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Enhanced osteointegration on tantalum-implanted polyetheretherketone surface with bone-like elastic modulus

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ABSTRACT

Polyetheretherketone (PEEK) possesses a similar elastic modulus as bones but yet suffers from bio-inertness and poor osteogenesis. In this work, tantalum ions are implanted energetically into PEEK by plasma immersion ion implantation (PIII) to form Ta_2O_5 nanoparticles in the near surface. Nano-indentation reveals that the surface elastic modulus of the Ta ion implanted PEEK is closer to that of human cortical bones. *In vitro* cell adhesion, alkaline phosphatase activity, collagen secretion, extracellular matrix mineralization, and real-time PCR analyses disclose enhanced adhesion, proliferation, and osteogenic differentiation of rat bone mesenchymal stem cells (bMSCs) on the Ta-PIII modified PEEK. *In vivo* evaluation of the cortico-cancellous rat femur model by means of micro-CT, sequential fluorescent labeling, and histological analysis after 8 weeks confirms significantly improved osteointegration. The bone-like elastic modulus and modified surface topography of the Ta-PIII modified PEEK synergistically induce osteogenic differentiation of bMSCs and the surface-modified materials have large potential in dental and orthopedic implants.

1. Introduction

Dental and orthopedic implants are hard tissue substitutes for impaired human bones in case of trauma, diseases, and aging [1–3] and polyetheretherketone (PEEK) is a prime candidate to replace traditional metallic implants made of titanium and its alloys [4–6]. Despite the good biocompatibility, titanium possesses a large elastic modulus of over 100 GPa whereas PEEK has an elastic modulus of about 5 GPa that is closer to that of cortical bones [6]. As a result of the mismatched elasticity between PEEK and human bones, there are concerns of osteanabrosis and bone resorption caused by stress shielding [7]. Besides, degradation of the mechanical properties of metallic implants caused by corrosion can be avoided due to the well-known good chemical resistance of PEEK [4]. Unfortunately, in spite of the above attractive properties, PEEK is normally bioinert thus impeding osteointegration *in vivo* after implantation.

Tantalum (Ta) is a biocompatible metal with excellent strength and anticorrosion properties even in an acidic medium. The excellent anticorrosion properties of tantalum originate from the stable Ta_2O_5 protective film formed on the surface [8–10] and the biocompatibility of Ta has also been demonstrated [11–13]. However, its large elastic modulus of over 186 GPa and density of 16.6 g/cm^3 make direct clinical application difficult. Hence, there has been much effort in lowering the elastic modulus and weight of tantalum products. For instance, tantalum has been used as an alloying

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constituent in titanium or niobium to reduce the weight of implants while enhancing the biocompatibility [14,15] and porous tantalum scaffolds with a small elastic modulus (from 3 to 25 GPa) have been produced to meet the practical requirements [16–19]. Nevertheless, potential deformation of porous tantalum under high pressure limits its use as a load-bearing bone substitute.

During the healing process, the surface properties of the biomaterials play a crucial role in the cell–implant interactions at the implant/tissue interface [20,21]. In this respect, surface modification is an effective way to tailor the surface mechanical and biological properties while preserving the favorable bulk characteristics of the materials. Among the various surface modification techniques, plasma immersion ion implantation (PIII) is a non-line-of-sight method that is suitable for biomedical products [22,23]. By introducing certain elements and functional groups into the biomaterials, specific bio-functions can be achieved [24–26]. For instance, our recent work indicated that the *in vitro* osteogenic activity of PEEK and carbon-fiber-reinforced PEEK was enhanced by altering the surface nanostructure using PIII [27,28]. However, the relationship between the surface mechanical properties altered by PIII and corresponding biological effects is not well understood. It has been suggested that the heat produced on the atomic scale during PIII consolidates the coating and improves the adhesion between the coating and substrate [29,30]. With regard to polymers, the thermal effects, namely heat accumulation, can be magnified due to the poor thermal conductivity. Consequently, carbonization, crosslinking, fusion, and/or resolidification can occur on the polymer surface thereby altering the surface elastic modulus and hardness. Gas ion PIII has been shown to improve the elastic recovery of polymers like PEEK [31] and polytetrafluorethylene (PTFE) [24] but no similar studies have been conducted for metal ion PIII.

In this work, tantalum plasma immersion ion implantation (Ta-PIII) is performed under different conditions to modify PEEK to take advantage of the favorable properties of PEEK and tantalum. The osteogenic properties of the Ta-PIII modified PEEK are determined systematically using rat bone marrow mesenchymal stem cells (bMSCs) *in vitro* and the materials are also inserted into the rat femur bones for 8 weeks to evaluate osteointegration *in vivo*.

2. Materials and methods

2.1. Sample preparation

Biomedical grade polyetheretherketone (PEEK) was machined into samples with different dimensions. Square samples ($10 \times 10 \times 1$ mm^3) were used for surface characterization, ion release tests, and *in vitro* studies on 24-well tissue culture plates, square samples ($20 \times 20 \times 1$ mm^3) were used in the real-time PCR tests, and cylindrical samples ($\phi 2 \times 10$ mm^3) were used in the *in vivo* animal evaluation. The samples were polished on one side to a near mirror finish and ultrasonically cleaned in acetone, ethanol, and ultra-pure water prior to PIII. Tantalum was implanted into the samples using a filtered cathodic arc source housing a 99.99% pure tantalum rod with a diameter of 10 mm. Before PIII, the chamber was evacuated to a pressure of 5×10^{-3} Pa. To attain stable ionization of tantalum, argon (Ar) was introduced to the cathodic arc source at a flow rate of 5 sccm (standard cubic centimeter per minute). The PEEK samples were placed on a rotating sample stage connected to a high voltage. By applying a pulsed negative high voltage, tantalum ions were implanted and the sample stage was continuously rotated to obtain uniform ion implantation. Table 1 lists the important parameters and corresponding sample designations.

