.3 CVS and SEM analyses

CVS and SEM analyses showed improved cytocompatibility of the PEEK surface after UV irradiation. In the CVS assay, there was significantly higher absorbance on UV1 and UV6 than on UV0 after 6, 12, and 24 h culture (Fig. 3). There were more adhered cells on UV1 and UV6 than on the UV0 PEEK surfaces ($p < 0.05$). SEM showed adhered cells on the UV1 and UV6 PEEK surfaces (Fig. 4).

4. Discussion

PEEK compatibility with osteocartilaginous tissues is relatively low in spite of its desirable biomechanical and stable chemical properties [1,2,5,6]. Biomaterial compatibility is influenced by hydrophobicity, surface roughness, and chemical properties [16,17]. Several studies have reported PEEK-associated processes such as composite formation, surface coating, and surface treatment [4,18-22]. Plasma surface treatment forms a hydrophilic organization on the PEEK surface, thus improving hydrophilicity and cytocompatibility [4]. Electron beam deposition, which is a surface coating technique, also improves PEEK cytocompatibility without influencing its mechanical properties [19]. Roughness and surface chemistry in particular control protein adsorption to the extracellular matrix, which is responsible for successful adhesion of endogenous cells to the biomaterial and for stable bonding of the implant to the surrounding tissue [4].

One of the major mechanisms for improving PEEK hydrophilicity and cytocompatibility by UV-irradiation is surface modification in the form of carbonyl and hydroxyl structures on the PEEK surface, as shown by FTIR. UV irradiation is effective for various kinds of materials, and the formation of functional groups by UV irradiation is thought to be equivalent to those formed by plasma treatment [23]. UV irradiation alters the molecular structure of starch-based surfaces, improving their hydrophilicity and cytocompatibility [23]. UV irradiation also improves the molecular structure and cytocompatibility of titanium surfaces [24]. In our study, UV irradiation improved the

hydrophilicity and cytocompatibility of PEEK surfaces (Fig. 1, 3). Our technique is simpler and costs less than other surface treatment or coating methods. Our results suggest 1 h UV-irradiation may be sufficient to improve PEEK surface biocompatibility.

It is important to note that PEEK covalent bonds degrade at 172 nm UV. However, in this study it was observed that only the surface response is altered, potentially leaving the mechanical strength of the material intact. Thus, this experiment examines only the initial stability, and long-term clinical stability needs to be examined in vivo in the future.

In conclusion, the molecular structure and cytocompatibility of PEEK surfaces was improved by UV irradiation. Our results suggest UV irradiation may be useful for improving cell adhesion to PEEK surfaces.

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References

[1] Rivard CH, Rhalmi S, Coillard C. In vivo biocompatibility testing of peek polymer for a spinal implant system: a study in rabbits. J Biomed Mater Res. 2002; 62:488.

[2] Toth JM, Wang M, Estes BT, et al. Polyetheretherketone as a biomaterial for spinal applications. Biomater. 2006; 27:324.

[3] Morrison C, Macnair R, MacDonald C, Wykman A, Goldie I, Grant MH. In vitro biocompatibility testing of polymers for orthopaedic implants using cultured fibroblasts and osteoblasts. Biomater. 1995; 16(13):987.

[4] Briem D, Strametz S, Schröder K, Meenen NM, Lehmann W, Linhart W, et al. Response of primary fibroblasts and osteoblasts to plasma treated polyetheretherketone (PEEK) surfaces. J Mater Sci Mater Med. 2005; 16:671.

[5] Kurtz SM, Devine JN. PEEK biomaterials in trauma, orthopedic, and spinal implants. Biomaterials. 2007; 28:4845.

[6] Edwards SL, Werkmeister JA. Mechanical evaluation and cell response of woven polyetheretherketone scaffolds. J Biomed Mater Res Part A. 2012; 100(12):3326.

[7] Comyn J, Mascia L, Xiao G, Parker BM. Plasma-treatment of polyetheretherketone (PEEK) for adhesive bonding. Int J Adhesion Adhesives. 1996; 16(4):97.

[8] Hegemann D, Brunner H, Oehr C. Plasma treatment of polymers for surface and adhesion

improvement. Nucl Instrum Meth B. 2003; 208:281.

[9] Comesaña R, Quintero F, Lusquiños F, Pascual MJ, Boutinguiza M, Durán A, Pou J. Laser cladding of bioactive glass coatings, Acta Biomater 2010; 6(3):953.

