Biomaterials xxx (2014) 1–18

Contents lists available at ScienceDirect

Biomaterials

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

Polyetheretherketone/nano-fluorohydroxyapatite composite with antimicrobial activity and osseointegration properties

Lixin Wang [a], Shu He [b,d], Xiaomian Wu [c,d], Shanshan Liang [e], Zhonglin Mu [e], Jie Wei [f], Feng Deng [c], Yi Deng [d,**], Shicheng Wei [b,c,d,*]

[a] Department of Stomatology, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China
[b] Department of Oral and Maxillofacial Surgery, Laboratory of Interdisciplinary Studies, School and Hospital of Stomatology, Peking University, Beijing 100081, China
[c] Chongqing Key Laboratory for Oral Diseases and Biomedical Sciences, Chongqing Medical University, Chongqing 401147, China
[d] Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
[e] The Affiliated Hospital, Hainan Medical College, Hainan 571199, China
[f] Key Laboratory for Ultrafine Materials of Ministry of Education, Engineering Research Center for Biomedical Materials of Ministry of Education, East China University of Science and Technology, Shanghai 200237, China

ARTICLE INFO

Article history:
Received 20 February 2014
Accepted 22 April 2014
Available online xxx

Keywords:
Polyetheretherketone
Fluorohydroxyapatite
Antimicrobial
Bioactivity
Osseointegration
Implant

ABSTRACT

Lack of antibacterial activity and binding ability to natural bone tissue has significantly limited polyetheretherketone (PEEK) for many challenging dental implant applications. Here, we have developed a polyetheretherketone/nano-fluorohydroxyapatite (PEEK/nano-FHA) biocomposite with enhanced antibacterial activity and osseointegration through blending method. Smooth and rough surfaces of PEEK/nano-FHA biocomposites were also prepared. Our results showed that *in vitro* initial cell adhesion and proliferation on the nano-FHA reinforced PEEK composite were improved. In addition, higher alkaline phosphatase activity and cell mineralization were also detected in cells cultured on PEEK/nano-FHA biocomposites, especially for rough PEEK/nano-FHA surfaces. More importantly, the as-prepared PEEK/nano-FHA biocomposite could effectively prevent the proliferation and biofilm formation of bacterial. For *in vivo* test, the newly formed bone volume of PEEK/nano-FHA group was higher than that of bare PEEK group based on 3D microcomputed tomography and 2D histomorphometric analysis. These reports demonstrate that the developed PEEK/nano-FHA biocomposite has increased biocompatibility and antibacterial activity *in vitro*, and promoted osseointegration *in vivo*, which suggests that it holds potential to be applied as dental implant material in dental tissue engineering applications.

1. Introduction

The most popular orthopedic/dental materials are metals such as titanium (Ti) and its alloys due to their excellent corrosion resistance, high mechanical strength, as well as cytocompatibility [1,2]. However, there are concerns regarding release of harmful metal ions and radiopacity of metal alloys *in vivo*. Moreover, the elastic moduli of metal alloys mismatch mechanical properties between metals and human bones resulting in bone resorption [3,4]. In fact, serious post-operative complications such as osteolysis, allergenicity, and loosening as well as eventual implant failure may occur [5]. To overcome these limitations and minimize negative post-implantation biological reactions, substitutes for metals are extensively pursued.

Polyetheretherketone (PEEK), a semi-crystalline and non-resorbable thermoplastic polymer, exhibits excellent mechanical properties, thermal stability and environmental resistance [6]. It also is non-toxicity and has low elastic modulus (3–4 GPa) compared to titanium and other metal alloys, which reduces the extent of stress shielding that is often observed in titanium-based metallic implants [7,8]. From the processing perspective, PEEK can be fabricated readily by conventional plastic processing equipments, repeatedly sterilized and heat contouring to fit the

* Corresponding author. School and Hospital of Stomatology, Peking University, No.22 Zhong-Guan-Cun South Road, Hai-Dian District, Beijing 100081, China. Tel./fax: +86 10 82195780.
** Corresponding author. Academy for Advanced Interdisciplinary Studies, Peking University, No. 5 Yi-He-Yuan Road Hai-Dian District, Beijing 100871, China. Tel./fax: +86 10 62753404.
E-mail addresses: dengyibandeng@pku.edu.cn (Y. Deng), sc-wei@pku.edu.cn, weishicheng99@163.com (S. Wei).

http://dx.doi.org/10.1016/j.biomaterials.2014.04.085
0142-9612/

shape of bones [9]. In recent decade, much attention has been given to PEEK composite in the biomedical field, particularly in the area of load-bearing orthopedic/dental applications [6,10] due to its unique properties mentioned above. After being inserted, a PEEK implant can support the body weight without the side effects of stress protection and its mechanical properties, which include elasticity, stiffness, tensile strength and resistance to distortion, abrasion and fatigue, are within the proper range to coexist with human bone [11,12]. In addition, bone recovery around the implant can be easily observed from outside the body because of the radiolucency of PEEK [13]. As a result, interbody fusion cages made from PEEK are widely used to treat degenerative spine diseases, to maintain mechanical stability during segmental fusion, and often to act as a carrier for orthopedic graft materials [14,15].

