

Preparation of Osteoconductive Polyetheretherketone Implant by Surface Modification

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**Preparation of Osteoconductive Polyetheretherketone Implant
by Surface Modification**

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Preparation of Osteoconductive Polyetheretherketone Implant by Surface Modification

Thesis

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Chapter 1

Introduction

1. History and disadvantages of titanium (Ti) implants

Endosseous implantable materials have improved human health by replacing lost teeth and dysfunctional joints such as dental implants and hip [1]. Metallic implants, particularly Ti and Ti alloys have been widely used as orthopedic and dental implants due to their superior mechanical properties and biocompatibility. However, Ti and Ti alloys have too high elastic modulus, which do not meet with natural bone. In the case of orthopedic implants, the difference in elastic modulus cause a stress shielding on the peri-implant bones, leading absorption of adjacent bone and prosthetic loosening. Another disadvantage of Ti/Ti alloys implants is their magnetic property that causes artifacts in computed tomography (CT) images, including limits the ability of magnetic resonance imaging (MRI) examination to the patient, eventhough their magnetic property are limited [2]. The Ti/Ti alloys based implants can also induce allergic reaction [3]. Therefore, it is required to find the other materials to replace metallic implants.

2. Polyetheretherketone (PEEK) as a candidate for orthopedic and dental implants

PEEK was first developed by a group of British scientists in 1978 [4]. Initially, it was intended for engineering applications such as turbine blades and aircraft. PEEK is a semi-crystalline polymer that composed of aromatic structure with combination of ether and ketone functional groups (Fig. 1.1) [5]. Its chemical structure gives the PEEK stable chemical and physical properties such as excellent resistant to chemicals (except for concentrated sulfuric acid), good wear resistance and thermal stability [4-7].

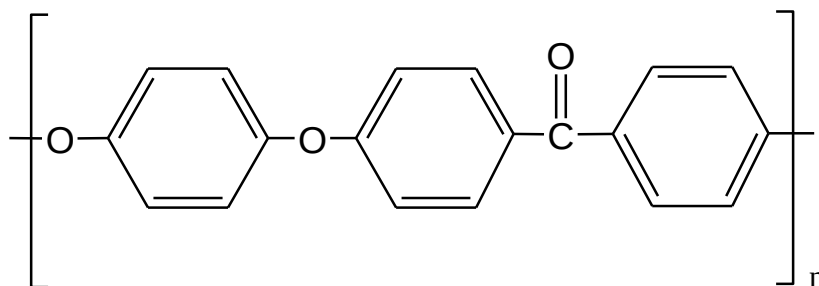


Fig. 1.1 Chemical structure of PEEK.

Since its first commercialization in the 1980s, PEEK has emerged as a candidate to replace metal implants due to its mechanical properties (close to human cortical bone), *in vivo* stability, and biocompatibility [8-10]. For example, PEEK exhibits the elastic modulus of approximately 8.3 GPa, which is close to that of human cortical bone (17.7 GPa) and much lower than that of commercially available metal implants such as Ti alloy (116 GPa) and Co-Cr alloy (210 GPa)[11-14]. In addition, PEEK demonstrates

excellent resistance to various sterilization methods such as gamma radiation, steam and ethylene oxide sterilizations [15, 16].

3. Current strategies to improve the osteoconductivity of PEEK

PEEK is bioinert or does not bond chemically to surrounding bone tissue during implantation [2]. For that reason, many methods have been developed to produce osteoconductive PEEK such as surface composite preparation, surface coating, physical and chemical treatments.

3.1 Composite preparation

Various PEEK composites have been developed to gain osteoconductivity (**Table 1**). Generally, PEEK is blended with osteoconductive materials such as calcium phosphate (CaP) or others. CaP biomaterials play a role as a filler, while PEEK play a role as a matrix. Abu Bakar et al. developed a hydroxyapatite (HA)/PEEK composite with good biological responses [17]. However, they reported that the introduction of HA decreased the tensile strength. Other researchers reported the preparation of PEEK composite with various other materials as fillers. For example, Ma et al. fabricated calcium silicate (CS) reinforced PEEK composite [18]. This CS/PEEK composite promoted apatite-like formation in simulated body fluid (SBF) solution, suggesting the osteoconductive potential of the composite. Ti and Ti oxide (TiO_2) were also added to PEEK and showed osteoconductivity [19, 20]. Various issues arise when PEEK is being compounded with CaP biomaterials or other materials such as the decreases in

mechanical properties, poor interfacial adhesion and fatigue damage during long-term loading, finally leads to implant failure. To overcome these problems, researchers have tried to use nano-sized fillers particles or powders. Wang et al. prepared a nano HA/PEEK composite using nano-sized HA particles [21]. This new HA/PEEK exhibited good mechanical properties without debonding between HA filler and PEEK matrix. Despite superior properties over conventional PEEK composite, nano CaP/PEEK composite remains several disadvantages. For example, nano-sized powders have a tendency to aggregate during composite preparation [22]. Powders agglomeration will result in the inhomogeneity of the powders dispersion. Inhomogeneous distribution of fillers affects the end properties of the composite. Overall, despite successful improvement of the osteoconductivity of PEEK implants, the superior mechanical properties of the original polymer were reduced. Therefore, other methods to improve osteoconductivity of PEEK implants are recommended.

Table 1. Various types of PEEK composites.

PEEK composites	Fillers	Result on bioactivity	References
Hydroxyapatite (HA)/PEEK	HA powders	<i>In vitro</i> in SBF solution: Induced apatite-like layer formation. <i>In vivo</i> in rat and pig femurs: Higher bone formation surrounding implant.	[23-25]
	HA nanoparticles	<i>In vitro</i> by MG-63 osteoblasts: Improved cell attachment and ALP activity.	[26]
β -tricalcium phosphate (β -TCP)/PEEK	β -TCP particles	<i>In vitro</i> by normal human osteoblasts: Failed to improve cell proliferation.	[27]
Ti/PEEK	Ti powders	<i>In vitro</i> by MC3T3-E1 cell line: Enhanced cell morphology, viability and ALP activity.	[16]
TiO ₂ /PEEK	TiO ₂ nanoparticles	<i>In vitro</i> by MG-63 osteoblasts: Improved cell attachment and cell spreading. <i>In vivo</i> in beagle dog tibia: Higher bone volume/tissue volume.	[17]
Calcium silicate (CS)/PEEK	CS nano-powders	<i>In vitro</i> by MC3T3-E1 cell line: Improved cell attachment, cell spreading, proliferation, ALP activity and mineralization as well as differentiation-related genes expression. <i>In vitro</i> in SBF solution: Enhanced apatite-like formation.	[15]

3.2 Surface treatment

3.2.1 Coating of osteoconductive materials

Various materials have been used to coat the surface of PEEK, including HA, TiO₂ and diamond-like carbon (Table 2). Among them, HA is the most popular materials used to coat PEEK surface. HA is known for its osteoconductivity. It has been extensively used to coat metallic implants such as Ti and its alloy [28]. The ability of HA to bond to bone is much greater than that of PEEK implants. Thus, the osteoconductivity of PEEK implants were significantly increased after HA coating. Despite excellent result on the improvement of osteoconductivity, HA coating remains several disadvantages. For example, it has been reported that coated HA can detach from the PEEK surface due to the weak bonding strength at the interface, which disrupts the integration between bone and implant [29]. Furthermore, detached HA particle debris facilitates bone resorption by osteoclasts, leading to osteolysis and implant failure [30, 31].

Table 2. Various types of coating materials and methods on PEEK implant.

Coating materials	Coating methods	Result on bioactivity	References
Hydroxyapatite (HA)	Cold spray	<i>In vitro</i> by human bone marrow: Enhanced ALP activity and mineralization. <i>In vivo</i> in minipig lumbar intervertebral disc space: Higher bone-implant contact (BIC).	[32]
	Vacuum plasma spray	<i>In vivo</i> in sheep pelvis: Higher pull-out test, BIC not significant.	[33]
	Gas plasma spray	<i>In vitro</i> in HBSS solution: Lower roughness (the author claimed to be the sign of biocompatibility).	[34]
	Chemical deposition	<i>In vivo</i> in rabbit tibia: Higher removal torque.	[35]
	Radio-frequency magnetron sputtering	<i>In vitro</i> by CRL 1486 human embryonic palatal mesenchyme (HEPM) cells: Higher cell attachment and proliferation.	[36]
Titanium (Ti) /Titanium dioxide (TiO ₂)	Vacuum plasma spray/ Physical vapor deposition	<i>In vivo</i> in sheep tibia: Higher removal torque and bone apposition.	[37]
	Sol-Gel	<i>In vitro</i> by rabbit bone marrow: Enhanced cell viability, ALP activity and osteogenic differentiation-related genes. <i>In vivo</i> in rabbit tibia: Higher detachment test, larger BIC.	[38]
	Electron beam deposition	<i>In vitro</i> by MC3T3-E1 cell line: Enhanced cell viability, morphology and ALP activity. <i>In vivo</i> in rabbit leg: Larger BIC.	[39]
Diamond-like carbon	Plasma immersion ion implantation and deposition technique	<i>In vitro</i> by human fetal-osteoblast cell line: Improved cell viability/proliferation, ALP activity and OCN expression.	[40]

3.2.2 Physical treatment

Various physical surface treatments have been reported to produce osteoconductive PEEK implants such as plasma treatments, UV irradiation and surface texturing. Among them, plasma treatments have been widely reported. Plasma treatment produces a hydrophilic polymer surface by introducing some functional groups such as $-OH$, $-NH_2$ and $-COOH$. For example, Awaja et al. treated PEEK surface with plasma composed of (CH_4/O_2) gas mixture [41, 42]. The treatment greatly improved cell adhesion and spreading. They found that CH_4/O_2 gases plasma treatment deposited an oxygen enriched film. This film has high surface energy and thus hydrophilicity which in turn resulted in the higher cell adhesion. Another researcher reported the use of two plasma process (NH_4/Ar and H_2/Ar gases) to treat PEEK and found increase of proliferation and differentiation of primary osteoblasts and fibroblasts [43]. Unfortunately, *in vivo* evaluation has not been performed.

It is known that roughened Ti prepared by sandblasting method has better osteoconductivity and bone-implant integration compared to machined surface [44, 45]. Similarly, Ti with porous structure on its surface showed excellent osteoconductivity and bone integration [46]. This *in vivo* success has inspired the researchers to apply that concept on PEEK implant by making its surface roughened or porous. For example, Zhao et al. successfully developed three-dimensional (3D) porous and nano-structured network on PEEK and found that it showed osseointegration and cytocompatibility

[47]. Evans et al. also fabricated surface porous PEEK and demonstrated osseointegration [48]. Surface texturing has also been reported to induce osseointegration of PEEK implants. Khoury et al. developed Accelerated Neutral Atom Beam (ANAB) technique to prepare nano-textured PEEK surface without changing its chemistry [49]. The level of texturing is in the range of 10-50 nm. This method also produced shallows with the depth of up to 5 nm. This nano-textured PEEK showed osseointegration in a rat calvarial defect model. It was reported that the proliferation and differentiation of bone cells improved by roughness of implant surface [45]. After implantation, bone cells are migrated to implant surface through transitory fibrin matrix before they forms new bone at the implant surface. Surface roughness (Ra) of the implant has profound effect on the formation of transitory fibrin matrix by providing more available surface and maintaining the anchorage of the fibrin matrix [50]. *In vitro* evaluation using osteoblasts indicated that rough surface induces the expression of differentiation markers such as alkaline phosphatase (ALP) and osteocalcin [51, 52]. Rough surface was also reported to improve bone nodule formation on Ti implant [53].

3.2.3 Chemical treatment

Various chemical treatments have been employed to lead PEEK osteoconductive. For example, PEEK was chemically modified to produce a hydroxylated polymer (PEEK-OH) by reduction reaction [54]. Carboxylated and aminated PEEK also have been reported to be successfully fabricated [55, 56]. Becker et al. used amine pre-treated

PEEK to covalently graft RGD-peptide onto it [57]. This RGD-peptide grafting onto PEEK surface resulted in higher osteoblasts adhesion and proliferation. Recently, Zheng et al., fabricated $-OH$, $-COOH$, and $-PO_4H_2$ modified PEEK through silanization technique [58]. After the treatment, the modified PEEK demonstrated the ability to form apatite layer, which indicated the osteoconductive potential of the polymer. MC3T3-E1 cell responses on apatite deposited PEEK was much higher compared with bare PEEK. Generally, the main advantage of chemical treatment of PEEK is the maintaining of the mechanical properties before and after treatment. Unfortunately, no *in vivo* test has been reported for chemically treated PEEK.

4. Introduction of calcium and phosphate ions on bone implants and their effect on osteoconductivity

The effect of calcium (Ca) ion on the osteoconductivity of bone implants, particularly Ti has been widely studied. Nishiguchi et al., reported that alkali- and heat-treated Ti showed excellent osteoconductivity compared to bare Ti [59]. Although the detailed mechanism on how the material becomes osteoconductive is still unclear. Takadama et al., proposed a mechanism based on X-ray photoelectron spectroscopy (XPS) study during apatite formation in simulated body fluid (SBF) solution (Fig. 1.2) [60]. They reported that during immersion in SBF solution, Ti-OH was formed. The formation of Ti-OH made the Ti surface became negatively charged. The negative charged Ti surface attracted Ca ions. These Ca ions created positive charge on Ti

surface, which then attracted phosphate and other negative charged species from the solution to form an apatite-like layer at the later. This apatite-like formation on material may have relation to obtain osteoconductivity.

