In vivo bioactivity of porous polyetheretherketone with a foamed surface

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The in vivo bioactivity of porous polyetheretherketone (PEEK) with a foamed surface was evaluated using rabbit femoral bone. Cylindrical porous PEEK scaffolds, with pore diameter of 550 μm and porosity of 70%, were first prepared and immersed in 98% sulfuric acid, and then washed and immersed in 3 M potassium carbonate solution used as a foaming reagent. Numerous open pores of various sizes, as well as new functional groups, were visualized on the treated PEEK surface by scanning electron microscopy and X-ray photoelectron spectroscopy, respectively. Micro computed tomography (micro-CT) showed that the volumetric density of treated PEEK was higher than that of bare PEEK at 8 weeks after surgery (p<0.05). Additionally, von Kossa staining indicated ingrowth of mature new bone tissue at 4 weeks relative to the bare PEEK group. Our data indicate that surface-treated PEEK exhibited improved bioactivity in vivo.

Keywords: Porous polyetheretherketone, Bioactivity, Bone, In vivo

INTRODUCTION

Cortical bone harvested from the mandibular ramus provides an excellent source for veneer grafts1). However, most dental implants are cylindrical in shape and therefore not suitable for applications requiring a plate-shaped graft as they are unable to establish adequate contact between the dental implant and the graft bone. Grafts adapted to fit a round implant surface should provide a firmer and more secure attachment2).

Polymers have attracted significant attention as substitutes for metal alloys in orthopedic applications3-7). Polyetheretherketone (PEEK) is widely used as a structural polymer for numerous applications in orthopedics and dentistry5,8-10). Although it provides good biocompatibility, PEEK has a relatively low Young’s modulus that is closer to that of human cortical bone4). In addition, as PEEK is easy to process, it may be utilized for designing veneer grafts adapted to fit a round implant surface.

Porous PEEK, whose porosity and structure are similar to that of trabecular bone, is likely to possess mechanical properties similar to trabecular bone13). Porous PEEK additionally enables increased fixation to bone via ingrowth of bone tissue into pores; furthermore, solid-porous PEEK hybrids are envisaged to provide both structural integrity and fixation with bone20.

However, PEEK is a bioinert material whose fixation with bone is limited. Surface modification of PEEK such as application of bioactive coatings by plasma spray, as well as the use of PEEK composites with bioactive reagents, is known to increase the fixation of PEEK with bone13). In addition, direct bone contact to the implant has been achieved by incorporating bioactive materials, such as hydroxyapatite, into PEEK10). The chemical composition of surfaces obtained by wet treatment, which involves direct chemical reaction with solutions, is favorable for the generation of bone implants. Kasahara and Sawamura14) demonstrated that the formation of a layer consisting of open pores of varying diameters could be achieved on a solid plastic material surface using sulfuric acid and a foaming reagent.

The aims of this study were to fabricate a foamed layer on the surface of porous PEEK utilizing a novel technique based on the use of sulfuric acid and a foaming reagent, and to investigate the in vivo bioactivity of the resulting material.

MATERIALS AND METHODS

Porous PEEK with a foamed surface

Cylindrical porous PEEK scaffolds with a diameter of 4 mm, height of 7 mm, pore diameter of 550 μm, and porosity of 70% were generated using salt combined with PEEK powder and compression-molded PEEK (450G, Victrex plc, Thornton-Cleveleys, UK) (Figs. 1(a), (b)). The scaffolds were ultrasonically cleaned in ethanol and ultra-high purified water, and dried at 80°C for 5 h. The dried samples were immersed in 98% sulfuric acid for 5 min and then washed with water repeatedly until the pH value of the water returned to neutral. Finally, the samples were dried for 5 h at 120°C and sterilized.

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using ethylene oxide gas. Each immersion step was accompanied by stirring (Figs. 1(a), (b)).

**SEM observation**
The surface morphology of the materials was observed by scanning electron microscopy (SEM) using a 5-kV S-4800 scanning electron microscope (Hitachi High Technologies, Tokyo, Japan) after the sample was dried on an aluminum stub and sputter-coated with Pt-Pd.

