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A combination of CO_2 laser and plasma surface modification of poly(etheretherketone) to enhance osteoblast response

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ABSTRACT

Poly(etheretherketone) (PEEK) is a rigid semicrystalline polymer that combines excellent mechanical properties, broad chemical resistance and bone-like stiffness and is widely used in biomedical fields. However, the bio-inert surface of PEEK tends to hinder its biomedical applications when direct osteointegration between the implants and the host tissue is desired. In this work, we demonstrate a dual modification method, which combines the laser and plasma surface treatment to combine advantages of both chemical states and microstructures for osteoblasts responses. While the plasma treatment introduces surface carboxyl groups (—COOH) onto PEEK surface, the laser treatment constructs microstructures over the PEEK surface. Our results indicated that —COOH as well as microgrooves containing micropores or microcraters structure are constructed on PEEK surface and plasma treatment has no apparent effect on the morphology of microstructures produced by laser micromachining. Unexpectedly, the superior mechanical properties of PEEK were maintained irrespective of the treatment used. Compared to native PEEK and single treated PEEK, dual modified PEEK is more favorable for pre-osteoblasts (MC3T3-E1) adhesion, spreading and proliferation. Moreover, cell pseudopodia protrude into the micropores or microcraters, in favor of forming firmer bone-implant integration. Our study illustrates enhanced osteoblasts responses to dual treated PEEK surface, which gives beneficial information of its potential use in orthopedic or dental implants.

1. Introduction

Poly(etheretherketone) (PEEK) [1], a high performance engineering thermoplastic, is a rigid semicrystalline polymer that exhibits superior mechanical properties, high temperature durability and broad chemical resistance. Therefore, PEEK has been widely used in diverse number of fields including aerospace, automotive and marine industries. When used in biomedical applications, PEEK, approved by the U.S. Food and Drug Administration (FDA) in the late 1990s as an implantable biomaterial, offers a set of additional characteristics advantageous to its use as a biomaterial such as bone-like stiffness [2], repeated sterilization capacity [3], non-toxicity [4,5], natural radiolucency and readily shaped using machining. More importantly, PEEK has extremely low elastic modulus (3–4 GPa), and the modulus can be tailored to closely match cortical bone (18 GPa) by preparing carbon-fiber-reinforced (CFR) composites [1]. Therefore, the stress-shielding effect and peri-implant bone resorption often observed in titanium-based metallic implants can be minimized [6,7]. However, PEEK has a bio-inert surface characteristic which limits the cellular adhesion [8] and hence it does not contribute to the formation of a chemical bond with natural bone tissue, thus producing delayed or weak bone-implant integration. Therefore, there is a need to modify its surface characteristics to enhance the ability for cells to attach.

It is known that surface characteristics, including the surface roughness, wettability and chemical composition, are important factors governing the cell interaction with a substratum [9]. Surface modification may make it more attractive for osteoblasts adhesion, spreading and growth, and hence leads to improved bone-implant integration. Therefore, considerable efforts have been made to alter the surface properties of PEEK implants. On the one hand, osteoconductive hydroxyapatite coating was introduced onto PEEK surface using various physical and chemical methods, including thermal plasma spray deposition [10], aerosol deposition [11] and *in vitro* precipitation [12,13] to improve and accelerate direct bone-implant integration. On the other hand, immobilization of biomolecules such as cell-adhesive RGD-containing peptides leads to the formation of biomimetic PEEK surface to promote cell

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adhesion, spreading and proliferation [14,15]. However, these techniques have some drawbacks such as weak bonding, complex operation and time-consuming post-treatment.

Another pivotal strategy to improve implant-bone integration is to increase roughness of implant surface or introduce porosity to implant surface. Rough surfaces were found to produce better bone fixation than smooth surfaces *in vivo* studies [16]. Many techniques have hitherto been employed to increase roughness or fabricate porous structures on metal surfaces, including machining, sandblasting, laser micromachining, acid-etching and anodic oxidation [17]. Among these methods, laser micromachining is a promising alternative because of its high resolution, high operating speed, low cost, processing flexibility and keeping unaltered the bulk properties [18]. Moreover, there are some publications on modifying PEEK surface physical morphology by laser micromachining to investigate its effects on wettability or the response of osteoblast cells in recent years [18–20], which has enlightening significance to the follow-up studies.

In the literature, a wide range of surface functional groups including carboxyls (—COOH), hydroxyls (—OH) and amines ($-NH_2$) have been evaluated to improve cell adhesion and proliferation [21,22]. And several studies have demonstrated that the presence of surface —COOH promotes both improved osteoblasts adhesion and proliferation compared to unmodified controls [21–23]. However, it is difficult to functionalize PEEK surface on account of its inherent chemical inertness. Fortunately, plasma polymerization is a versatile method for surface modification of non-reactive substrates like PEEK, because plasmas can functionalize surfaces with various chemical groups without altering materials bulk properties [24]. To the best of our knowledge, there are no reports on surface-carboxylated PEEK by plasma polymerization, although there are some works on wet chemical treatment [25], silanization modification [15,26] and UV assisted photograft polymerization [27].