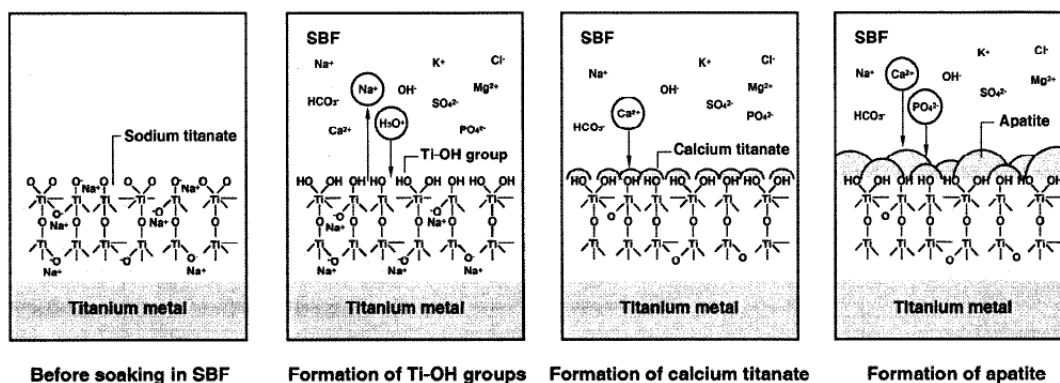


Fig. 1.2 Proposed mechanism of apatite formation on alkali and heat treated Ti in SBF solution [60]

If the above mechanism is correct, the introduction of Ca ions on implant surface would accelerate the apatite-like formation which predicts osteoconductivity.

Biologically, osteoblast lineage can be divided into 3 stages: (1) proliferation, (2) matrix maturation and (3) mineralization that marked by the deposition of bone-like apatite [61]. Various gene expressions are produced along those stages that are usually used as markers. Matrix maturation is marked by maximal expression of ALP and synthesis of collagen type I [62]. During the early stage of matrix mineralization, specific genes expression such as bone morphogenic protein (BMP), osteopontin and osteocalcin are also detected [63]. It has been reported that Ca ion increases BMP-2 and -

4 and collagen type I synthesis in human bone cells [64]. Meanwhile, phosphate ion increases osteopontin expression, which means that both ions have positive effect on cell mineralization [65]. An elevated concentration of phosphate ions in the culture medium increased runt-related transcription factor 2 (Runx-2) and bone-like nodule formation by MC3T3-E1 cells [66, 67].

5. Introduction of Ca on bone implants and its effect on osteoconductivity

Many studies have reported the effect of surface modification by Ca ion on improving osteoconductivity especially in Ti implants [68, 69]. Ca ion-modified Ti exhibited higher osteoconductivity both *in vitro* and *in vivo*. For example, our research group successfully immobilized Ca ions on Ti surface through bonding with phosphate ions that previously bonded to Ti surface [70]. *In vitro* evaluation by MC3T3-E1 demonstrated that our surface modification significantly improved cell responses including differentiation and mineralization. We also found that phosphate ions not only play as the binding site for Ca ions, but also contribute to the cell responses, differentiation and mineralization.

In this study, to gain osteoconductive PEEK, surface roughening and chemical treatment were selected to modify PEEK surface. Sandblasting was used to roughen the PEEK surface. For chemical treatment, we have demonstrated that Ca ion was useful to improve osteoconductivity of Ti implants [70]. It is expected that if Ca ion could be immobilized on the surface of PEEK, its surface would be osteoconductive. However,

Ca ion cannot immobilize on original PEEK because PEEK have no functional groups that show negative charge. In order to immobilize Ca ion, the first strategy is to modify PEEK surface with chemical group that has negative charges through chemical reaction with ozone (O_3) gas. Phosphate is a good candidate for this purpose since it has high affinity to Ca ion. Moreover, phosphate is also hoped to contribute to the osteoconductivity through synergetic effect with Ca [70]. For comparison, other surfaces such as O_3 +phosphate, O_3 +Ca and O_3 gas treated PEEK were also evaluated.

Based on this background, the main objective of the present research is to evaluate the feasibility of the proposed treatments for the preparation of osteoconductive PEEK. The effect of the proposed treatments on biological property of PEEK was also evaluated both *in vitro* and *in vivo*.

Chapter 2

Effect of surface roughness on osteoconductivity of PEEK implant

1. Introduction

As mentioned in chapter 1, Ra was reported to improve osteoconductivity of titanium. In the case of PEEK implant, however, only few studies were reported regarding the effect of Ra on creating osteoconductive PEEK implants as mentioned in chapter 1. One example is the use of accelerated neutral atom beam technology (ANAB) that can texture the PEEK surface with the texture level between 10-50 nm and up to 5 nm in depth (Fig. 2.1A). This nano-texture was able to increase human fetal osteoblastic (hFOB) cells and gave osteoconductivity *in vivo* to PEEK implants. Second example is the formation of porous structure on the surface of PEEK implants. Porous structured network could be produced by immersion in high concentration of sulfuric acid (Fig. 2.1B). The porous structure accelerated the proliferation and differentiation of MC3T3-E1 cells on PEEK. Furthermore, PEEK implants showed osteoconductivity evidenced from push-out strength and bone-implant contact.

Based on these findings, it is clear that surface texture is one of the important factors in creating osteoconductivity. In this study, we used sandblasting method to prepare roughened PEEK. Unlike the reported methods, sandblasting produces rough

PEEK surface without changing its chemical composition. For example, preparation of porous PEEK by concentrated sulfuric acid produces sulfonated PEEK (Fig. 2.1C). The increase of cell responses and *in vivo* results of sulfuric acid treated PEEK may also be influenced by sulfonate group of treated PEEK. Therefore, it is difficult to differentiate the effect of Ra and chemical treatment. On the other hand, the use of ANAB can produce nano-textured PEEK without changing its chemical composition. Although osteoconductive PEEK surface was successfully produced, it is a complicated and expensive method, thus its clinical application is different. In contrast, sandblasting method produces micro level of Ra. Interestingly, there is no paper that evaluates the effect of micro roughness on creating osteoconductive PEEK implant.

In order to investigate the effect of surface roughening at micro level by sandblasting on cell responses and *in vivo*, two different surfaces were prepared. Mirror polished PEEK was prepared as a control as its Ra is very small ($Ra \approx 0$), so that it will not affect both *in vitro* and *in vivo*. We hypothesize that micro roughness improves the rat bone marrow (RBM) cell responses and bone-bonding strength of PEEK implant. *In vitro* cell study and *in vivo* pull-out test were used to evaluate these two specimens.

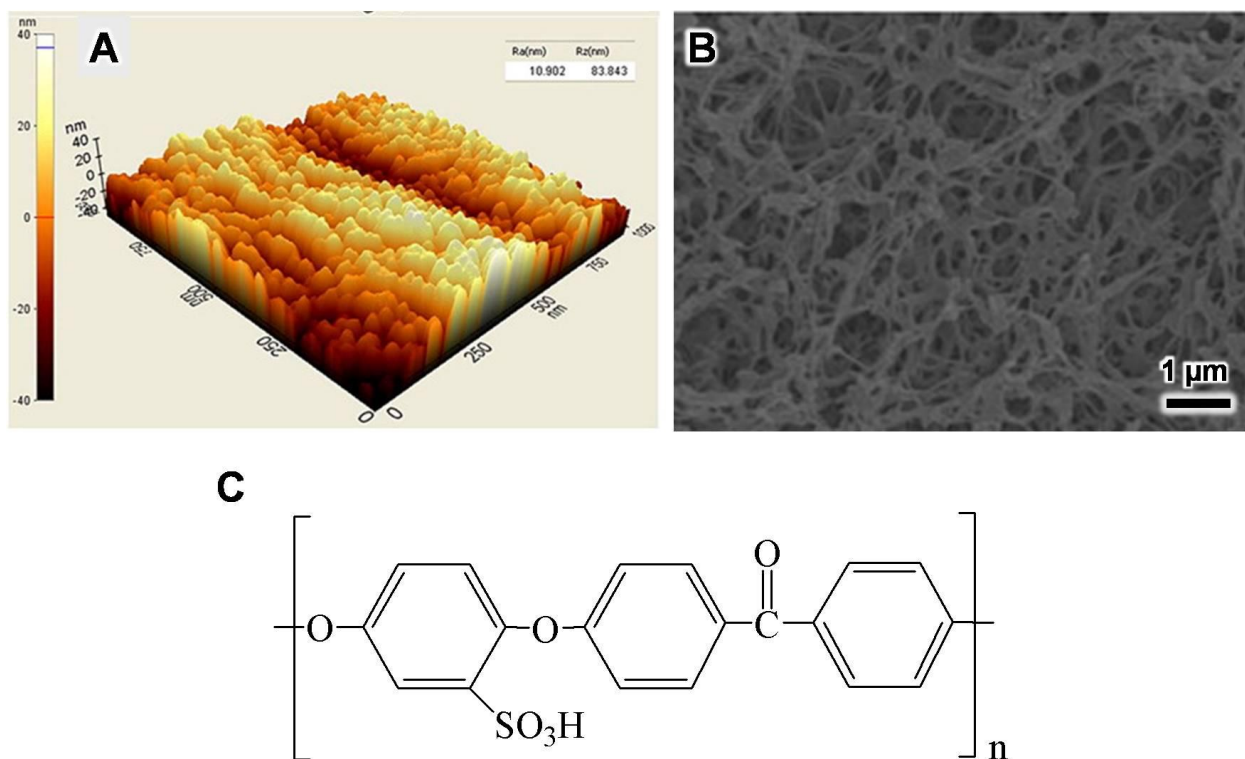


Fig. 2.1 Atomic force microscope (AFM) 3D image of nano-textured PEEK surface obtained by ANAB (A), SEM image of sulfonated PEEK surface (B) and Chemical structure of sulfonated PEEK (C).

2. Materials and methods

2.1 Sample preparation

Commercially available PEEK disc (14 mm diameter; 1 mm thickness) (Sun Brain Co. Ltd., Tokyo, Japan) was used as a specimen. Roughened PEEK (R-PEEK) implants were prepared by sandblasting using 60 grit alumina particles under following condition: 5 kg/cm² for 5-10 s (Hozan sandblast SG-106, Osaka, Japan). Alumina

particles were selected since it is also used to sandblast titanium implant, while 60 grit (medium size) was chosen as a preliminary size. The distance between the PEEK disk and the sandblast nozzle is approximately 5 cm. After sandblasting, the specimens were washed ultrasonically with distilled water and acetone, respectively. Specimens were then dried at room temperature for overnight. For animal experiment, PEEK rod (Sun Brain Co. Ltd.) was used. Sandblasting was performed by the same condition as the one used for PEEK disk. The PEEK rod was rotated to make the whole surface roughened.

Mirror polished PEEK (M-PEEK) implants were prepared by firstly polishing the PEEK disc with 1000 grit and 2000 grit SiC sandpaper followed by final polishing with 8000 grit alumina lapping film to make mirror surface. Specimens were washed ultrasonically with distilled water and acetone, respectively. Specimens were later dried at room temperature for overnight. For animal experiment, PEEK rods (1.4 mm diameter; 23 mm length) were polished by wrapping the rod with alumina lapping film until mirror polished surface with similar roughness as disc specimen was obtained.

2.2 Analysis of surface characteristics of roughened and mirror polished PEEK

Surface morphology of all specimens was observed by scanning electron microscope (SEM) (S-3100, Hitachi Co., Tokyo, Japan). Ra of specimens was measured by Ra tester (Mitutoyo Surftest SJ-400, Kawasaki, Japan). Surface hydrophilicity was indexed by measuring contact angle of water droplets (1.5 μ L) on the surface of

specimen using a contact angle meter (DM500, Kyowa Interface Science, Saitama, Japan).

2.3 Cell isolation and culture

Rat bone marrow (RBM) cells were isolated from Wistar rats. The rats were sacrificed according to the Kyushu University regulation for the care and use of laboratory animals. Femurs of 4-week-old Wistar rats were removed and cleaned from soft tissue. The epiphyses were cut and bone marrow was flushed out from medullary cavity with alpha minimal essential medium (α -MEM) (Wako, Osaka, Japan). RBM cells were cultured in α -MEM containing 15 % FBS (Thermo Scientific, NY, USA), 100 U/mL penicillin, 100 μ g/mL streptomycin, and 0.25 μ g/mL amphotericin B (Gibco, NY, USA). The cells were cultured at 37 °C in a humidified atmosphere containing 5 % CO₂ and 95 % air. The culture medium was renewed every 2 days. Cells were detached using 0.25 % trypsin–EDTA (Gibco) after 6 days or when the cells were almost confluent. Cells were seeded with a density of 4×10^4 cells/well on the test substrates in 24-well plates.

