Materials Letters 164 (2016) 60–63

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Dose-dependent enhancement of osteoblast cell adhesion, spreading and proliferation on plasma-carboxylated poly(etheretherketone) surface

Yanyan Zheng [a,b], Chengdong Xiong [a], Lifang Zhang [a,*]

[a] Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu 610041, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form
12 October 2015
Accepted 26 October 2015
Available online 26 October 2015

Keywords:
Poly(etheretherketone)
Plasma polymerization
Carboxyl groups
Surfaces
Osteoblast cell
Biomaterials

ABSTRACT

Poly(etheretherketone) (PEEK) possesses bone-like stiffness and excellent biocompatibility but yet suffers from bioinertness and poor osteoblasts adhesion. In this work, plasma polymerization was employed to produce carboxylated PEEK surface containing 5.9–13.4% –COOH groups, expressed as a percent of total carbon content. Pre-osteoblasts exhibited decreased cell adhesion and proliferation with increasing –COOH surface concentration, whereas pre-osteoblasts spreading increased with increasing –COOH surface density. Our results illustrate that optimization of –COOH surface content could produce significant enhancement in osteoblasts adherence, spreading and proliferation.

1. Introduction

Poly(etheretherketone) (PEEK) offers a set of characteristics superior for biomedical applications including a similar elastic modulus as bones, non-toxicity, broad chemical resistance, natural radiolucency and sterilization resistance [1]. In spite of these attributes, the bioinert nature of PEEK is not conductive to rapid bone cell attachment [2], and hence it does not actively participate in the formation of new bone, thus producing inferior bone-implant integration. Therefore, it is imperative to modify its surface characteristics to enhance the ability for cells to attach.

Although functionalization of PEEK surface cannot be readily achieved on account of its inherent chemical inertness, plasma polymerization is a versatile method for surface tailoring, because plasmas can functionalize various substrates surfaces including non-reactive substrates like PEEK with various chemical groups without altering materials bulk properties [3]. Moreover, under a given plasma polymerization condition, the functional groups density varies with polymerization time, thus providing a convenient control of surface density of functional groups. Others studies [4–6] and our previous studies [7–9] have shown that the presence of surface –COOH promotes improved osteoblast cell adhesion, spreading and proliferation compared to untreated controls. However, up to now, there is no report on the effect of –COOH surface density on osteoblast cell adhesion, spreading and proliferation. Herein, different density of –COOH was introduced on PEEK surface using plasma polymerization of acrylic acid (AAc), and the dose-dependent effects on pre-osteoblast cells (MC3T3-E1) responses in terms of cell adhesion, spreading and growth to plasma-carboxylated PEEK surface were explored.

2. Materials and methods

–COOH was introduced onto pristine PEEK (abbreviated as P-PEEK) surface using plasma polymerization of AAc as described in our previous studies [8,10]. 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 was carried out at 100 W for 2 min, 4 min or 6 min (abbreviated as PEEK-COOH2, PEEK-COOH4 and PEEK-

* Corresponding author. Fax: +86 28 8523 3480.
E-mail address: zhanglfcioc@163.com (L. Zhang).

http://dx.doi.org/10.1016/j.matlet.2015.10.124
0167-577X/

COOH6, respectively). After returning the chamber to atmospheric pressure, the samples with plasma-polymerized thin films were removed. The chemical structures of PEEK samples were investigated through static water contact angle studies and X-ray photoelectron spectroscopy (XPS, XSAM800) at a take-off angle of 20°. Average roughness (Ra) was measured by a Mahr Perthometer M1 surface profiler (Germany).

Pre-osteoblasts MC3T3-E1 cells were routinely grown and maintained in α-MEM medium (Hyclone) containing 10% fetal calf serum (Hyclone) in a humidified incubator (5% CO_2, 37 °C). Prior to cell culture, PEEK samples (P-PEEK, PEEK-COOH2, PEEK-COOH4 and PEEK-COOH6) were sterilized with epoxy ethane. The culture media was replenished every 2 days. The osteoblasts were seeded onto the substrates at a density of 1.03×10^5, 1.54×10^4 and 2.10×10^4 cells/well using 24-well plates as the holders for initial cell adhesion, cell proliferation and cell morphology studies, respectively. The osteoblasts adhesion after 4 h incubation and osteoblasts proliferation after 1, 3 and 5 days culture were examined by CCK-8 (Dojindo, Kumamoto, Japan) assay. The CCK-8 assay is described elsewhere [8]. Following 4 h, 1 and 3 days culture, the cells on the substrates were washed with PBS and fixed with 2.5% glutaraldehyde for 2 h, dehydrated with ethanol and dried under CO_2 critical point. Dried samples were sputter-coated with gold and then the morphology of the adhered cells were observed using SEM (JSM-6300, Japan).

For statistical analyses, all experiments were performed at least triplicate and *t*-test was employed for analysis. Statistical significance is indicated by $^*P<0.05$ against P-PEEK.