**X-ray photoelectron spectrum measurements**
X-ray photoelectron spectra (XPS) of the PEEK surfaces were obtained using a PHI X-tool (Ulvac-Phi, Kanagawa, Japan) equipped with an Al-Kα radiation source (15 kV; 53 W; spot size: 205 μm) at a pass energy of 224 eV (wide scan) or 112.00 eV (narrow scan), a step size of 0.100 eV, and a takeoff angle of 45°, with 20 scans. The measurements were conducted for three randomly selected points on each sample, and the concentrations of all functional groups on PEEK surfaces were calculated from the areas of the relevant spectral peaks.

**Animal studies**
Nine male Japanese white rabbits, weighting approximately 3.0 kg were used for the in vivo study. All animals were operated under general anesthesia. A 1-to-1 mixture of ketamine hydrochloride (Daiichi Sankyo, Tokyo, Japan) and xylazine (Byer Yakuhin, Osaka, Japan) was used for general anesthesia. The samples were sterilized using ethylene oxide gas before implantation. To implant the samples in the metaphysis of the femur, identical defects (diameter: 4 mm) were then made using a twist drill and the two sample types (bare PEEK and treated PEEK) were implanted into the femoral bone (1 sample in each leg). The animals were sacrificed after 4, 8, or 12 weeks post-operation. Three rabbits were operated for each implantation time and sacrificed under excess somnopentyl anesthesia (Kyoritsu Seiyaku, Tokyo, Japan). All procedures in this study were approved by the Animal Experiment Committee of Hamri (approval number: 14-039).

**Radiographic analysis**
The femoral bone samples were harvested for examination by micro-computed tomography (SMX-130CT, Shimadzu, Kyoto, Japan). Blocks of bone specimens were mounted on the turntable. The exposure parameters were 51 kV and 120 mA. Data obtained from a slice separation of 66.5 μm were stored at a resolution of 512×512 pixels. TRI/3D BON software (Ratoc, Tokyo, Japan) was used to generate a 3D reconstruction via the volume-rendering method for morphological assessment. In the 3D analysis, the total volume (TV; cm³) and bone volume (BV; cm³) were measured using TRI/3D-BON software based on the obtained values. Volumetric density (VD) was then calculated according to the following formula: VD (%) = BV/TV.

**Histological assessment**
The femoral bone samples were fixed in 4% paraformaldehyde for 16 h. Four-micrometer-thick non-decalcified frozen sections were obtained by the Kawamoto method and stained with hematoxylin and eosin (H&E), von Kossa, and tartrate-resistant acid phosphatase (TRAP). Sections were visualized using a BZ9000 All-in-One Fluorescence Microscope (Keyence, Tokyo, Japan). The percentage of the tissue-touching length that directly contacted the scaffold was measured in H&E histological images at 4 weeks post-operation. The contact ratio was measured from three visual fields in each specimen, and the average values were used.

**Statistical analysis**
The means and standard deviation for each parameter...
were compared between groups with by Student’s t test using Statcel2 statistical software (OMS Publisher, Tokorozawa, Japan). \( p<0.05 \) was considered to indicate statistical significance.

**RESULTS**

**SEM analysis**

SEM images of the surfaces of bare PEEK and treated PEEK (Fig. 2) are shown. SEM analysis of the treated PEEK group indicated small open pores with an average diameter of 5 \( \mu \)m or less and large open pores with an average diameter of 10 to 200 \( \mu \)m on the bulk as well as the porous surface.

**XPS analysis**

The wide XPS for the treated PEEK specimens is shown in Fig. 3. Carbon, oxygen, and low levels of unexpected contamination were found to be present on the sample surfaces (Fig. 3). In the narrow scan C1s spectra of the treated PEEK group, the functional groups present within the envelope were resolved into five peaks corresponding to the main C–C/C–H hydrocarbons, C–O

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**Fig. 2** Scanning electron micrographs of polyetheretherketone (PEEK).

**Fig. 3** Wide X-ray photoelectron spectra of treated PEEK.

**Fig. 4** Peak fit of C1s X-ray photoelectron spectra of (a) bare PEEK and (b) treated PEEK.
Fig. 5 Micro computed tomography (micro-CT) images at 4, 8, and 12 weeks after surgery.

Fig. 6 Volumetric density (in percent) of each group reflecting the quantity of new bone formed at 4, 8, and 12 weeks after surgery.

