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Enhanced osteoblast responses to poly ether ether ketone surface modified by water plasma immersion ion implantation

Heying Wang, Tao Lu, Fanhao Meng, Hongqin Zhu, Xuanyong Liu*

State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China

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ABSTRACT

Poly ether ether ketone (PEEK) offers a set of characteristics superior for human implants; however, its application is limited by the bio-inert surface property. In this work, PEEK surface was modified using single step plasma immersion ion implantation (PIII) treatment with a gas mixture of water vapor as a plasma resource and argon as an ionization assistant. Field emission scanning electron microscopy, atomic force microscopy and X-ray photoelectron spectroscopy were used to investigate the microstructure and composition of the modified PEEK surface. The water contact angle and zeta-potential of the surfaces were also measured. Osteoblast precursor cells MC3T3-E1 and rat bone mesenchymal stem cells were cultured on the PEEK samples to evaluate their cytocompatibility. The obtained results show that the hydroxyl groups as well as a "ravined structure" are constructed on water PIII modified PEEK. Compared with pristine PEEK, the water PIII treated PEEK is more favorable for osteoblast adhesion, spreading and proliferation, besides, early osteogenic differentiation indicated by the alkaline phosphatase activity is also up-regulated. Our study illustrates enhanced osteoblast responses to the PEEK surface modified by water PIII, which gives positive information in terms of future biomedical applications.

1. Introduction

As a substitute for titanium and its alloys, poly ether ether ketone (PEEK) was highlighted in the 1980s [1]. PEEK is a linear polyaromatic thermoplastic (chemical structure shown in Fig. S1) with a crystallinity of 30–35% typically and offers a set of characteristics superior for biomaterials including excellent mechanical properties [2], non-toxicity [3], good chemical and sterilization resistance [4], and natural radiolucency. More importantly, compared with titanium and its alloys, PEEK has a relatively low elastic modulus which is closer to that of cortical bone [5,6]. This contributes to the minimization of stress shielding effect and peri-implant bone resorption [7,8], avoiding the possible loosening of implants.

However, the stable chemical structure makes PEEK inert and keeps it from binding to bone directly [9], resulting in inferior osseointegration [10]. It is known that surface properties, both chemical and topographical, are important in tissue response and wound healing [11]. Surface modification may make it more attractive for osteoblast growth, which leads to improved bone integration. Various modification methods have been developed to alter the surface properties of PEEK [12–14]. Han et al. [15] coated PEEK surface with a titanium layer using electron beam deposition method. In vitro cellular responses were enhanced and the in vivo animal tests also showed a higher bone-in-contact ratio. Noiset et al. [16] employed wet-chemistry technique to selectively reduce PEEK and covalently fixed amine and carboxyl groups on the surface. The cultivation of $CaCo_2$ epithelial cells demonstrated the cellular adhesion and growth were improved. Nevertheless, these methods have some drawbacks such as poor bonding, complex operation and time-consuming post-treatment.

As an important surface modification technique, plasma immersion ion implantation (PIII) has drawn much attention due to its simple operation and non-light-of-sight characteristics which bode well for biomedical implants with a complex shape [17]. Moreover, surface properties can be selectively modified using PIII technique without affecting the bulk characteristics [18]. In the PIII process, the sample is immersed in a plasma and negative high voltage pulses are applied to it. When the sample is negatively biased, the electrons around it are repelled and a positive ion sheath is established. Ions are accelerated by the electric field in the sheath and implanted into the sample surface vertically from all directions. Surface physical states such as roughness and hydrophilicity can be adjusted flexibly by PIII method [19,20]. Meantime, by using

* Corresponding author. Tel.: +86 21 52412409.
E-mail address: xyliu@mail.sic.ac.cn (X. Liu).

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different plasma sources, different elements and chemical groups can be introduced into the surface [21,22].

It has been reported that surfaces fixed with hydrophilic functional groups [16] and short peptides such as RGD (Arg-Gly-Asp) [23], or packed with proteins [24] are favorable for cell adhesion and proliferation. Hydroxyl is a hydrophilic functional group, meantime, its chemical activity provides possibilities for further chemical modification of PEEK. Construction of hydroxyl groups using water PIII method has been reported in the literatures [25,26]. Moreover, there are some publications on modifying polymeric biomaterials involving PEEK by gas PIII in recent years [27,28], which has enlightening significance to the follow-up works.

In the present work, a simple PIII method with a gas mixture of water vapor as a plasma resource and argon as an ionization assistant is conducted to graft hydroxyl groups on PEEK and at the same time alter the physical states of the surface. The surface physical and chemical properties are examined and in vitro osteoblast responses are determined and discussed.