2.4 Cell proliferation

Cells were cultured on PEEK disks in a 24-well plate at an initial seeding density of 4×10^4 cells/well. Cell number was calculated after 3 and 7 days culture. Cell counting kit-8 (Dojindo, Kumamoto, Japan) was used to calculate the number of viable cells.

Absorbance at 450 nm was measured using a microplate reader (Infinite M200, TECAN, Port Melbourne, Victoria, Australia).

2.5 Alkaline phosphatase (ALP) activity and bone nodule

Cells were seeded on PEEK disks in a 24-well plate at a density of 4×10^4 cells/well and cultured for 7 and 14 days. Cells were differentiated in α -MEM supplemented with 15 % FBS, 100 U/mL penicillin, 100 μ g/mL streptomycin 0.25 μ g/mL amphotericin B, 10 mM sodium β -glycerophosphate (Wako) and 10 nM dexamethasone (Wako). Medium was renewed with fresh differentiation medium every 2-3 days. ALP activity was quantified using the Lab Assay ALP Kit (Wako). Briefly, medium was removed from the well followed by washing with PBS. Subsequently, 400 μ L of cell lysis buffer M (Wako; containing 20 mM Tris-HCl, 200 mM sodium chloride, 2.5 mM magnesium chloride and 0.05 % NP-40 substitute) was added to each well. Twenty-microliter of cell lysate was incubated with buffer solution containing disodium *p*-nitrophenylphosphate at 37 °C for 15 min. Subsequently, stop solution (0.2 mol/L NaOH) was added. Absorbance at 405 nm was measured using a microplate reader (TECAN). The amount of protein in lysate was measured using Bio-Rad Protein Assay (Bio-Rad Laboratories Inc., CA, USA). ALP activity was determined by normalizing the activity unit with the total amount of cellular protein.

Bone nodule was evaluated by calcified nodule staining kit (Cosmo Bio Co., Ltd., Tokyo, Japan). Briefly, medium was removed from the well, followed by PBS washing

for 3 times. The cells were then fixed by -20°C methanol at room temperature for 30 min. After methanol removal and distilled water washing, 400 µL of chromogenic substrate solution was dispensed to each well. The plate was incubated at room temperature for 5 min. Each well was washed by substrate-containing buffer solution until washing solution became transparent.

2.6 Animal experiment

12-week-old male Wistar rats were used to investigate the effect of smooth and rough PEEK surfaces *in vivo*. The animal experiment was performed according to the Kyushu University regulation for the care and use of laboratory animals. Anesthesia was introduced by intraperitoneal injection of ketamine and xylazine. In this study, the bone marrow cavity lengthwise to the femur was used as the implantation site. The surgical area was shaved and cleaned with iodine before the surgery. An incision was made on the lateral side of the patella, followed by lifting the patella and the ligament to the medial side, to expose the intercondylar area. A hole was made between the condyles by drilling with a dental reamer (1.43 mm in diameter) to the bone marrow cavity lengthwise to the femur. Sterile saline was used to clean the debris. Subsequently, the PEEK rod was inserted in the bone marrow cavity. The PEEK rod was placed by which the tip does not disturb the joint surface. The patella and ligament were backed to the original position and then closed with the silk sutures.

After 4 weeks, the rats were sacrificed according to the guidelines of Kyushu University regulation for the care and use of laboratory animals. Tissue was taken and embedded (half of the bone) in self-curing acrylic resin to firm the position. The tissue position was adjusted by which it is vertically aligned with the load-cell. The tissue was slightly cut to expose the hole of the implant. A stainless wire was connected from the implant hole to the load cell. For pull-out strength measurement, a universal testing machine (Autograph AGS-J, Shimadzu, Japan) with a calibrated load-cell of 1 kN was used. Cross-head speed was set to 10 mm/min. The maximum load, which is defined as the load when the implant is loosening, was recorded. The graphs, which produced from the machine, were then transformed into a table for statistical analysis.

2.7 Statistical analysis

The means and standard deviation of the data were calculated. The statistical significance between test groups was evaluated using Student's t-test. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1 Surface characteristics

Fig. 2.2 shows the SEM images of PEEK surface after mirror polish (A, B) and after sandblasting (C, D). Mirror polished PEEK exhibited very smooth surface with a

Ra of 0.06 μm . In contrast, sandblasted PEEK exhibited rough surface with a Ra of 2.26 μm .

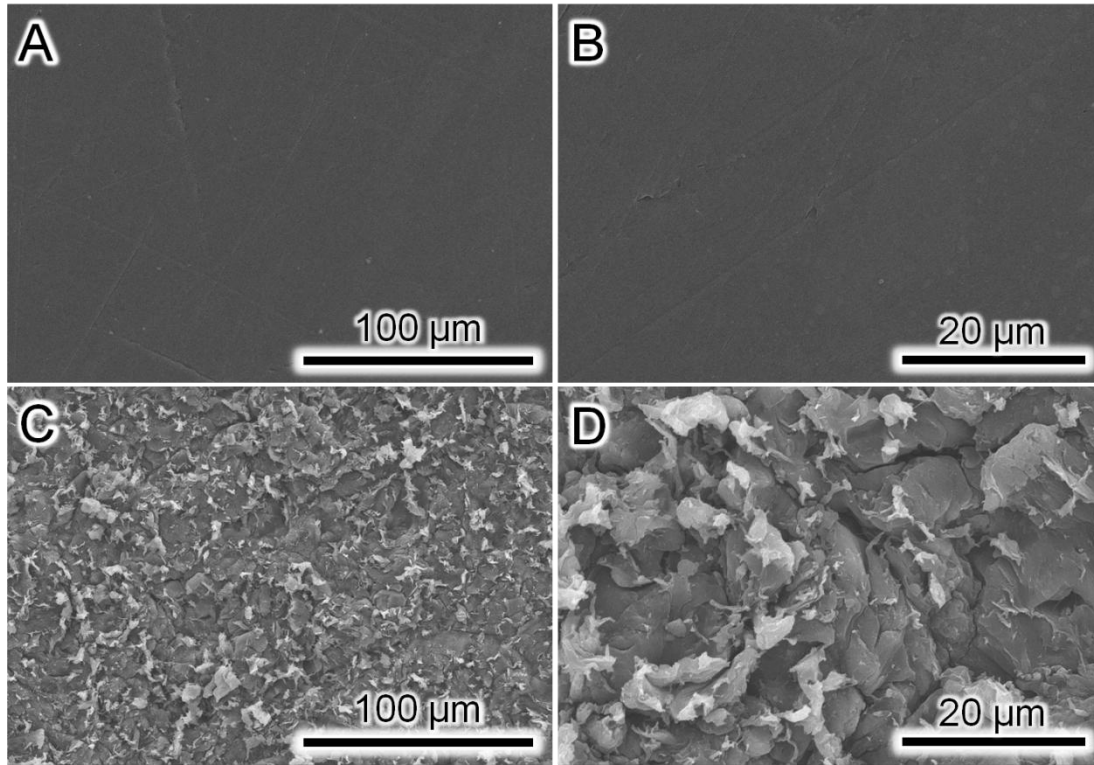


Fig. 2.2 SEM images of mirror polished PEEK (A,B) and roughened PEEK (C,D).

3.2 Surface hydrophilicity and roughness

Fig. 2.3A showed the results of water contact angle of mirror polished and roughened PEEK implants. The contact angle of roughened and mirror polished PEEK were $116.3 \pm 3.7^\circ$ and $81.2 \pm 1.1^\circ$, respectively. Ra of mirror polished and roughened PEEK were shown in Fig. 2.3B. It showed that roughened PEEK was significantly rougher compared with the mirror polished PEEK.

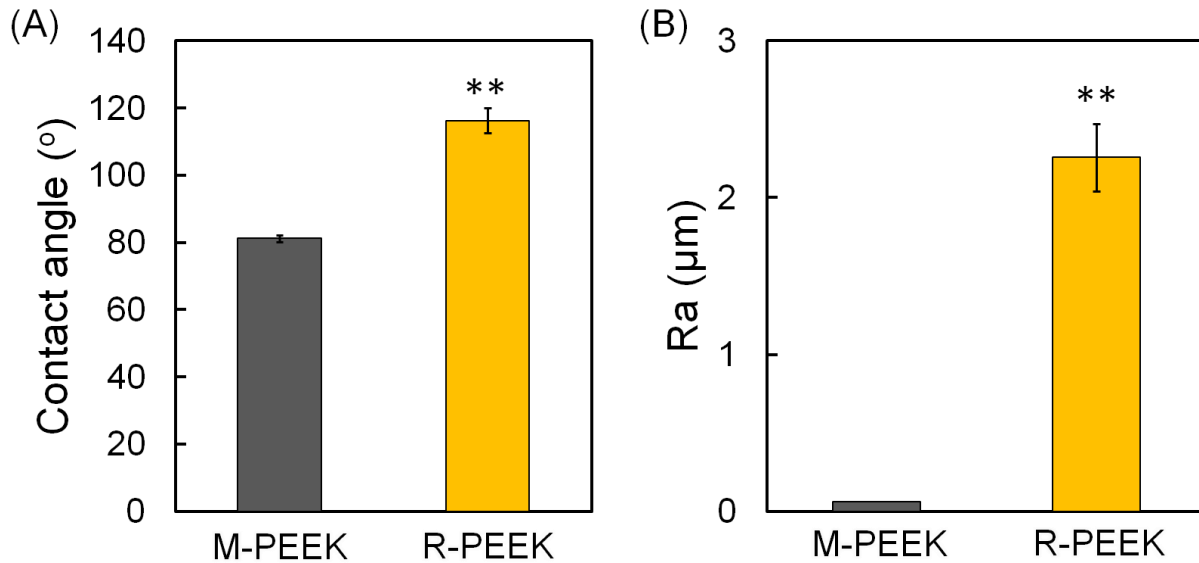


Fig. 2.3 Water contact angle (A) and Ra (B) of M-PEEK (mirror polished PEEK) and R-PEEK (roughened PEEK) implants. Data are mean $\pm$ SD (n = 3). Student's t-test; ** $p < 0.01$.

3.3 Cell proliferation

As shown in Fig. 2.4, at day 3, the number of cells on roughened PEEK was significantly higher compared with the mirror polished PEEK. At day 7, the roughened PEEK still showed higher cell proliferation, but no significant difference could be found between roughened and mirror polished PEEK specimens.

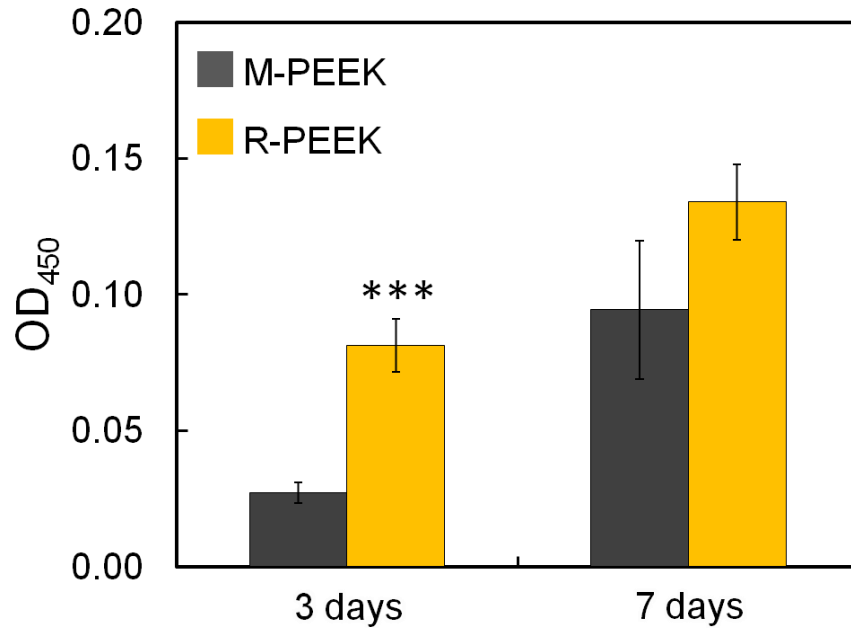


Fig. 2.4 Cell proliferation on mirror polished and roughened PEEK. Data are mean $\pm$ SD (n = 4). Student's t-test; *** $p < 0.001$.

3.4 ALP activity and bone nodule

Fig. 2.5 showed the ALP activity of RBM cells cultured on roughened and mirror polished PEEK substrates. Ra tends to increase the ALP activity of RBM cells evidenced from higher ALP activity of cells that grow on roughened PEEK compared with mirror polished one. However, the value was not statistically significant.

Bone nodule formations were shown in Fig. 2.5. Bone nodule was found to be much larger on roughened PEEK compared with that on mirror polished PEEK. At day 28, roughened PEEK surface was almost covered by nodule. Meanwhile, most of the mirror polished PEEK surface was still not covered by nodule.