[10] Laurens P, Sadras B, Decobert F, Arefi-Khonsari F, Amouroux J. Laser induced surface modifications of poly(ether ether ketone): influence of the excimer laser wavelength. J Adhes Sci Technol. 1999; 13(9):983.

[11] Laurens P, Ould Bouali M, Meducin F, Sadras B. Characterization of modifications of polymer surfaces after excimer laser treatments below the ablation threshold. Appl Surf Sci 2000; 154:211.

[12] Laurens P, Sadras B, Decobert F, Arefi-Khonsari F, Amouroux J. Enhancement of the adhesive bonding properties of PEEK by excimer laser treatment. Int J Adhesion Adhesives 1998; 18:19.

[13] Riveiro A, Soto R, Comesaña R, Boutinguiza M, del Val J, Quintero F, Lusquiños F, Pou J. Laser surface modification of PEEK. Appl Surf Sci. 2012; 258:9437.

[14] Giancaterina S, Rossi A, Rivaton A, Gardette JL. Photochemical evolution of poly(ether ether ketone). Polymer Degrad Stabil. 2000; 68(1):133.

[15] Saotome K, Morita H, Umeda M. Cytotoxicity test with simplified crystal violet staining method using microtitre plates and its application to injection drugs. Toxicol in vitro. 1989; 3:317.

[16] Lampin M, Warocquier-Clérout, Legris C, Degrange M, Sigot-Luizard MF. Correlation between substratum roughness and wettability, cell adhesion, and cell migration. J Biomed Mater Res. 1997; 36(1):99.

[17] Anselme K, Noel B, Hardouin P. Human osteoblast adhesion on titanium alloy, stainless steel, glass and plastic substrates with same surface topography. J Mater Sci Mater Med. 1999; 10:815.

[18] Kim S, Lee KJ, Seo Y. Polyetheretherketone (PEEK) surface functionalization by low-energy ion-beam irradiation under a reactive O2 environment and its effect on the PEEK/copper adhesives. Langmuir. 2004; 20:157.

[19] Han CM, Lee EJ, Kim HE, Koh YH, Kim KN, Ha Y, Kuh SU. The electron beam deposition of titanium on polyetheretherketone (PEEK) and the resulting enhanced biological properties. Biomater. 2010;1:3465.

[20] Yu S, Hariram KP, Kumar R, Cheang P, Aik KK. In vitro apatite formation and its growth kinetics on hydroxyapatite/polyetheretherketone biocomposites. Biomater. 2005; 26(15):2343.

[21] Kim IY, Sugino A, Kikuta K, Ohtsuki C, Cho SB. Bioactive composites consisting of PEEK and calcium silicate powders. J Biomater Appl. 2009; 24(2):105.

[22] Ma R, Weng L, Bao X, Song S, Zhang Y. In vivo biocompatibility and bioactivity of in situ synthesized hydroxyapatite/polyetheretherketone composite materials. J Appl Polym Sci. 2012; 127(4):2581.

[23] Pashkuleva I, Marques AP, Vaz F, Reis RL. Surface modification of starch based biomaterials by oxygen plasma or UV-irradiation. J Mat Sci Mat Med. 2010; 21(1):21.

[24] Aita H, Att W, Ueno T, Yamada M, Hori N, Iwasa F, et al. Ultraviolet light-mediated photofunctionalization of titanium to promote human mesenchymal stem cell migration, attachment, proliferation and differentiation. Acta Biomater 2009; 5:3247.

Figure legends

Fig. 1

H_2O contact angle. The average H_2O contact angle on UV0 (A), UV1 (B), and UV6 (C) PEEK surfaces was 87.1° ± 3.4°, 60.9° ± 4.1°, and 61.4° ± 5.1°, respectively (n = 5). UV-irradiation reduced the H_2O contact angle in the UV1 and UV6 groups (D); * $p < 0.05$

Fig. 2

FTIR spectroscopy. UV1 and UV6 showed additional peaks around 3370 cm^{-1} (A) and 1720 cm^{-1} (B), indicating OH and C=O stretching.