2. Materials and methods

2.1. Specimen fabrication

The samples were one-side polished 10 mm square PEEK plates with a thickness of 1 mm. Before placed onto the sample stage, the samples were ultrasonically cleaned to remove contaminations. The pressure of the vacuum chamber was 5×10^{-3} Pa and water vapor was bled into it till the pressure increased to 1×10^{-2} Pa. The vacuum chamber was heated to 100 °C to avoid vapor liquidation. In order to aid water vapor to ionize, argon was introduced into the vacuum chamber at a flow rate of 30 sccm to form a ($Ar + H_2O$) mixture, then a bias voltage of −800 V was applied. Other parameters are listed in Table S1. To eliminate the interference of argon and highlight the effects of water vapor, a comparison set was designed: the samples were prepared as above, but omitting the introduction of water vapor into the vacuum chamber. The PEEK samples after undergoing ($Ar + H_2O$) mixture and argon PIII are designated as AW-PEEK and A-PEEK, respectively.

2.2. Surface characterizations

The surface morphology of the samples was examined by field emission scanning electron microscopy (FE-SEM; S-4800, HITACHI, Japan) and atomic force microscopy (AFM; Digital Instruments, USA). The AFM was operated in contact mode on a scanned area of 5 μm × 5 μm, and the arithmetic average roughness (Ra) of the samples was also estimated.

2.3. Contact angle measurement

The surface wettability was measured by contact angle measurement (Automatic Contact Angle Meter Model SL200B, China) using sessile distilled water droplets at 26 °C. Generally, polymers such as PEEK will go through a process named “aging” spontaneously when they are treated with high-energy particles and then kept in air. In the aging process, hydrophilicity of the treated surface will decrease, which is unfavorable for keeping the hydrophilicity of modified surfaces [29]. In order to inhibit the aging of PEEK polymer after modification [30], we stored the PIII-treated samples in water and measured the contact angles at 0, 4, 10 and 15 days, respectively. After being stored in water for 2 weeks, they were put back to atmospheric environment and the contact angles at 0, 4, 10, 15 days were measured again to examine the effect of the inhibition. For comparison, the contact angles of samples stored in atmospheric environment were also measured at the same time points. The three kinds of storage medium mentioned above are designed as H_2O, H_2O–air, and air, respectively. The measurement was conducted five times for each sample set and the results are expressed as means ±standard deviation (SD).

2.4. X-ray photoelectron spectroscopy

The surface chemical states were determined by X-ray photoelectron spectroscopy (XPS; PHI 5802, Eden Prairie, MN) with a Mg Kα (1253.6 eV) source.

A chemical derivatization method was applied to label the hydroxyl groups for detection. With the assistance of pyridine and benzene, hydroxyl groups were reacted with trifluoroacetic anhydride at 25 °C for 1.5 h to be labeled with fluorine [31]. The reaction formula is shown in Fig. S2.

2.5. Surface zeta-potential measurements

The surface zeta-potential was measured by Surpass electrokinetic analyzer (Anton Parr, Austria). For each sample set, two specimens with size of 20 mm × 10 mm were fixed aspectant on sample holders. A KCl solution (0.001 M) was used as the medium and the pH value was adjusted by HCl and NaOH. During the streaming current measurements, the zeta-potential resulting from the motion of ions in the diffusion layer was measured according to Helmholtz–Smoluchowski equation,

$$\zeta = \frac{dI}{dP} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times \frac{L}{A}$$

in which ζ is the zeta-potential, dI/dP represents the slope of the streaming current versus pressure difference, η, ε_0 and ε denote the viscosity, vacuum permittivity and dielectric constant of the electrolyte solution, L and A are the length and cross-section of the streaming channel, respectively. For statistical accountability the zeta-potential was measured four times at each pH value and the results are expressed as means ±SD.

2.6. Osteoblast responses to surfaces

2.6.1. Cell culture

Osteoblast precursor cells MC3T3-E1 (Cells Resource Center, Shanghai Institutes for Biological Sciences) were seeded on the samples at a density of 5.0×10^4 cells per well using 24-well tissue culture plates (Costar, USA) as holders. Before cell seeding, all the specimens were sterilized with 75% ethanol for 2 h. The cultures were incubated in a humidified atmosphere of 5% CO_2 at 37 °C and the medium was refreshed every three days.

Rat bone mesenchymal stem cells (BMSC, Cells Resource Center, Shanghai Institutes for Biological Sciences) derived from male Wistar rats were seeded on the sterilized samples at a density of 5.0×10^3 cells per well and incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 14 days. The medium was refreshed every three days.

2.6.2. Cell proliferation and viability

The cell proliferation and viability of MC3T3-E1 were determined quantificationally using the alamarBlue™ (AbD Serotec Ltd., UK) assay that measured the accumulative metabolic activity. Five specimens were tested for each incubation period (1, 4 and 7 days). Accumulation of reduced alamarBlue™ was determined by absorbance measurements at extinction wavelengths of 570 nm and 600 nm and calculated according to the following formula:

$$\frac{117,216 \times A^{\lambda_1} - 80,586 \times A^{\lambda_2}}{155,677 \times A'^{\lambda_2} - 14,652 \times A'^{\lambda_1}} \times 100\%$$