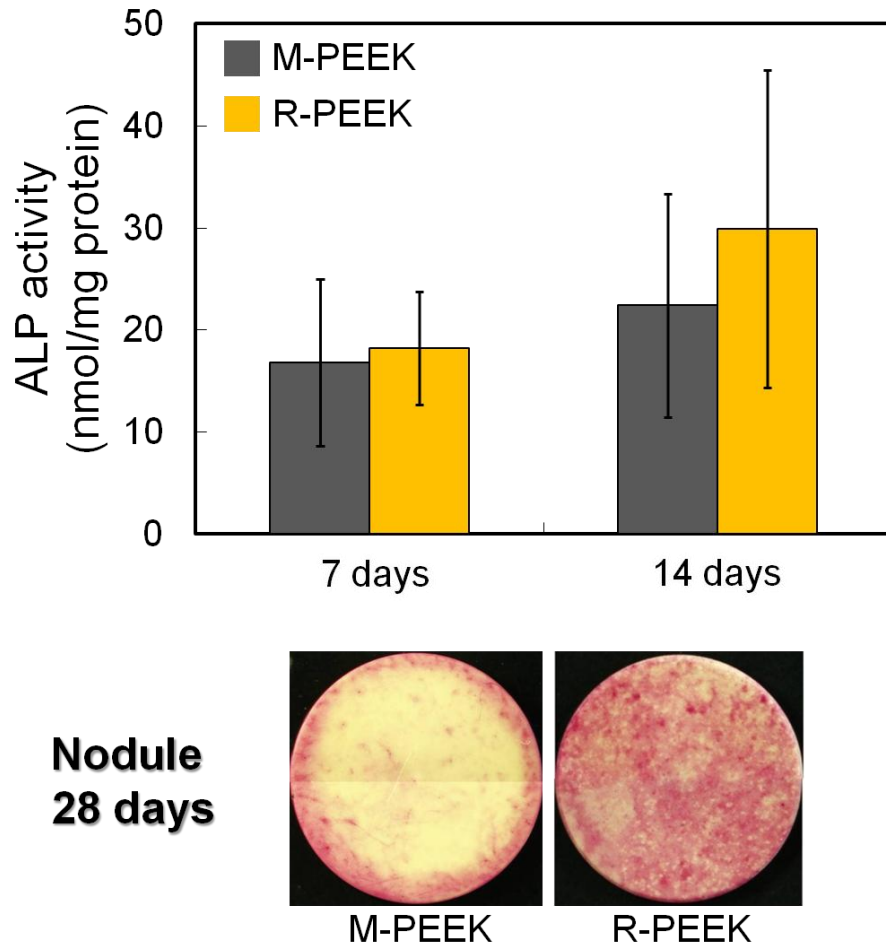


Fig. 2.5 ALP activity of RBM cells on mirror polished and roughened PEEK substrates (Data are mean $\pm$ SD (n = 4)) and bone nodule at 28th day.

3.5 *In vivo* pull-out force

Fig. 2.6 demonstrated the result of pull-out force test obtained from *in vivo* using rat animal model. Roughened PEEK implant showed significantly higher pull-out force compared with the mirror polished PEEK.

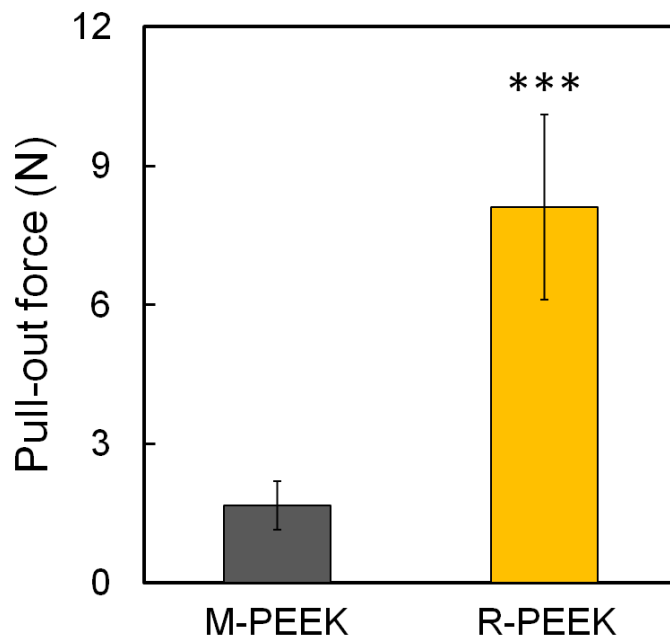


Fig. 2.6 Pull-out force of mirror polished and roughened PEEK implants after implantation for 4 weeks. Data are mean $\pm$ SD (n = 6). Student's t-test; *** $p < 0.001$.

4. Discussion

Both roughened and mirror polished PEEK implants were successfully prepared. Based on contact angle result, it was shown that roughened PEEK surface is more hydrophobic compared with mirror polished PEEK surface. The result was in accordance with Ourahmoune et al. (2014), where sandblasting significantly affects hydrophobic behavior of PEEK [71]. The peaks and valleys created on the surface after sandblasting act as barriers to the spreading of water droplet, resulting in the higher contact angle [72]. In case of very smooth surface such as mirror polished PEEK, the water droplet spreads relatively without barrier which in turn decrease contact angle.

In this study, the effect of Ra on the RBM cells responses and pull-out force was evaluated. It was reported that ALP activity increases with increasing cell numbers when mesenchymal stem cells are cultured on microdomains at various cell densities [73]. It indicated that the interactions between cells are important to achieve efficient differentiation of mesenchymal stem cells into mature osteoblasts. As shown in Fig. 2.4, roughened PEEK exhibited higher cell proliferation compared with mirror polished PEEK. In the case of ANAB treated PEEK, proliferation of human mesenchymal stem cells was upregulated [74]. The results suggested that cell-cell interactions would be achieved earlier on roughened PEEK compared with those on the mirror polished PEEK. As the result (Fig. 2.5), mineralization of RBM cells on roughened PEEK was enhanced. Although, roughened PEEK has more hydrophobic characteristic than mirror polished PEEK. However, roughened PEEK demonstrated better cell proliferation and mineralization. These results indicated that Ra has more effect than surface hydrophilicity in the case of PEEK implant.

Initial implant stability is an important factor to achieve implant osseointegration which defines the implant success [75]. Osseointegration is a direct connection between implant and living bone. The failure in implant stability will cause excessive micromotion that promotes fibrous tissue formation at the implant interface, leading to implant failure [76]. Ra has been reported to significantly improve implant stability especially in the case of titanium and its alloy [77, 78]. However, no report about the

pull-out force of sandblasted PEEK. Pull-out test is one of the methods to evaluate the implant stability as the result of osteoconductivity [79]. In this study, roughened PEEK exhibited higher pull-out force compared with mirror polished PEEK. *In vitro* results might explain the reason of high pull-out strength in roughened PEEK. As shown in Fig. 2.5, cells produced larger bone nodule on roughened PEEK which indicated the potential of its surface to support bone formation. As the result, roughened PEEK surface would become osteoconductive which eventually increased implant-bone stability. The effect of mechanical interlocking to implant stability has also been previously reported on roughened Ti implant [80]. Therefore, mechanical interlocking was also believed to have been contributed to the increased of pull-out strength of roughened PEEK implant.

5. Conclusion

Roughened and mirror polished PEEK implants have been successfully fabricated. Roughened PEEK prepared by sandblasting method showed higher contact angle compared with mirror polished PEEK. Higher water contact angle of roughened PEEK was possibly due to peaks and valleys produced after sandblasting that barrier the spreading of water droplet. The RBM cells attached on roughened PEEK exhibited higher proliferative activity compared with those on mirror polished PEEK. Bone nodule formation was also greatly larger on roughened PEEK which indicated the

enhanced cell differentiation. However, Ra seems to have less effect on ALP activity. Pull-out force of roughened PEEK was significantly higher compared with that of mirror polished PEEK. Based on these results, roughened PEEK implant is better than smoothened PEEK in creating osteoconductive PEEK.

Chapter 3

Chemical modification of roughened PEEK implant

1. Introduction

The effect of micro-roughness on creating osteoconductive PEEK implant was discussed in chapter 2. It was found that surface micro-roughening using sandblasting method improved RBM cell proliferation and mineralization. Furthermore, *in vivo* pull-out test results suggested that micro-roughening significantly increased pull-out force of PEEK compared with mirror polished PEEK implant. These results demonstrated that micro-roughening can be used to produce osteoconductive PEEK implant. It is expected that the osteoconductivity of roughened PEEK implant would be further improved by combination with chemical modification. As mentioned in chapter 1, various chemical modifications have been reported such as polymer reduction and silanization. In this study, phosphate and/or Ca ion were used to modify roughened PEEK surface. Chemical modification of PEEK using phosphate and/or Ca have never been reported, even though their role for improving osteoconductivity is widely known. Furthermore, our proposed method is simpler when compared to the previous reported methods for chemical modification of PEEK. Therefore, evaluation of the effect of phosphate and/or Ca on the preparation of osteoconductive PEEK is required.

Previously, Sakaguchi has successfully immobilized Ca ion on the surface of Ti implant through hydroxyl (OH) group obtained by ozone treatment. The immobilization of Ca ion significantly improved the osteoconductivity of Ti implant. Moreover, as described in chapter 1, we have investigated the effect of Ti surface functionalization using phosphate on improving its osteoconductivity, where both phosphate and Ca ions were co-immobilized on Ti surface. Co-immobilization of phosphate and Ca ion produced higher MC3T3-E1 cells responses compared with those of only phosphate or Ca ion. These results demonstrated that both phosphate and Ca ions play significant roles on improving osteoconductivity.

Based on these findings, phosphate and Ca were used to modify PEEK surface. PEEK is a chemically stable polymer. In order to bind phosphate and Ca onto PEEK surface, ozone (O_3) gas treatment was used to produce OH-group-modified PEEK. The O_3 gas was chosen since it is a strong oxidizer and has been reported to produce various kinds of oxidized groups such as carboxyl and OH as it reacts to PEEK. The use of phosphate and/or Ca for PEEK surface modification has never been reported, therefore their effects are unknown. Therefore, our purpose is to evaluate the effect of phosphate and Ca on creating osteoconductive PEEK implant. To achieve this goal, we prepared 5 kinds of specimens, namely bare PEEK, ozone gas treated PEEK, phosphate treated PEEK, Ca treated PEEK and combination between phosphate and Ca treated PEEK.

2. Materials and methods

2.1 Sample preparation

Commercially available PEEK disc (14 mm diameter; 1mm thickness) (Sun Brain Co., Ltd., Japan) was used as a specimen. All PEEK specimens were sandblasted by the same condition as described in chapter 2, before subjected to chemical treatment. The roughened PEEK specimens were treated by O_3 gas at room temperature (RT) for 1 h. This O_3 gas treated PEEK specimen was later denoted as O_3 . The O_3 specimens were immersed in 20 ml of dichloromethane (DCM) containing 0.28 ml of triethylamine and 0.18 ml of phosphorylchloride ($POCl_3$) followed by shaking at RT for 24 h to prepare phosphate treated PEEK (denoted as "P"). After reaction, the specimens were washed by acetone for 2 times, followed by deionized water with shaking for 30 min and acetone for 2 times. The specimens were dried by flowing N_2 gas. This specimen was denoted as O_3+P . The O_3+P specimens were immersed in 10 mM $CaCl_2$ solution at RT for 30 minutes to prepare phosphate and Ca treated PEEK (denoted as O_3+P+Ca). The specimens were washed by deionized water for 3 times, followed by acetone before drying with nitrogen gas flow. To fabricate Ca treated PEEK (O_3+Ca), the O_3 gas treated specimens were immersed in 10 mM $CaCl_2$ at RT for 30 min. Similarly, the specimens were washed by deionized water for 3 times and acetone before drying with N_2 gas flow.

2.2 Analysis of surface characteristics

The chemical compositions of the surfaces were characterized by XPS (K-alpha, Thermo Fisher Scientific, East Grinstead, UK). The contact angle of water droplets (1.5 μ L) placed on the samples was measured using a contact angle meter (DM500) to determine surface hydrophilicity. SEM (S-3100) was used to analyze the surface morphology before and after treatment. Ra of specimens was measured by Ra tester (Mitutoyo Surftest SJ-400).

2.3 Cell study

Procedures for cell isolation, culture and assays were similar with the one described in chapter 2.

2.4 Pull-out force

The O₃+P+Ca specimen was subjected to pull-out test since it showed the best *in vitro* result. For comparison, bare and O₃+P specimens were also evaluated. Procedures for pull-out force test were similar with the one described in chapter 2.

2.5 Histology

Similar to pull-out test, O₃+P+Ca, O₃+P and bare were evaluated. First, the tissue was taken after 2 and 4 weeks of implantation. The tissue was fixed in 70 % ethanol. Dehydration steps were employed with a series of ethanol. Then, the tissue was embedded in polymethylmethacrylate (PMMA) through polymerization of methylmethacrylate solution (Nacalai tesque inc., Tokyo, Japan) by dimethyl 2,2'-

azobisisobutyrate (Wako) as the initiator at 37°C for 7 days. Tissue sectioning was made lengthwise to the femur. Subsequently, tissue section was stained using Villanueva-Goldner staining. Procedures for the staining are described as follows:

- i. Villanueva bone staining solution (Wako) for 30 min and then washed by 70 % ethanol.
- ii. Weigert's iron hematoxylin solution (Muto pure chemical Co. Ltd., Tokyo, Japan) for 10 min, followed by washing with water.
- iii. Ponceau fuchsin solution (Muto) for 30 min, followed by washing with 1% acetic acid solution.
- iv. Phosphotungstic acid-phosphomolybdic acid mixture solution (Muto) for 15 min, followed by washing with 1% acetic acid solution.
- v. Naphthol Green B solution (Wako) for 30 min, followed by washing with water, 70% ethanol and 99.5% ethanol respectively.
- vi. Saffron solution (Chem-Impex International inc., Illinois, USA) for 15 min, followed by washing with 99.5% ethanol.