3. Results and discussion

High-resolution C1s and O1s XPS spectra are shown in Fig. 1, along with accompanying peak assignments. In comparison with P-PEEK, high-resolution C1s spectra of plasma-carboxylated PEEK exhibited a new contribution at 288.8 eV, typical of carboxyl groups, and in accord with prior data of plasma-polymerized AAc film [11]. Clearly, there is a progressive increase of –COOH surface concentration from 5.9% to 13.43% as the plasma polymerization time increases from 2 min to 6 min (Table 1). Additionally, the steady increase of the C–C/C–H component at 284.8 eV (Table 1) is indicative of the increase in polymer cross-linking with increasing the time of plasma polymerization. The shake-up satellite peak observed in the high-resolution C1s spectra of P-PEEK at 291.6 eV characterizes the presence of $\pi\rightarrow\pi^*$ transitions arising from

Table 1
Concentrations of different C-species and O-species for PEEK samples obtained from high-resolution C1s and O1s spectra.

B.E.(eV)	Attribution	Relative composition of carbon and oxygen species (%)			
		P-PEEK	PEEK-COOH2	PEEK-COOH4	PEEK-COOH6
284.8	C–C/C–H	68.5	56.88	58.97	61.68
286.1	C–O	15.74	22.19	24.89	17.60
287.1	C=O	7.38	9.44	8.93	7.29
288.8	COOH	–	5.90	7.21	13.43
291.6	π	8.37	5.60	–	–
531.6	O=C	30.89	–	–	–
532.2	O–C*/O=C/O–H	–	36.68	70.35	65.93
533.5	(O–C#)O=C–O–H	69.11	63.32	29.65	34.07

electrons in the aromatic ring and its intensity is dependent on the magnitude of π-electron conjugation. In comparison with P-PEEK, the intensity of the shake-up satellite peak of PEEK-COOH2 decreases but not disappears. Therefore, the thickness of plasma-deposited polyacrylic acid film on PEEK-COOH2 may be less than 100 Å (XPS analyzes approximately the uppermost 100 Å of a surface). In addition, the carbonyl oxygen and the hydroxyl oxygen in –COOH appears at 532 eV and 533.5 eV, respectively [12]. Therefore, the first peak at 532.2 eV of the high-resolution O1s spectra of PEEK-COOH2 should attribute to O–C*/O=C/O–H, and the second peak at 533.5 eV corresponds to O–C#/O=C–OH. However, the shake-up satellite peak disappears in the high-resolution C1s spectra of PEEK-COOH4. That is, the thickness of plasma-deposited AAc coating on PEEK-COOH4 should be more than 100 Å. Hence, the chemical state reflected by XPS spectra is all the chemical state of the plasma-deposited polyacrylic acid coating on PEEK-COOH4. Thus, the first peak at 532.2 eV of the high-resolution O1s spectra of PEEK-COOH4 should comprise O–C*/O=C/O–H, and the second peak at 533.5 eV only corresponds to O=C–OH, and the same as the high-resolution O1s spectra of PEEK-COOH6. –COOH contributes equally to the content of the peaks at 532.2 eV and 533.5 eV. And the concentrations of O–C and O=C produced by fragmentation and rearrangement reactions in the plasma gradually decrease (Table 1). Thus, the concentration difference between the component at 532.2 eV and the component at 533.5 eV becomes smaller as the time of plasma polymerization increases form 4 min to 6 min (Table 1). And this also corroborates the density of –COOH increases with the time of

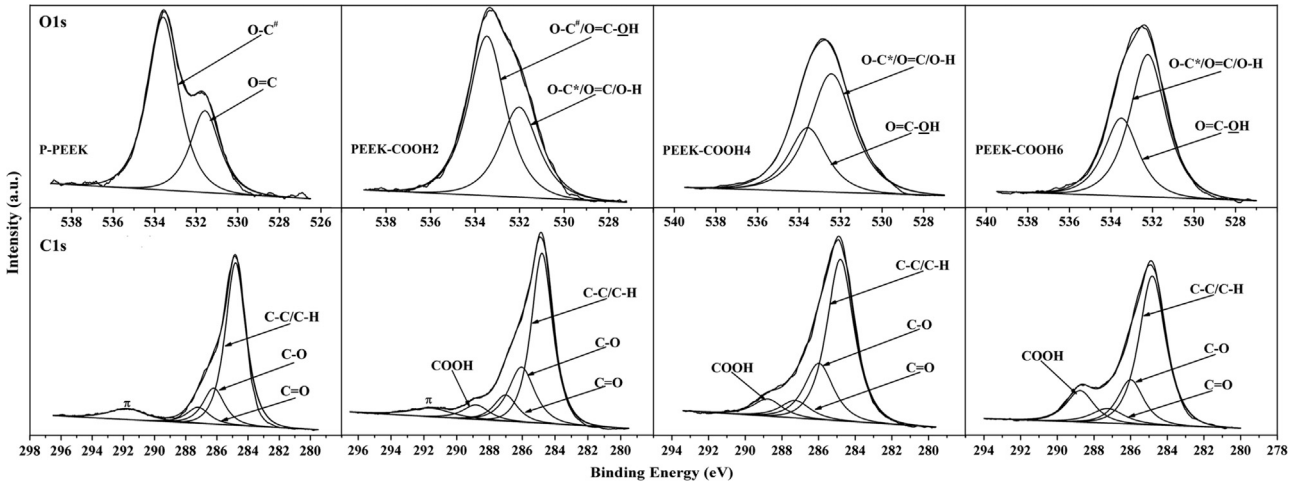

Fig. 1. XPS C1s spectra (bottom panel) and O1s spectra (top panel) for PEEK specimens. O–C# and O–C* represent the O–C bonds existing in backbone molecular chain of PEEK represents and existing in plasma-deposited AAc film on PEEK surface, respectively.