In this current work, therefore, we introduced a dual treatment method which combines the laser micromachining and plasma polymerization to obtain microstructured and carboxylated PEEK surfaces. The surface physical and chemical properties are examined and *in vitro* pre-osteoblast cells (MC3T3-E1) responses in terms of cell adhesion, spreading and proliferation are determined and discussed.

2. Experimental

2.1. Microstructuring by laser modification

PEEK membranes (APTIV® 1000, thickness ~240 μm) were obtained from Victrex (Lancashire, England). Prior to use, in order to obtain pristine surface (named P-PEEK), the PEEK films were immersed in refluxing acetone for 48 h, rinsed twice with acetone, and then dried under vacuum at 60 °C for 3 h. The laser micromachining was carried out at 10.6 μm wavelength irradiation and at a pulse frequency 5 kHz using a LM-24 CO_2 laser marking machine (Chengdu Leip Technology Co., Ltd.). Laser output power was fixed to 1 W. The laser treatment was done in air and at an angle of incidence with respect to the surface normal of 0°. The PEEK sample was placed at the focus of the laser system which was 100 mm away from the output facet of the laser system. After laser surface treatment, the samples were cleaned in refluxing acetone for 24 h and dried under vacuum at 60 °C for 24 h. The surface-microstructured PEEK was denominated as PEEK-L.

2.2. Plasma polymerization

—COOH was introduced onto PEEK surface using plasma polymerization of acrylic acid (AAc, Aldrich, >99%). Distilled AAc was injected into a plasma-induced grafting reactor (Suzhou OPS Plasma Technology Co., Ltd., DJ-01). Vacuum before glow discharge was 8 Pa and the working temperature was 20 °C. The PEEK substrates were pretreated with argon gas at 32 Pa and a plasma power of 300 W for 3 min. The plasma was generated using a radio-frequency generator (Suzhou OPS Plasma Technology Co., Ltd., DT-04) operating at 13.56 MHz. Then, the stainless steel reaction chamber was evacuated to 7 Pa and, subsequently, AAc monomers were grafted using the grafting reactor, while maintaining the vacuum at 35 Pa. Plasma polymerization lasted about 4 min at 100 W. After returning the chamber to atmospheric pressure, the samples with plasma-polymerized thin films were removed. Dual- and single-treatment specimens were named PEEK-L-COOH and PEEK-COOH, respectively.

2.3. Characterization

2.3.1. X-ray photoelectron spectroscopy (XPS)

The chemical components of the surface-modified PEEK were measured by XPS (XSAM800, Kratos Ltd., UK). The photoelectrons were exited with an X-ray source using Al Kα (1468.6 eV). Measurements were taken at a take-off angle of 20° with respect to the sample surface with an analyzed area of 100 mm^2. Survey scans over a binding energy range of 0–1100 eV were performed for each substrate at pass energy range of 100 eV, followed by high-resolution XPS measurement (pass energy 50 eV) for quantitative determination of binding energy and atomic concentration. Electron binding energies were calibrated to the reference of C1s set at 284.8 eV. The peak areas were determined with a linear background subtraction. Data analysis and curve fitting were done using XPS PEAK95 Version 3.1 software. Repeatability of the peak positions was ±0.2 eV.

2.3.2. Water contact angle

The water static contact angle measurements were taken using a goniometer (Krüss DSA100) by placing 3 μl of distilled water on PEEK surface. Each sample was measured at three separate locations and reported values are an average of these measurements with the associated standard deviation.

2.3.3. Differential scanning calorimetry (DSC)

Thermal analysis was performed using a TA Instruments DSC (Q20) with a Universal Analysis 2000, operating under ultrahigh-purity nitrogen flow. The samples were loaded in aluminum pans, heated to 380 °C for 5 min to erase all previous thermal history, and then cooled to 25 °C at a cooling rate of 10 °C/min. After cooling, the samples were reheated from 25 °C to 380 °C at a heating rate of 10 °C/min. The glass transition temperature and melting point temperature of PEEK derivatives were read from the second heating traces. The crystallinity of PEEK derivatives were determined from the relation between the apparent melting enthalpy of the functionalized PEEK ΔH_m and the extrapolated value of the enthalpy corresponding to the melting of a 100% crystalline unmodified PEEK, taken as 130 J g^{-1} [28].

2.3.4. X-ray diffraction (XRD)

The X-ray diffractometer (XRD, Philips, X'Pert Pro) with a monochromatic Cu Kα radiation ($\lambda = 0.1542$ nm) was operated at 30 kV and 20 mA. The 2θ range was 5–50° and an XRD profile was recorded in step-scan intervals of 0.05° in a continuous scan mode.

2.3.5. Scanning electron microscopy (SEM)

The sample surface was studied using field emission scanning electron microscope (FE-SEM, S-4200, Hitachi, Japan). The samples