After complete staining, the tissue section was then embedded on glass slide. Histological image was taken by an All-in-One Fluorescence Microscope (BZX710, Keyence, Osaka, Japan).

2.6 Statistical analysis

The means and standard deviation of the data were calculated. One way ANOVA was performed. If necessary, a post-hoc Fisher's LSD test was conducted to perform multiple comparison tests. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1 Surface characteristics

O₃ is a highly oxidative gas that oxidizes polymers. It was reported that O₃ reacted with saturated hydrocarbons, aldehydes, ethers and acetals. As mentioned in chapter 1, PEEK polymer composed of aromatic hydrocarbon with ether and ketone functional groups. O₃ reacted with PEEK through oxidation mechanism to produce oxidized PEEK molecules including OH group (Fig. 3.1), that is consistent with previous report [81]. Later, the OH group reacts with POCl₃ to produce phosphate modified PEEK. Ca ions can also be electrostatically immobilized through binding with OH and/or phosphate groups on modified PEEK.

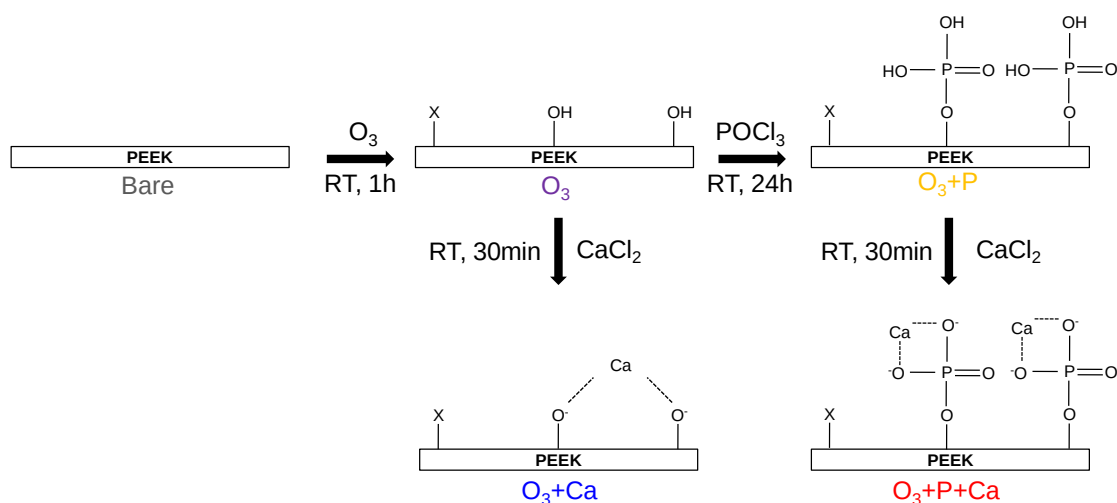


Fig. 3.1 Scheme of chemical modification of PEEK.

O₃, a strong oxidizer, makes PEEK surface ablated [82]. Therefore, it is necessary to evaluate O₃ exposure time. PEEK was then subjected to O₃ gas treatment for 1 h and 3 h. Fig. 3.2 demonstrated the surface morphology of PEEK, 1 h and 3 h after O₃ gas treatment. Surface morphology of PEEK treated with O₃ gas for 1 h was relatively similar to bare. However, the surface morphology was changed, 3 h after O₃ gas treatment, indicating that the surface was ablated. Therefore, in this study, 1 h O₃ gas treatment was employed.

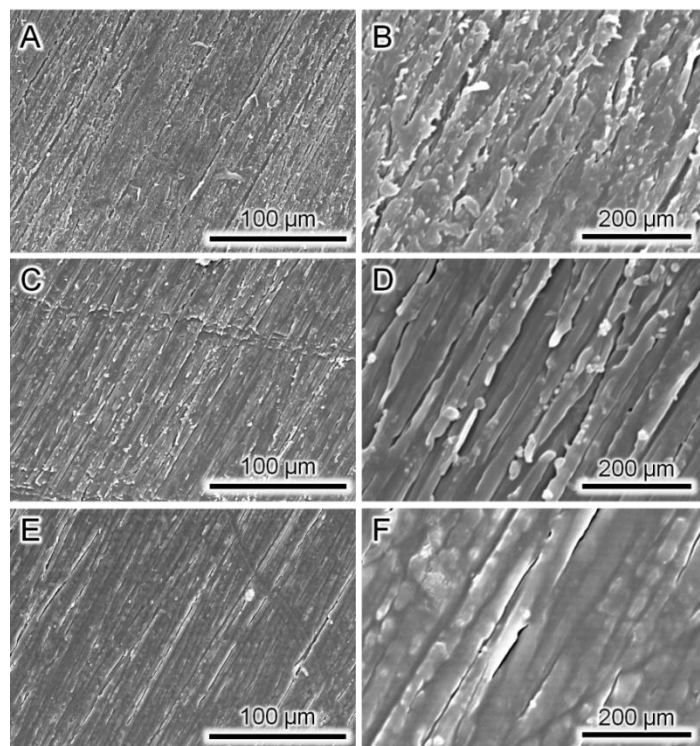


Fig. 3.2 Surface morphology of machined PEEK (A, B), after 1h ozone treatment (C, D) and after 3h O₃ gas treatment (E, F).

Fig. 3.3 shows the SEM images of PEEK surface before and after chemical treatments. There is no significant difference between bare PEEK surface and treated PEEK surfaces. Among the treated PEEK surfaces, similar results were also found where the surface morphologies did not change so much.

Fig. 3.4 shows the Ra of the investigated PEEK surfaces. The Ra value between bare and the treated PEEK surfaces were almost identical.

Fig. 3.5 demonstrates the water contact angle results measured from bare and the treated PEEK surfaces. The water contact angle of bare surface decreased about half

after O₃ gas treatment. The water contact angle was slightly higher as the O₃ surface was further treated with phosphate, Ca or phosphate and Ca.

XPS analysis of surface chemical composition of the specimens was presented in Fig. 3.6. The O1s peak obtained from bare PEEK consists of two components. Low binding energy (≈ 531.41 eV) component was ascribed for C=O (ketone) and high binding energy (≈ 533.26 eV) for C-O (ether). After O₃ gas treatment, the O1s peak was intensified and broadened. Peak fitting analysis shows that the O1s peak consists of three components; two peak components were from C-O and C=O groups similar to bare PEEK, while a new peak at ≈ 534.19 eV was ascribed for phenolic OH. This showed that the OH group was formed. After POCl₃ treatment of O₃ modified PEEK, the P2p peak was newly generated. When O₃ modified PEEK was treated with CaCl₂, the Ca2p peak was detected. Similarly, Ca can be bonded onto phosphate modified PEEK. The amount of Ca on O₃+Ca and O₃+P+Ca was comparable (≈ 0.6 at%). Also, the P2p peak intensity of O₃+P+Ca specimen was relatively similar with that of O₃+P specimen, indicating the Ca treatment did not decrease phosphate content.

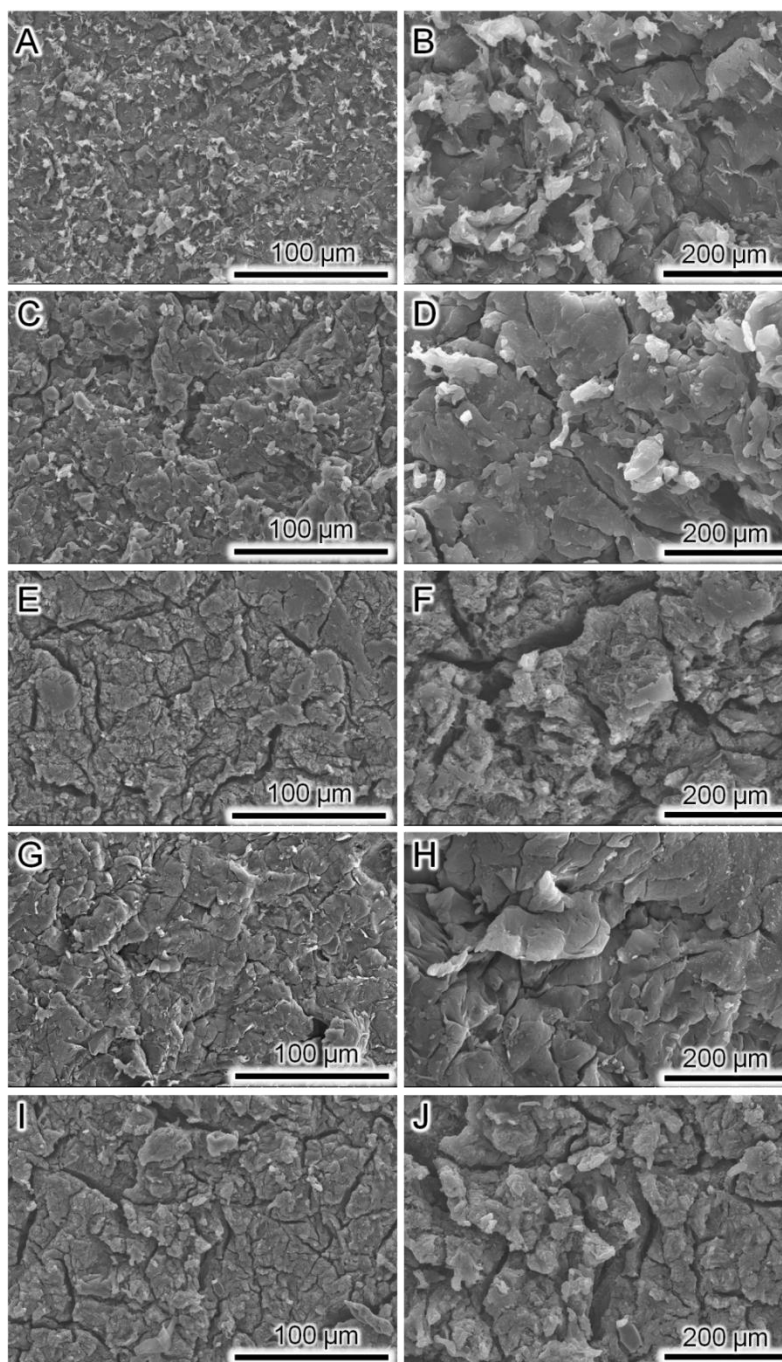


Fig. 3.3 SEM images of Bare PEEK (A, B), O₃ (C, D), O₃+P (E, F), O₃+Ca (G, H) and O₃+P+Ca (I, J).

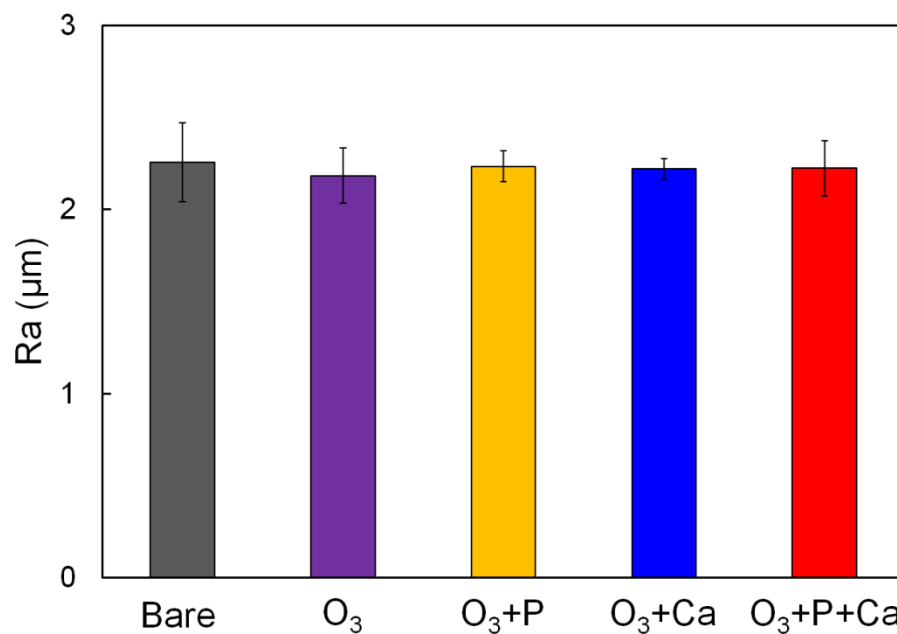


Fig. 3.4 Surface roughness of PEEK surfaces before and after chemical treatments. Data are mean $\pm$ SD (n = 3).

