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Enhanced osteogenic activity of poly ether ether ketone using calcium plasma immersion ion implantation

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ABSTRACT

As a promising implantable material, poly ether ether ketone (PEEK) possesses similar elastic modulus to that of cortical bones yet suffers from bio-inertness and poor osteogenic properties, which limits its application as orthopedic implants. In this work, calcium is introduced onto PEEK surface using calcium plasma immersion ion implantation (Ca-PIII). The results obtained from scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) confirm the modified layer with varying contents of calcium are formed on PEEK surfaces. Water contact angle measurements reveal the increasing hydrophobicity of both Ca-PIII treated surfaces. *In vitro* cell adhesion, viability assay, alkaline phosphatase activity and collagen secretion analyses disclose improved the adhesion, proliferation, and osteo-differentiation of rat bone mesenchymal stem cells (bMSCs) on Ca-PIII treated surfaces. The obtained results indicate that PEEK surface with enhanced osteogenic activity can be produced by calcium incorporation.

1. Introduction

Orthopedic implants have been increasingly required for impaired human bones caused by trauma, disease, aging or congenital defects [1]. Poly ether ether ketone (PEEK) is recently becoming a prime candidate to replace traditional metallic implants such as titanium and titanium alloys [2]. Despite of the good biocompatibility, titanium implants possess much higher elastic moduli (over 100 GPa), while PEEK has an adjustable elastic modulus close to that of cortical bone which can mitigate concerns over the risks of osteanabrosis and bone resorption caused by stress shielding as a result of the elasticity mismatch between the implants and human bones [3–5]. Besides, potential toxicity of ion release and declining mechanical properties of metallic implants caused by corrosion can be avoided due to the well known good chemical resistance of PEEK [3,6,7].

However, the excellent stability originated from the chemical structure makes PEEK bioinert, which impedes osteointegration after implantation thereby severely hampering clinical adoption as orthopedic implants [2,3]. To improve its osteogenic properties, calcium compounds have been introduced into PEEK in numerous ways, as calcium is one of the main constitutes in human bones. Moreover, studies have demonstrated that moderate amount of extracellular calcium ions plays a critical role in regulating proliferation and osteo-differentiation of osteoblasts and bone mesenchymal stem cells (bMSCs) [8]. For example, hydroxyapatite has been added into PEEK to improve bioactivity both *in vitro* and *in vivo* [9]. Poly ether ether ketone/β-tricalcium phosphate (PEEK/β-TCP) composites have also been developed to promote osteoblast proliferation [10]. Nevertheless, in contrast to the improved osteogenic properties, mechanical properties of PEEK compounds have been more or less sacrificed [11,12].

During the healing process, surface properties play a crucial role in the early cell and bacterial behavior at the implant/tissue interface [13,14]. Therefore, surface modification offers an effective way to enhance the surface mechanical and biological properties meanwhile preserving the advantageous bulk properties of the materials. Among the various surface modification techniques, plasma immersion ion implantation (PIII) is a non-line-of-sight method that has been widely applied to microelectronics, aerospace engineering, precision manufacturing, and biomedical engineering [15–17]. By introducing different elements and functional groups, surface properties such as cytocompatibility, antibacterial activity, and mechanical properties can be selectively tailored [4,18–22]. For instance, our previous work introduced silver nanoparticles on titanium by silver-PIII to enhance both biocompatibility and antibacterial activities [19]. Krupa et al.

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

	PEEK	Ca-1	Ca-2
Cathodic arc pulse duration (μs)	–	500	500
High voltage pulse duration (μs)	–	500	500
Pulsing frequency (Hz)	–	7	7
Implantation voltage (kV)	–	15	30
Implantation time (min)	–	60	60
Pulsed arc current (A)	–	0.2	0.2
Pulsed arc voltage (kV)	–	13.1	13.1

implanted calcium and phosphorus onto titanium and improved the bioactivity and corrosion resistance [20].

Although a number of studies have already devoted to modifying titanium surface with PIII technique [19,23,24], surface modification of PEEK with PIII technique and the corresponding bMSC response to modified PEEK surface have been few studied [25,26]. In this work, Ca ions were implanted onto PEEK surface by calcium plasma immersion ion implantation (Ca-PIII). The effects of Ca-PIII process on the surface structure and surface composition were investigated. Furthermore, the osteogenic properties of the Ca-PIII modified PEEK surface were assessed *in vitro* using rat bMSCs.