Fig. 3

CVS assay. Significantly higher absorbance in the UV1 and UV6 groups indicated greater cell adhesion; * $p < 0.05$

Fig. 4

SEM analysis. UV0 (A-C), UV1 (D-E), UV6 (G-H). Cultivation time: 6 h (A, D, G), 12 h (B, E, H), 24 h (C, F, I). More adhered cells were observed on the UV1 and UV6 vs. the UV0 surfaces after 6 h culture; Bars, 100 µm

Figure 1

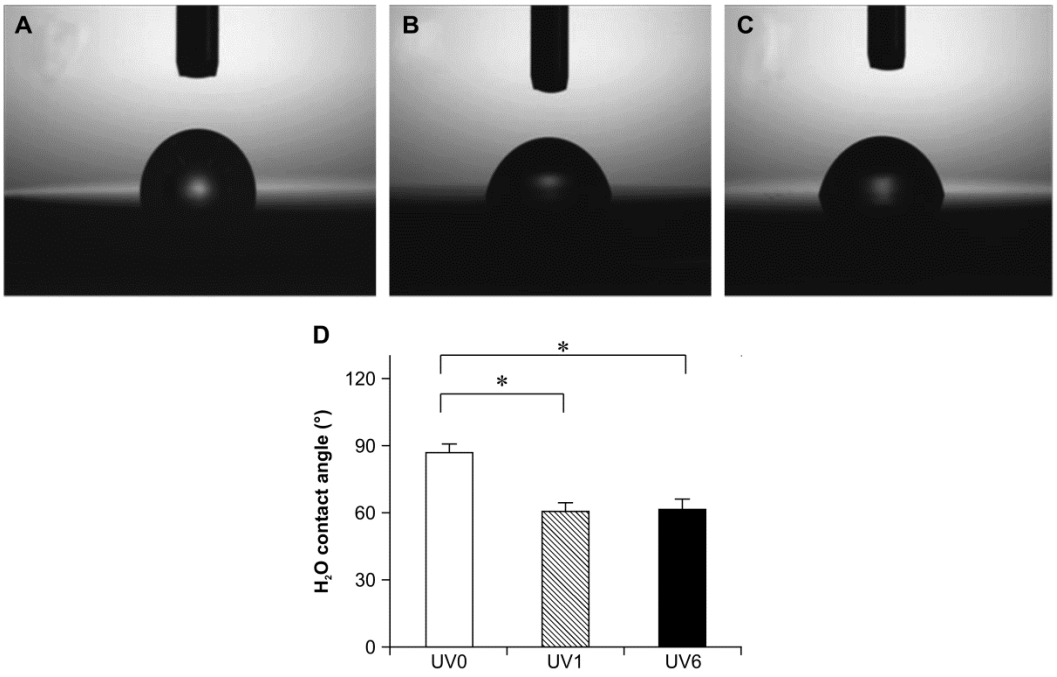

Figure 2

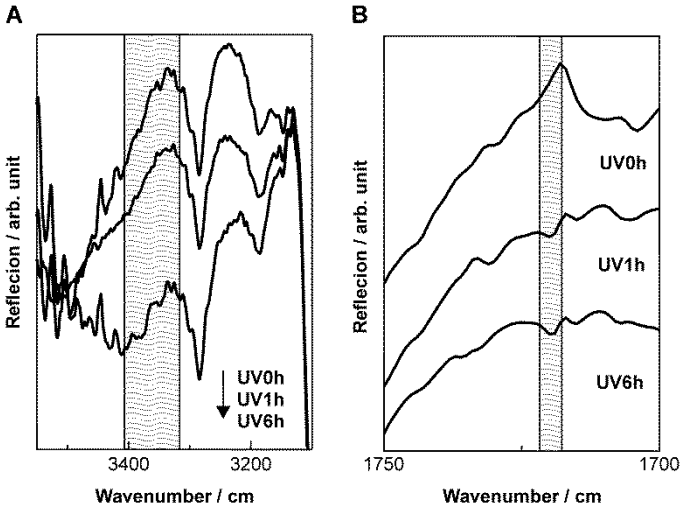

Figure 3

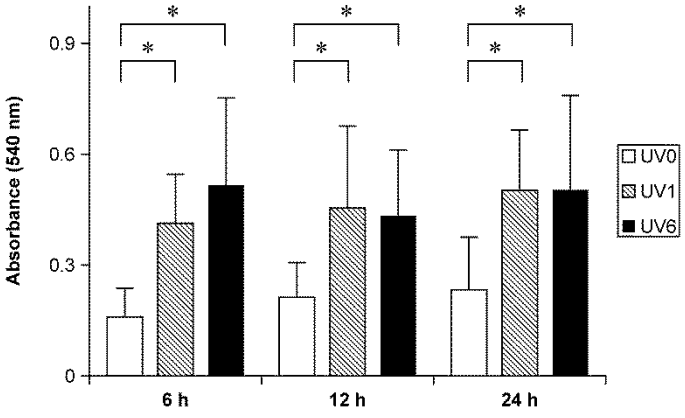

Figure 4