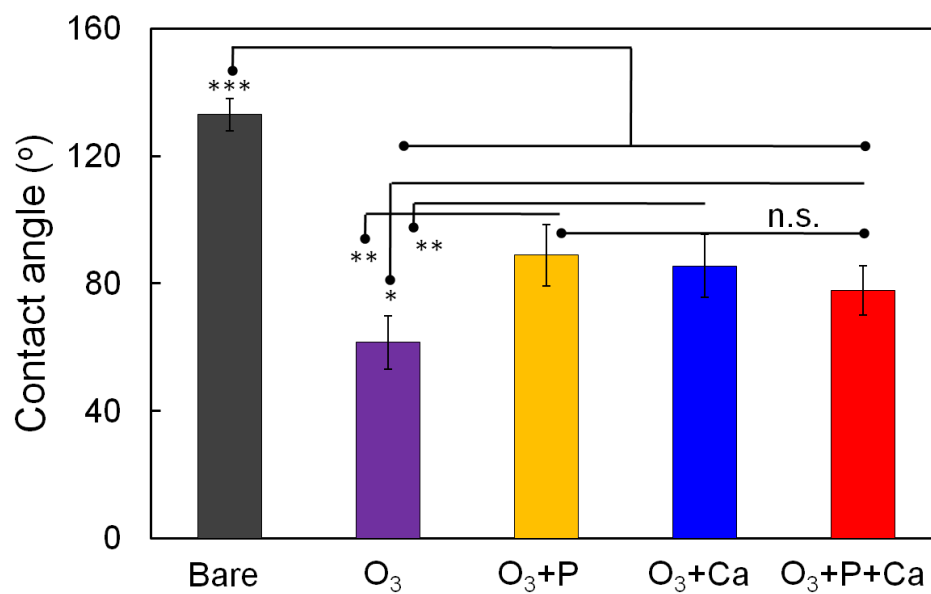


Fig. 3.5 Contact angle of PEEK surfaces before and after chemical treatments.

Data are mean $\pm$ SD (n = 3). One-way ANOVA with multiple comparisons;

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. not significant.

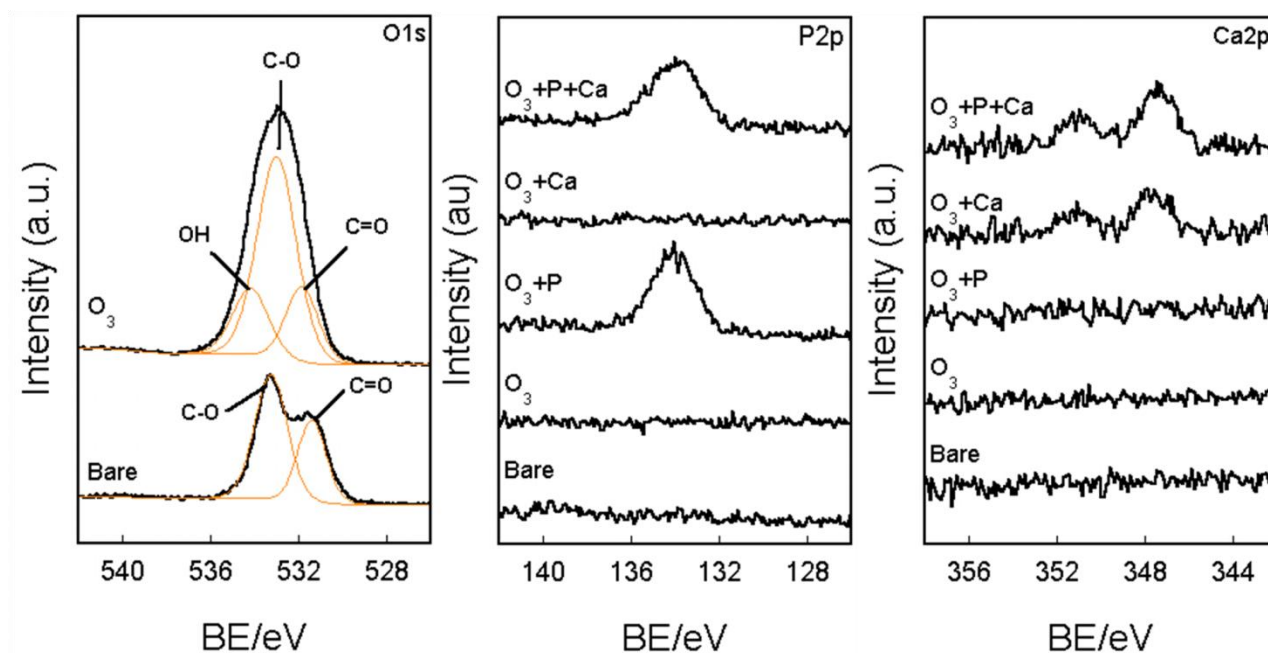


Fig. 3.6 XPS peaks of O1s, P2p and Ca2p before and after chemical

3.2 Proliferation and differentiation

The number of cell was found to be higher 3 days after cell culture in both Ca-modified specimens such as O₃+Ca and O₃+P+Ca (Fig. 3.7). O₃+P+Ca specimen demonstrated the highest cell proliferation than O₃+Ca at day 3. However, the O₃ specimen has lower cell proliferation compared with bare PEEK, while O₃+P specimen has similar number of cells at day 3. The number of cells was further increased 7 days after cell culture. Both Ca immobilized specimens such as O₃+P+Ca and O₃+Ca showed higher cell number compared with O₃+P and O₃ alone. Importantly, O₃+P+Ca showed greater cell proliferation than O₃+Ca.

ALP activity and bone nodule are the important markers for osteobalstic cell differentiation. The O₃+P+Ca specimen showed the highest ALP activity followed by

O₃+Ca, O₃+P, O₃ and bare PEEK (Fig. 3.8). Cell mineralization was measured by bone nodule 20 days after cell culture (Fig. 3.9). O₃+P+Ca specimen shows the most bone nodule formation followed by O₃+P and O₃+Ca, 20 days after cell culture. At day 28, bone nodule formation on all specimens were significantly larger compared with those at day 20 (Fig. 3.10). Surface of O₃+P+Ca and O₃+P specimens were almost completely covered by bone nodule, but other specimens still did not. These results demonstrated that both Ca immobilization and phosphate surface functionalization activated proliferation and differentiation.

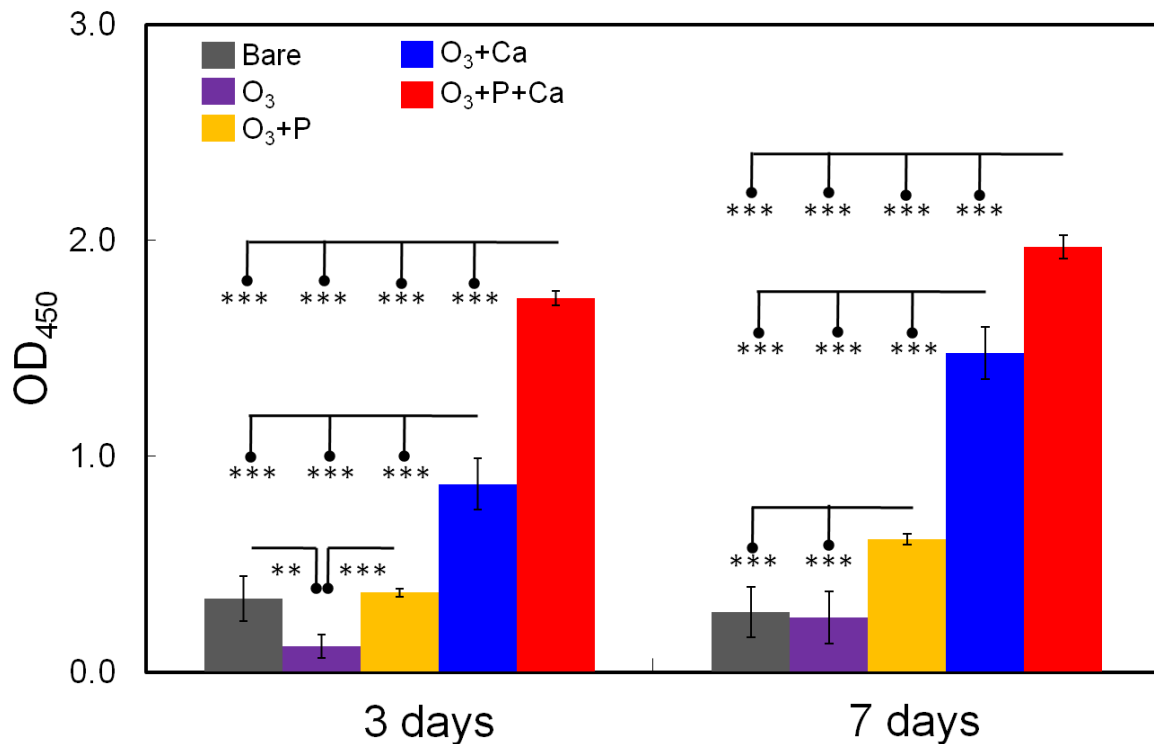


Fig. 3.7 Cell proliferation of PEEK after chemical treatments. Data are mean $\pm$ SD (n = 4). One-way ANOVA with multiple comparisons; *** p < 0.001; ** p < 0.01.

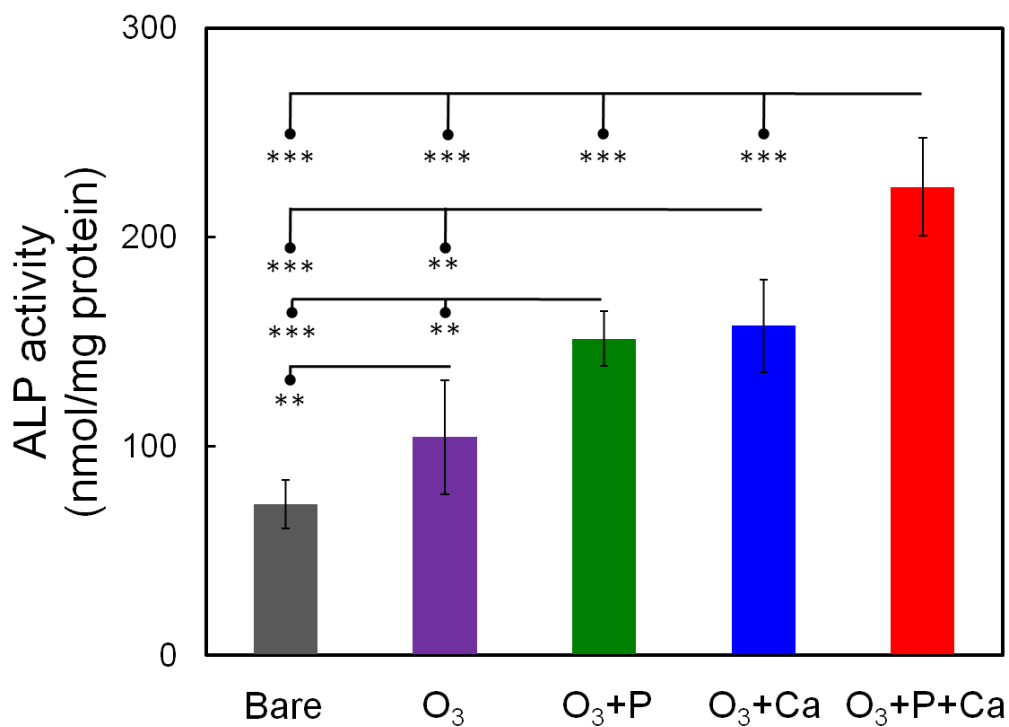


Fig. 3.8 ALP activity of PEEK after chemical treatments. Data are mean $\pm$ SD (n = 4).

One-way ANOVA with multiple comparisons; *** $p < 0.001$; ** $p < 0.01$.

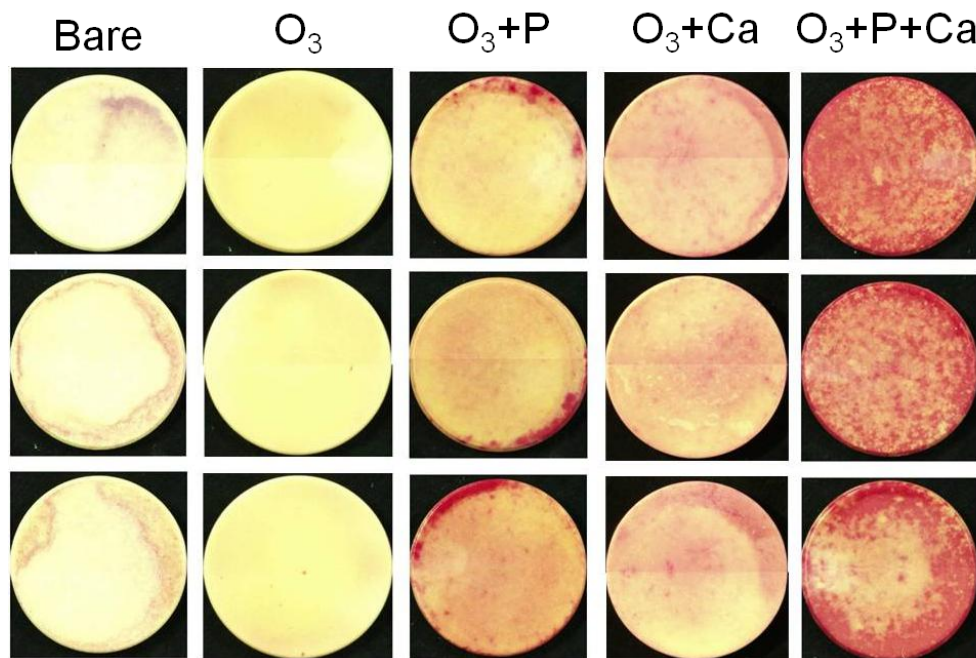


Fig. 3.9 Bone nodule of PEEK after chemical treatments at 20 days.