Table 1
Main conditions used in tantalum plasma immersion ion implantation.

	PEEK	Ta-30	Ta-120
Cathodic arc pulse duration (μs)	–	500	500
High voltage pulse duration (μs)	–	500	500
Pulsing frequency (Hz)	–	7	7
Implantation voltage (kV)	–	30	30
Implantation time (min)	–	30	120

2.2. Surface characterization

2.2.1. Surface structure and chemical characterization

The surface morphology was examined by field-emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Japan) at different magnification without a conductive coating. The surface chemical states and elemental depth profiles were determined by X-ray photoelectron spectroscopy (XPS, Physical Electronic PHI 5802) equipped with a monochromatic Al K_α source in City University of Hong Kong. The Ta depth profiles were acquired by XPS in conjunction with argon ion bombardment at a sputtering rate of about 4 nm/min.

The surface elastic modulus and hardness were determined by nanoindentation (Nano Indenter G200, Agilent, USA). The indentation depth range was 20–100 nm and the elastic recovery curves were acquired at a loading of 1.3 mN. At least eight indents were made to improve the statistics.

2.2.2. Ion release

Two pieces of each sample were incubated in 5 mL of phosphate buffered saline (PBS 1 M, pH = 7.4) for 7, 14, 21, and 28 days at 37 °C without stirring. At a prescribed time, all the 5 mL solution was withdrawn and analyzed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES, JY2000-2, France) to determine the amount of released tantalum. The withdrawn solution was replaced by the same volume of fresh PBS.

2.3. In vitro studies

2.3.1. Cell culturing

The rat bone mesenchymal stem cells (bMSCs: provided by Stem Cell Bank, Chinese Academy of Science, Shanghai, China) were isolated from the bone marrow. The bMSCs were cultured in the α-minimum essential medium (α-MEM, Gibto-BRL, USA) with 10% fetal bovine serum (FBS, Hyclone, USA), 1% antimicrobial of penicillin, and streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The α-MEM was refreshed every 3 days during cell culturing and the experiments were carried out with the bMSCs before passage five. All the samples were sterilized with 75% alcohol for 3 h and rinsed twice with sterile PBS before cell seeding.

2.3.2. Cell adhesion

The bMSCs were seeded onto the samples on a 24-well plate at a density of 5×10^4 cells per well. After culturing for 1, 3, and 24 h, the samples were taken out and put on another 24-well plate. The samples were rinsed twice with PBS and fixed with 3% glutaraldehyde overnight. Ethanol with different concentrations of 30, 50, 75, 90, 95, 100, and 100% v/v was used sequentially to dehydrate the samples for 10 min. The samples were dried and sputter coated with platinum before SEM observation (SEM, Hitachi S-3400, Japan).

2.3.3. Cell proliferation and viability

The alamarBlue™ (AbD Serotec Ltd, UK) assay was employed to quantitatively determine the cell proliferation and viability on the samples. The bMSCs were seeded on the samples (four replicates) on 24-well plates at a density of 2.5×10^4 cells per well. After 1, 4, and 7 days, the culture medium was replaced by 0.5 mL of the fresh medium with 5% alamarBlue™ in each well. After incubation for 4 h, 100 μL of the medium was transferred to a 96-well plate for measurement. The amount of reduced alamarBlue™ was determined on an enzyme-labeling instrument (BIO-TEK, ELX 800) at wavelengths of 570 nm and 600 nm. The operation and calculation of cell proliferation followed the instruction of the alamarBlue™ assay.

2.3.4. Alkaline phosphatase activity

The bMSCs were seeded on the samples (four replicates) on 24-well plates at a density of 1×10^4 cells per well (culturing for 7 days) or 0.5×10^4 cells per well (culturing for 14 days). In the quantitative alkaline phosphatase (*ALP*) assay, after culturing for 7 and 14 days, a Bio-Rad protein assay kit (Bio-Rad, USA) was employed to calculate the total protein content and the results were adjusted with a series of BSA (Sigma) standards by measuring the optical density (OD) values of the absorbance at 570 nm. After incubation with p-nitrophenyl phosphate (Sigma) at 37 °C for 30 min, the *ALP* activity was calculated and adjusted with a series of 4-Nitrophend NaOH (0.02 M) solutions by measuring the OD values at 405 nm. The *ALP* levels were normalized to the total protein content and described as μM/mg total proteins.

For *ALP* staining, the samples cultured for 7 or 14 days were rinsed twice with PBS, immersed in citrate buffered acetone for cell fixation for 30 s, and rinsed with ultra-pure water for 45 s. A mixture of naphthol AS-MX phosphate (Sigma–Aldrich) and fast blue RR salt (Sigma–Aldrich) was used in alkaline-dyeing. The samplers were dyed with the alkaline-dye mixture for 30 min and rinsed thoroughly with ultra-pure water for 2 min. Finally, the samples were immersed in Mayer's Hematoxylin Solution (Sigma–Aldrich) for 10 min and rinsed with ultra-pure water for 3 min. The stained specimens were observed by fluorescence microscopy (Olympus IX 71, Olympus, Japan).

2.3.5. Collagen secretion

Collagen secretion of the bMSCs on the samples was quantified using Sirius Red staining method. The bMSCs were seeded on the samples (four replicates) on 24-well plates at a density of 1×10^4 cells per well (cultured for 7 days) or