Histological analysis
We studied bone regeneration and metabolism using the von Kossa staining method, where the dark brown-stained regions represent the bone tissue. Our findings indicate that mature bone tissue was present inside the pores at week 4 in the treated PEEK group; however, this was not observed in the control group. At 8 and 12 weeks, bone tissue was also observed inside the

Microradiography and volumetric density (VD) analysis
New bone formation post-surgery was evaluated at the prescribed time points by micro-CT. Figure 5 shows cross-sections of the femurs containing the implants at 4, 8 and 12 weeks after surgery. The corresponding percent changes in bone volume are shown in Fig. 6. PEEK could not be visualized by micro-CT, as it is X-ray transparent. An impermeable structure was observed in both the cortical bone and the cancellous bone of both groups; the amount of newly formed bone continuously increased such that the entire defect was filled with new bone at 8 weeks post-surgery in the treated PEEK group. The volumetric density (VD) of each group was determined at 4, 8, and 12 weeks (Fig. 6) post-surgery. The VD of the treated PEEK group was higher than that of the bare PEEK group at 8 weeks ($p<0.05$). There were no significant differences in VD between the two groups at 4 and 12 weeks ($p>0.05$).
pores and surrounding material in the control group; however, the area of bone tissue was lower than that in the treated PEEK group (Fig. 7). Cross-sections of the bone defect sites obtained 4, 8, and 12 weeks after surgery were stained with von Kossa, H&E, and TRAP stains (Figs. 8, 9 and 10). At 4 weeks, von Kossa and H&E staining indicated penetration of the newly formed bone tissue into the forming layer, as well as bonding of the porous network of the treated PEEK. Therefore, the present material is expected to enable stronger adhesion.
for improved fixation of the implant. H&E staining showed that osteoblasts, which are responsible for new bone formation, were also present around both bare and treated PEEK (Fig. 8(e)). TRAP staining revealed the presence of numerous osteoclasts on the forming layer of treated PEEK (Fig. 8(f)). At 8 and 12 weeks, newly formed bone tissue was observed in the bare PEEK; however, the bare PEEK showed gaps between the new bone and the surface of the material. Osteoclasts cells by stained TRAP were observed in both groups (Figs. 9 and 10). Moreover, no inflammation or necrosis was observed in both PEEK groups over the course of the study (Figs. 8, 9 and 10). The proportion of tissue-touching length (contact ratio) was measured using H&E histological
DISCUSSION

Porous metals with pore sizes similar to those of trabecular bone (~700 μm) possess mechanical properties that make them suitable for use in orthopedic applications such as bone augmentation\(^1\). However, porous metals are susceptible to corrosion; in addition, the occurrence of cell ingrowth is difficult to monitor using medical imaging technologies such as MRI\(^1\). The potential for osseoconductivity was previously compared between solid and porous PEEK in cortical and cancellous bone sites, using a sheep model\(^1\). Histological analysis\(^1\) demonstrated adjacent tissue integration and bone ingrowth on the porous PEEK samples; however, in the images, a gap was observed at the interface between the solid PEEK implant and the tissue. In previous studies, salt (NaCl) porogens were used in combination with various processing methods to produce porous polymer structures\(^{16-19}\). The present cylindrical porous PEEK scaffolds were prepared using salt combined with PEEK powder and compression molded PEEK.

Wet surface chemistry has been used to chemically modify PEEK to create surface-functionalized PEEK. One of the known methods includes immersion of a base made of plastic in a corrosive solution such as concentrated sulfuric acid\(^{20-23}\). Zhao \textit{et al.}\(^{22}\) demonstrated that a 3D porous and nanostructured network with bio-functional groups fabricated on PEEK by sulfonation and subsequent water immersion induces pre-osteoblast functions such as initial cell adhesion, proliferation, and osteogenic differentiation \textit{in vitro}, and also substantially enhances osseointegration and bone-implant bonding strength \textit{in vivo}. However, most of the pores are sized between 0.5 and 1.0 μm in the sulfonation-only treatment of Zhao \textit{et al.}\(^{22}\). White and Shors\(^{24}\) demonstrated a relationship between pore size and tissue elements that favored good bone ingrowth: a pore size of 10 μm allowed ingrowth of cells, and a pore size of 15–50 μm and >150 μm favored fibrous tissue formation and new bone formation, respectively. In the present study, the combined use of sulfuric acid and 3 M K\(_2\)CO\(_3\) solution enabled the fabrication of a foamed surface with open pores of various sizes on the surface of the material. When sodium carbonate was used as the foaming agent, the diameter of the pores ranged from 0.1 to 200 μm. In addition, the use of sulfuric acid resulted in formation of an inner wall that connected the large open pores with the small pores\(^{14}\).