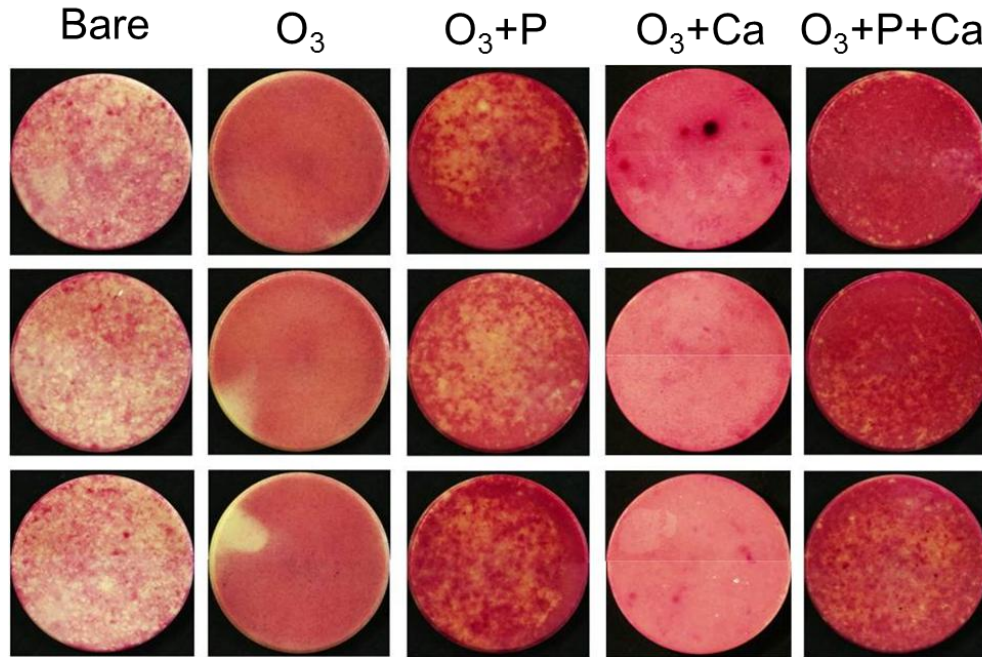


Fig. 3.10 Bone nodule of PEEK after chemical treatments at 28 days

3.3 Histology and Bone-implant contact (BIC)

Figs. 3.11 - 13 showed the histological results of O_3+P+Ca specimen after 2 and 4 weeks implantation, and quantification of bone-implant contact (BIC) was shown in Fig. 3.14. BIC was mostly observed in cancellous part of the femur rather than the cortical part (Figs. 3.11 and 3.12). At 2 weeks implantation, BIC was hardly found in bare PEEK (Fig. 3.13A). However, increased BIC was observed in O_3+P (Fig. 3.13C). Furthermore, O_3+P+Ca showed much greater BIC than both specimens (Fig. 3.13E). After 4 weeks implantation, the BIC of O_3+P+Ca specimen was increased and maintained as the highest compared with those of O_3+P and bare specimens (Figs. 3.13B, D, and F).

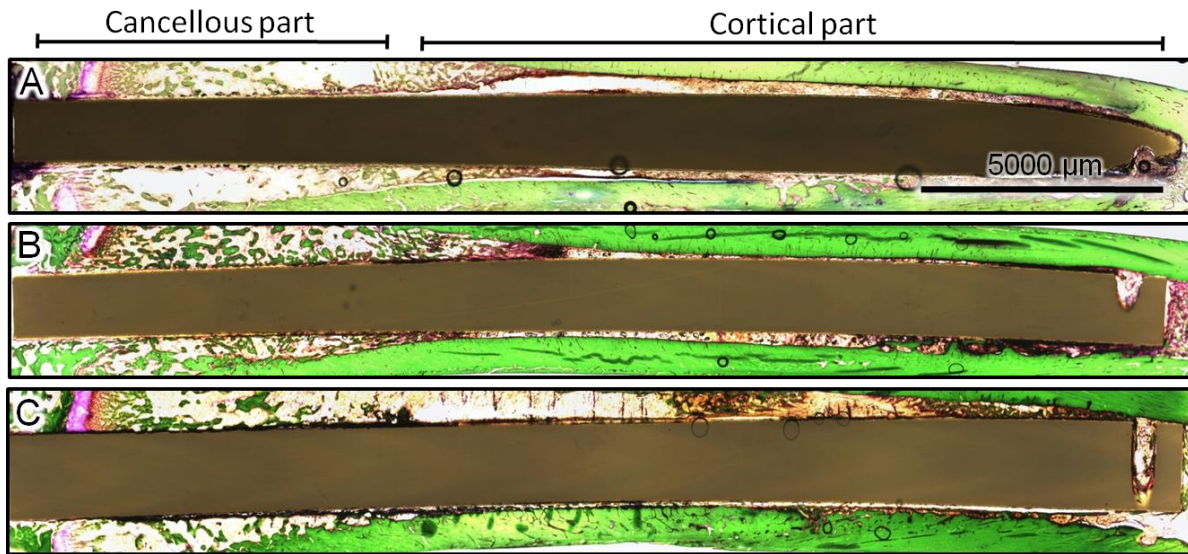


Fig. 3.11 Histological image of bare (A), O_3+P (B) and O_3+P+Ca (C) after 2 weeks implantation.

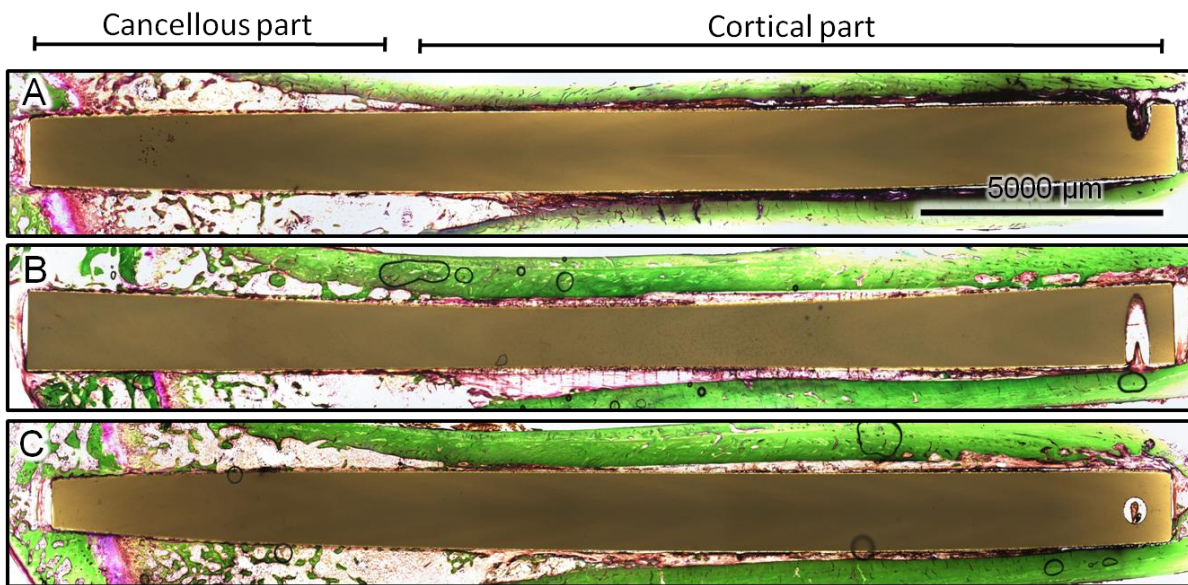


Fig. 3.12 Histological image of bare (A), O_3+P (B) and O_3+P+Ca (C) after 4 weeks implantation.

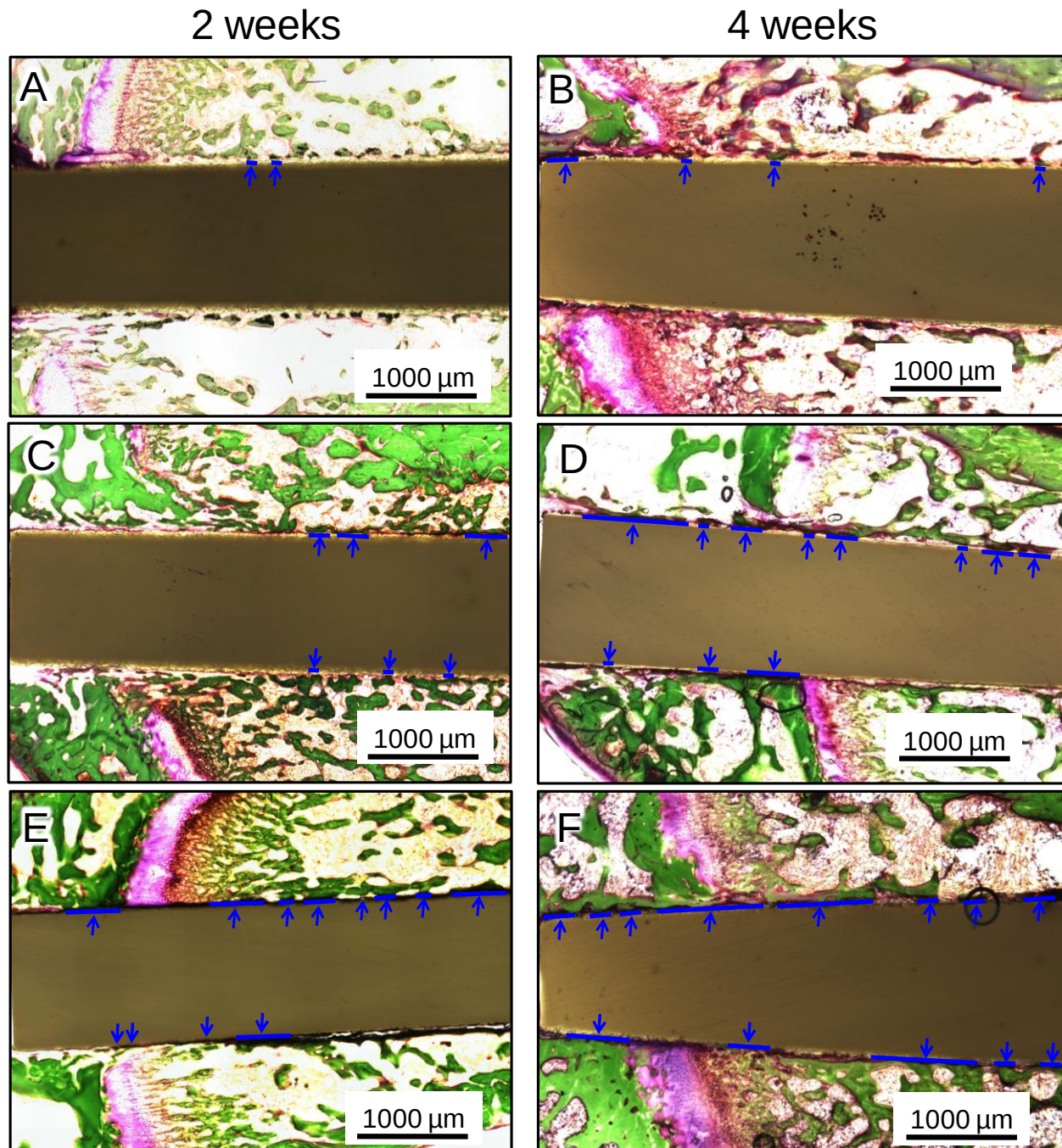


Fig. 3.13 Histological image of bare 2w (A), bare 4w (B), O₃+P 2w (C), O₃+P 4w (D), O₃+P+Ca 2w (E) and O₃+P+Ca 4w (F). Images were taken from cancellous part of the rat femur. Blue line indicates bone contact.