2. Materials and methods

2.1. Sample preparation

Biomedical grade poly ether ether ketone (PEEK) was used in this work. 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. The samples were one-side polished to a near mirror finish and ultrasonically cleaned in acetone, ethanol and ultra-pure water prior to PIII treatment. Calcium cathode rod was obtained from a large piece of 99.99% pure calcium bulk material and machined to the dimension of 10 mm diameter with 30 mm length. The calcium rod was kept in an inert environment to prevent oxidation before using. Table 1 lists the important parameters and the corresponding sample designation. Briefly, calcium was implanted into all samples using a magnetic filtered cathodic arc source housing the calcium rod. Before PIII process, the main chamber was vacuumed to a pressure of 5×10^{-3} Pa. PEEK samples were placed on the sample stage connected to high voltage. Calcium was controllably triggered, ionized and positively charged using a high-voltage pulsed source. By applying a pulsed negative high voltage, calcium ions were implanted and the sample stage was continuously rotated in order to obtain uniform ion implantation.

2.2. Surface characterizations

2.2.1. Surface structure and chemical characterizations

The surface morphology of all samples was examined using field-emission scanning electron microscopy (FE-SEM, Hitachi S-4800, Japan) at different magnification without applying any conductive coating.

The surface chemical states were determined by X-ray photoelectron spectroscopy (XPS) (Physical Electronic PHI 5802 equipped with a monochromatic Al Kα source) in City University of Hong Kong.

To determine the surface wettability, static water contact angle was measured (Automatic Contact Angle Meter Model SL200B, Solon information technology Co., Ltd., China) by sessile drop method, using ultra-pure water as media. Each data was acquired from the average of three different areas on each sample.

2.2.2. Ion release

One piece of each sample was incubated in 10 mL phosphate buffered saline (PBS 1 M) for different immersion time (7, 14, 28, and 56 days) at 37 °C without stirring. At a prescribed time, all the solution was withdrawn and analyzed using inductively-coupled plasma atomic emission spectroscopy (ICP-AES, JY2000-2, France) analysis to determine the amount of released calcium ions. The withdrawing solution was replaced with the same volume of fresh PBS.

2.3. In vitro *studies*

2.3.1. Cell culturing

The bone mesenchymal stem cells (bMSCs, provided by Stem Cell Bank, Chinese Academy of Sciences, Shanghai, China) were isolated from the bone marrow of six-week-old male Fisher 344 rats. 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

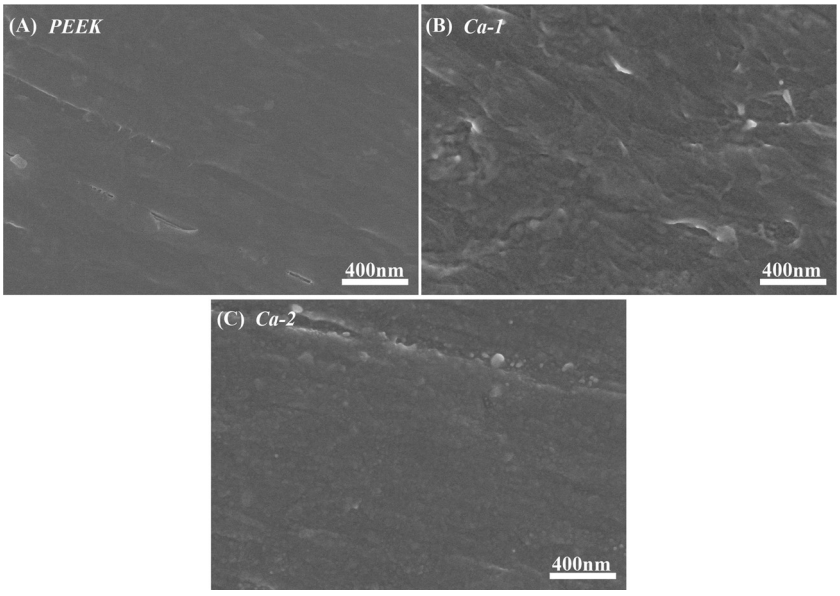

Fig. 1. Surface morphology of PEEK (A), Ca-1 (B) and Ca-2 (C).