A sulfur peak was not detected in the wide XPS spectra for the treated PEEK surface. Sulfuric acid retained on the surface may be removed using distilled water. The C1s spectra of the bare PEEK was fitted to three peaks with binding energies of aromatic C–C, C–O, and C=O, which were identical to those of bare PEEK\(^{26}\). The treated PEEK showed a decrease in the concentration of aromatic carbons, whereas the amount of the total oxygen-containing functional groups was increased. The reasons for this finding are not known; however, it was considered that atomic oxygen reacted with oxygen species resulting in the formation of oxygen-containing functional groups on the surface of treated PEEK. A new peak at 288.7 eV (O–C=O) was observed in the fitted curves of the treated PEEK. This finding indicates that the chemical treatment performed in this study introduced a new functional group to the surface of treated PEEK. The concentration of the C=O group also increased. It has been reported\(^{26}\) that the modification of PEEK surfaces by plasma treatment with higher concentrations of C=O and O=C=O functional groups promotes initial human osteoblast cell attachment. In addition, the expression of alkaline phosphatase, a phenotypic marker of osteoblast differentiation, was observed in the early phase. Our findings indicate that the surface modification of PEEK, as performed in this study, may improve cell-material interaction.

At 4 weeks post-surgery, an impermeable structure was observed in both groups via micro-CT analysis; however, bone tissue could not be detected in the bare PEEK group by von Kossa staining. In contrast, the VD value of the treated PEEK group was higher than that of the control group at 8 weeks. Numerous open pores of various sizes were found to be present on the surface of the treated PEEK, which attracted osteoblasts from native bone, thereby promoting new bone formation and bone remodeling at the early time points. Additionally, obvious bone ingrowth into numerous pores was observed in the treated PEEK, confirming that the porous implant surface was securely anchored to the native bone. Moreover, carbonates were used as the foaming agent and a sulfur peak, corresponding to sulfuric acid, was not detected; these findings indicate that the treated PEEK does not produce observable toxic effects in the surrounding tissues.

![Fig. 11](image)

Fig. 11 The proportion of the tissue-touching length that directly contacted the scaffold was measured using H&E histological images at 4 weeks post-operation. The contact ratio for treated PEEK was significantly higher than that for bare PEEK \((p<0.05)\) (Fig. 11).
CONCLUSION

Porosity in biomaterials enables better integration and interaction with the surrounding bone tissue. Our results suggest that PEEK with a foamed surface exhibits improved bioactivity in vivo, with no observable toxicity. PEEK materials with a foamed surface are more suitable for orthopedic and dental applications and have great clinical potential. Further in vitro studies are necessary to evaluate the bioactive mechanism of the surface treatment process described here.