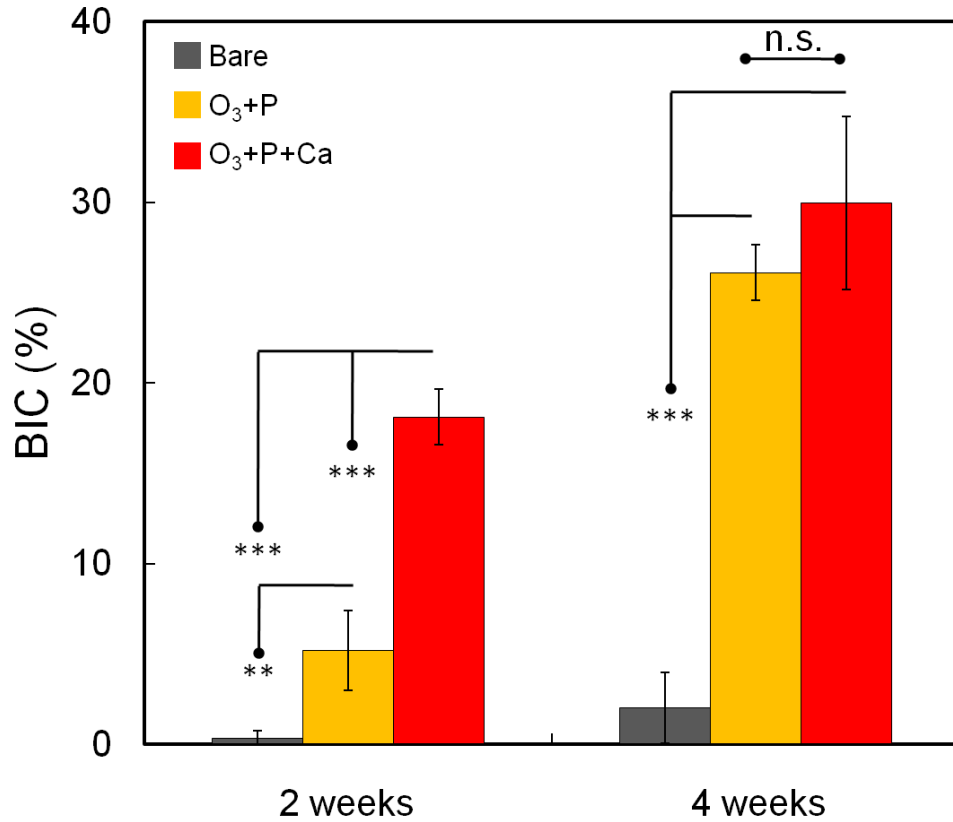


Fig. 3.14 Bone-implant contact of the specimens after 2 and 4 weeks implantation. Data are mean $\pm$ SD (n = 3). One-way ANOVA with multiple comparisons; *** p < 0.001; ** p < 0.01; n.s. not significant).

3.4 Pull-out force test

Bone-implant bonding of O₃+P+Ca specimen was evaluated by measuring its pull-out force (Fig. 3.15). First, roughened PEEK exhibited higher pull-out force compared with mirror polished PEEK as described in chapter 2. Pull-out force of roughened PEEK was further increased after modified with phosphate and Ca (O₃+P+Ca) at both 2 and 4 weeks implantation. At week 2, O₃+P+Ca specimen showed

higher pull-out force compared with O₃+P. However, BIC of O₃+P+Ca and O₃+P become similar at week 4. It was noteworthy that O₃+P+Ca demonstrated higher pull-out force when compared with machined Ti (dash line).

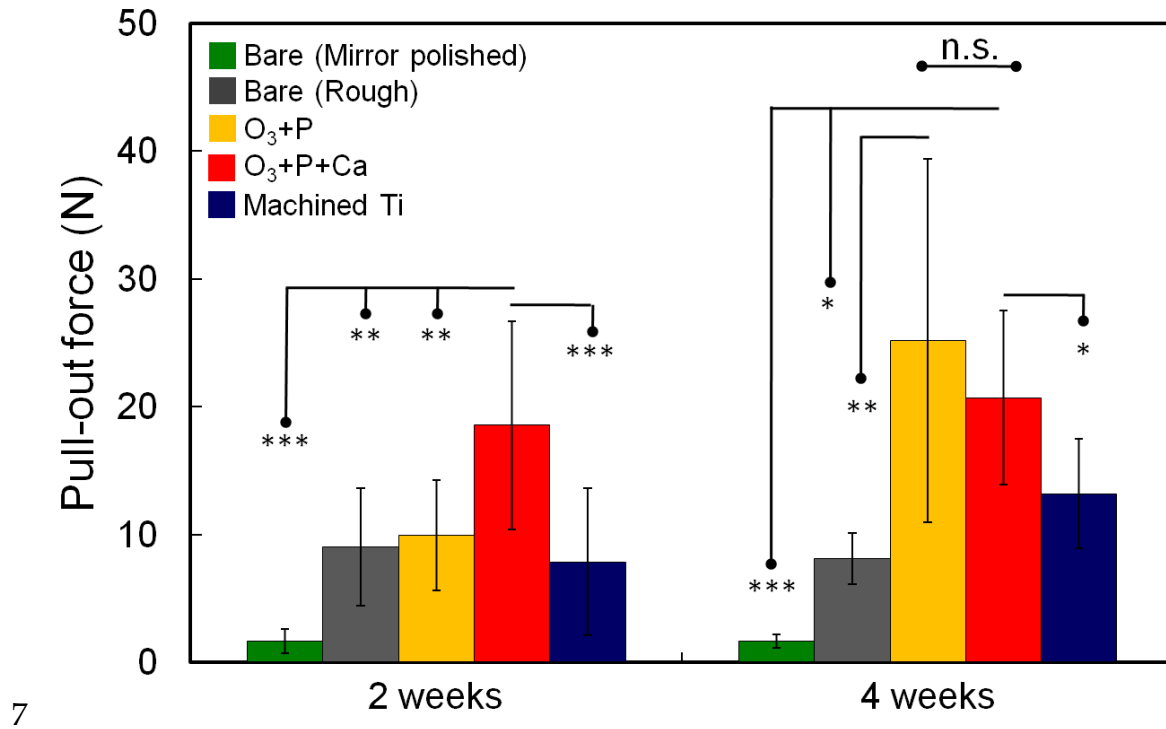


Fig. 3.15 Pull-out force of O₃+P+Ca at 2 and 4 weeks implantation. Data are mean $\pm$ SD (n = 6). One-way ANOVA with multiple comparisons; *** p < 0.001;

** p < 0.01; * p < 0.05; n.s. not significant.

4. Discussion

PEEK implant surface has been chemically modified with phosphate and Ca. Initially, PEEK was treated by ozone to produce oxidized PEEK surface containing OH group. This OH group was then used to bind the phosphate through reaction with

POCl₃. Subsequently, Ca ions could be immobilized through electrostatic interaction with phosphate.

Roughened PEEK surface has a hydrophobic characteristic as described in chapter 2. However, after O₃ gas treatment, the surface became more hydrophilic evidenced from the low water contact angle value. The increase of hydrophilicity is due to the formation of hydrophilic OH group after O₃ gas treatment. Similarly, O₃+P, O₃+Ca and O₃+P+Ca were all more hydrophilic compared with bare PEEK.

In cell study, O₃+P+Ca demonstrated the highest cell differentiation evidenced from the greatest ALP activity and bone nodule formation among the other samples. Previous report showed that the interactions between cells are fundamental in the process of cell differentiation as discussed in chapter 2. When mesenchymal stem cells are cultured on microdomains at various cell densities, ALP activity increases with the increasing of cell numbers [83]. As shown in Fig. 3.7, the greatest proliferation was found on O₃+P+Ca, suggesting that cell-cell interactions would be achieved earlier compared with the other samples. Therefore, one explanation for how the O₃+P+Ca induces more cell differentiation is its superior cell proliferation compared to those of other samples. Another possibility is that Ca and phosphate on PEEK activates cell differentiation. It was known that both Ca and phosphate increases the osteogenic activities of mesenchymal cells via distinct pathways [84, 85]. Elevated concentration of Ca increases ALP and osteocalcin expression, while phosphate increases runt-related

transcription factor 2 (Runx-2) and osteopontin expression [84-87]. Notably, all modified surfaces showed greater ALP activity and bone nodule formation, indicating both Ca and phosphate on PEEK activates cell differentiation. However, effect of Ca and phosphate on cell differentiation is found to be different. When compared with O_3+P and O_3+Ca , bone nodule formation of O_3+P was larger than that of O_3+Ca , although O_3+P had lower proliferation. This means that phosphate surface has greater osteoconductivity than that of Ca surface, although detail mechanism remains unknown. More importantly, O_3+P+Ca has the greatest osteoconductivity compared with other modified samples mainly because Ca and phosphate synergistically facilitate cell differentiation.

Bone-implant integration is the key for implant success. Osseointegration is the term that firstly introduced by Bränemark to explain such a phenomena [88]. Bränemark defines osseointegration as direct structural and functional connection between the ordered, living bone and the surface of load carrying implants. Histologically, osseointegration is defined in *Dorland's Illustrated Medical Dictionary* as the direct anchorage of an implant by the formation of bony tissue around the implant without the growth of fibrous tissue at the bone-implant interface. Albrektson and Johnson (2001) reported that osseointegration is achieved by direct bone-implant contact as the result of osteoconduction of an osteoconductive surface. It means that osteoconductivity is the prerequisite for osseointegration [89]. Biomechanically,

osseointegration can be defined as attachment resistant to shear as well as tensile forces as described by Steinmann et al (1986) cited by Albrektsson and Jacobsson (1987) [90] and Skripitz and Aspenberg (1998) [91]. As described in chapter 2, pull-out test is one of biomechanical tests that commonly used to evaluate the osseointegration.

PEEK does not bond chemically to bone as described in chapter 1. It was reported that fibrous tissue interface formed during implantation which indicated low degree of osseointegration [92]. Cheng (2013) demonstrated that the formation of fibrous tissue interface in bare PEEK was larger compared to that in plasmapore coated PEEK, indicating that bare PEEK had lower BIC [93]. Meanwhile, pull-out force of plasmapore coated PEEK was higher compared to that of bare PEEK, indicating higher osseointegration. The result demonstrated that the pull-out force correlated with BIC in which the higher BIC, the higher pull-out force.

In this study, BIC of O₃+P+Ca specimen was higher compared with both O₃+P and bare PEEK at 2 weeks implantation. Similarly, pull-out force of O₃+P+Ca specimen was also higher compared with both O₃+P and bare PEEK at 2 weeks implantation. At week 4, both O₃+P+Ca and O₃+P showed relatively similar BIC. As the result, their pull-out force was also relatively similar. Interestingly, the pull-out force of O₃+P+Ca was higher than that of machined Ti which is known for its osteoconductivity. These results demonstrated that chemical modification of PEEK using phosphate and Ca was able to produce osteoconductive PEEK through synergistic effect.

5. Conclusion

Chemical treatment of roughened PEEK using phosphate and/or Ca was successfully prepared. *In vitro* evaluation of the treated PEEK revealed that chemical modification by phosphate and Ca to roughened PEEK greatly improved RBM cell proliferation and mineralization, better than phosphate-modified PEEK and Ca-modified PEEK. *In vivo* evaluation of phosphate+Ca-modified PEEK showed highest BIC and pull-out force compared with both O₃+P and bare PEEK, 2 weeks after implantation. It also demonstrated that pull-out force of mirror-polished PEEK could be 10-times increase through surface roughening and chemical modification (Ca and P) on PEEK. In conclusion, chemical modifications by Ca and P to roughened PEEK have a great potential to create osteoconductive PEEK .

Chapter 4

General summary

In this thesis, surface roughening and chemical modification using phosphate and/or Ca were used to prepare osteoconductive PEEK. The results are summarized as follows:

In chapter 2, surface roughening was prepared by sandblasting method using alumina particles. Micro rough surface ($R_a \approx 2.3 \mu\text{m}$) was obtained. Mirror polished PEEK ($R_a \approx 0$) was prepared as a control. *In vitro* study revealed that micro rough surface was able to increase RBM cell proliferation and bone nodule formation compared with mirror polished surface, while ALP activity was comparable. *In vivo* pull-out force test showed that the pull-out force of roughened PEEK was about four times higher compared with that of mirror polished PEEK. These results demonstrated that surface roughening using sandblasting method was able to accelerate cells differentiation and improve pull-out force.

In chapter 3, chemical modification of roughened PEEK using phosphate and/or Ca was prepared to further increase its osteoconductivity. Briefly, PEEK was first treated with O_3 gas to produce OH groups on its surface. Phosphate (P) was bond to OH group on O_3 PEEK (O_3+P) by immersing in POCl_3 solution. Ca could be modified into O_3

and O₃+P PEEK through electrostatic interaction by immersion of both PEEK (O₃+Ca and O₃+P+Ca, respectively) in CaCl₂ aqueous solution. XPS analysis showed that Ca and P were successfully modified onto roughened PEEK surface. RBM cells on chemically modified PEEK exhibited higher proliferation, ALP activity and bone nodule formation than those on bare PEEK. The greatest cell responses were observed on O₃+P+Ca specimens. The O₃+P+Ca and O₃+P (a negative control) were then further evaluated *in vivo* using rat model. The O₃+P+Ca specimen showed highest bone-implant contact (BIC) and pull-out force compared with both O₃+P and bare PEEK specimens 2 weeks after implantation. It was demonstrated that pull-out force of mirror-polished PEEK could be 10-times increase through surface roughening and chemical modification (Ca and P) on PEEK.

In conclusion, chemical modifications by Ca and P to roughened PEEK have a great potential to create osteoconductive PEEK.

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