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REFERENCES

1) Hwang KG, Shim KS, Yang SM, Park CJ. Partial-thickness cortical bone graft from the mandibular ramus: A non-invasive harvesting technique. J Periodontol 2008; 79: 941-944.
2) Kim SG, Song JY, Lee YC. Modified veneer bone graft with the concomitant installation of a dental implant: Technical note. Oral Maxillofac Surg 2011; 15: 189-192.
3) Baba S, Inoue T, Hashimoto Y, Kimura D, Ueda M, Sakai K, Matsumoto N, Hiwa C, Adachi T, Hojo M. Effectiveness of scaffolds with pre-seeded mesenchymal stem cells in bone regeneration —Assessment of osteogenic ability of scaffolds implanted under the periosteum of the cranial bone of rats. Dent Mater J 2010; 29: 673-681.
4) Hunter A, Archer CW, Walker PS, Blunn GW. Attachment and proliferation of osteoblasts and fibroblasts on biomaterials for orthopaedic use. Biomaterials 1995; 16: 287-295.
5) Kurtz SM, Devine JN. PEEK biomaterials in trauma, orthopedic, and spinal implants. Biomaterials 2007; 28: 4845-4869.
6) 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. Biomaterials 1995; 16: 987-992.
7) Sagomonyants KB, Jarman-Smith ML, Devine JN, Aronow MS, Gronowicz GA. The in vitro response of human osteoblasts to polyetheretherketone (PEEK) substrates compared to commercially pure titanium. Biomaterials 2008; 29: 1563-1572.
8) Pokorný D, Fulín P, Štolf M, Jahoda D, Landor I, Sošna A. Polyetheretherketone (PEEK). Part II: Application in clinical practice. Acta Chir Orthop Traumatol Cech 2010; 77: 470-478.
9) Silhampitag P, Chaipareon P, Tattakorn K, Banjongprasert C, Takahashi H, Arksornmukit M. Effect of surface pretreatments on resin composite bonding to PEEK. Dent Mater J 2016; 35: 668-674.
10) Toth JM, Wang M, Estes BT, Scifert JL, Seim Iii HB, Turner AS. Polyetheretherketone as a biomaterial for spinal applications. Biomaterials 2008; 27: 324-334.
11) Jarman-Smith M, Brady M, Kurtz SM, Cordaro NM, Walsh WR. Porosity in polyaryletherketone. PEEK Biomaterials Handbook 2011; 181: 4.
12) Kaczorowski W, Szymanski W, Batory D, Niedzielski P. Tribological properties and characterization of diamond like carbon coatings deposited by MW/RF and RF plasma —Enhanced CVD method on poly (ether-ether-ketone). Plasma Process Polym 2014; 11: 878-887.
13) Tan K, Chua C, Leong K, Chesh C, Cheang P, Bakar MA, Cha S. Scaffold development using selective laser sintering of polyetheretherketone-hydroxyapatite biocomposite blends. Biomaterials 2003; 24: 3115-3123.
14) Kasahara S, Sawamura T. Surface foamed article, biological implant and method of producing the same. 2009; PCT/JP2008/002717.
15) Kawamoto T, Kawamoto K. Preparation of thin frozen sections from nonfixed and undecalcified hard tissues using Kawamoto’s film method. Skeletal Development and Repair 2012; 1130: 148.
16) Cai Q, Yang J, Bei J, Wang S. A novel porous cells scaffold made of polylactide-dextrans blend by combining phase-separation and particle-leaching techniques. Biomaterials 2002; 23: 4483-4492.
17) El-Kady AM, Rizk RA, Abd El-Hady BM, Shafaa MW, Ahmed MM. Characterization, and antibacterial properties of novel silver releasing nanocomposite scaffolds fabricated by the gas foaming/salt-leaching technique. J Genet Eng Biotechnol 2012; 10: 229-238.
18) Fslabani M, Elvassore N. Gas anti-solvent precipitation assisted salt leaching for generation of micro- and nano-porous wall in bio-polymeric 3D scaffolds. Mater Sci Eng C Mater Biol Appl 2012; 32: 1632-1639.
19) Kim TG, Chung HJ, Park TG. Macroporous and nanofibrous hyaluronic acid/collagen hybrid scaffold fabricated by concurrent electrospinning and deposition/leaching of salt particles. Acta Biomater 2008; 4: 1611-1619.
20) Daoust D, Devaux J, Godard P. Mechanism and kinetics of poly (ether ether ketone) (PEEK) sulfonation in concentrated sulfuric acid at room temperature Part 1. Qualitative comparison between polymer and monomer model compound sulfonation. Polym Int 2001; 50: 917-924.
21) Ouyang L, Zhao Y, Jin G, Lu T, Li J, Qiao Y, Ning C, Zhang X, Chu PK, Liu X. Influence of sulfur content on bone formation and antibacterial ability of sulfonated PEEK. Biomaterials 2016; 83: 115-125.
22) Zhao Y, Wong HM, Wang W, Li P, Xu Z, Chong EY, Yan CH, Yeung KW, Chu PK. Cytocompatibility, osseointegration, and bioactivity of three-dimensional porous and nanostructured network on polyetheretherketone. Biomaterials 2013; 34: 9264-9277.
23) Zhou L, Qian Y, Zhu Y, Liu H, Gan K, Guo J. The effect of different surface treatments on the bond strength of PEEK composite materials (DEMA-D-13-00481). Dent Mater 2014; 30: e209-215.
24) White E, Shors E. Biomaterial aspects of Interpore-200 porous hydroxyapatite. Dent Clin North Am 1986; 30: 49-67.
25) Luo H, Xiong G, Ren K, Raman SR, Liu Z, Li Q, Ma C, Li D, Wan Y. Air DBD plasma treatment on three-dimensional braided carbon fiber-reinforced PEEK composites for enhancement of in vitro bioactivity. Surf Coat Technol 2014; 242: 1-7.
26) Poulsin AH, Richards RG. Surface modification techniques of polyetheretherketone, including plasma surface treatment. PEEK Biomaterials Handbook 2011: 145.