Although PEEK implants have been used frequently in recent medical operations, PEEK itself does not directly bind to bone due to its chemical and biological inertness and displays much lower mechanical properties than those of human bone [6,16]. Subsequently, to improve the bioactivity of PEEK, many researchers have successfully made PEEK-hybrid materials using hydroxyapatite (HA), because HA is a constituent of living bone [17]. Simple HA-filled PEEK mixtures have shown improved cell and tissue responses than pure PEEK in previous studies [10,18–20], but critical implant failures also often occur due to lack of antibacterial activity on the implant–abutment interface.

It is well-known that microbial infection is one of the main causes of implant failure [21,22]. These device-associated infections can progress rapidly as planktonic bacteria first adhere to an implant interface and ultimately evolve into biofilms. Lack of antibacterial activity on the implant–abutment interface often causes undesirable complications such as oral infections, inflammatory reactions, destruction of the adjacent tissue, implant loosening or even detachment [23]. To enhance the antibacterial property of PEEK, Ag-doped or Ag-decorated HA coating was deposited onto the surface of PEEK implant by other groups and some prospective results were achieved [24,25]. Excess release of silver nanoparticles, however, inhibits osteoblasts growth [26] and cause many severe side effects [27,28], such as cytotoxicity and internal organ injury, though silver ions are known to have a broad spectrum of antimicrobial activities. Therefore, there is a pressing need to reduce and even eliminate the infection on PEEK implants without impairing the cytotoxicity.

Nano-fluorohydroxyapatite (nano-FHA) is a bioactive calcium phosphate with chemical and crystallographic similarity to that of natural apatite in bones and dentals, and has been currently used in hard tissue engineering for bone regeneration and as bioactive coatings on Ti-based alloys for orthopedic/dental applications to improve the integration between the implants [29,30]. Compared to pure HA, FHA has much higher physic-chemical stability and possesses higher osteogenic activity to bone cells [31]. Furthermore, the effects of FHA on oral bacteria and plaque due to the release of fluorine ions, which can act as an antimicrobial agent, are well documented by a considerable amount of literature [32,33]. Despite the attractive advantages and progress in preparation of novel PEEK composites, the employment of nano-FHA as nano reinforcement in bioactive PEEK-based composites conferring PEEK with both antimicrobial activity and osseointegration for load-bearing dental applications, to our knowledge, has not been reported. Moreover, based on the clinical outcome and histological evidence from retrieved implants, a rough surface can promote ingrowth of soft and hard tissue into the materials, thereby creating more biological anchorage to improve the stability of the implant [34]. Hence, in the present study, a pilot and comprehensive work was conducted on the fabrication of PEEK/nano-FHA implants with smooth and rough surfaces, and we also systematically evaluated the biofunctionalities of the PEEK/nano-FHA *in vitro* and *in vivo*. The PEEK/nano-FHA biocomposites with enhanced antimicrobial activity and osseointegration could be a promising candidate for dental implant.

2. Materials and methods

2.1. Materials

Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%, Sinopharm Chemical Reagent Co. Ltd., Beijing, China), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 99%, Sinopharm Chemical Reagent Co. Ltd., Beijing, China) and ammonia fluoride solution (NH_4F, 99%, Shanghai Sanaisi Reagent Co. Ltd., Shanghai, China) were selected as Ca-precursor, P-precursor and F-precursor, respectively. Ammonia solution ($NH_3{\cdot}H_2O$) was also supplied from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China), and medical graded polyetheretherketone (PEEK powders, 450G, diameter approximately 2–3 mm, molecular weight about 5000) with the density about 1.30 g/cm^3 was purchased from Victrex Co. Ltd. (United Kingdom). All other chemicals were of analytical reagent grade and were used as received unless otherwise noted. All aqueous solutions were prepared with de-ionized water (D.I. water).

2.2. Synthesis of nano-FHA

Nano-FHA crystals were prepared in our laboratory *via* chemical precipitation reaction between aqueous $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$, with NH_4F as the precursor for F during which F would substitute OH^-. 5 wt% F content was proposed in this study. Briefly, $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ were dissolved in D.I. water separately according to a Ca/P molar ratio of 1.67, and NH_4F was added to $(NH_4)_2HPO_4$ solution. The pH of each solution was adjusted to 10 by adding ammonia. Then, a solution of $Ca(NO_3)_2$ was slowly added drop-wise into $(NH_4)_2HPO_4$-NH_4F mixed solution with continuous stirring. Crystal growth occurred when kept at 60 °C for 8 h, and the pH value of the supernatant was maintained in the range of 10–10.5 using ammonia in whole experiments. The reaction of FHA ($Ca_{10}(PO_4)_6F_x(OH)_{2-x}$) can be expressed by the reaction:

$$10Ca^{2+} + 6HPO_4^{2-} + (8-x)OH^- + xF^- \rightarrow Ca_{10}(PO_4)_6(OH)_{2-x}F_x + 6H_2O$$

After reaction, FHA slurry was aged at ambient temperature for 24 h, and the precipitates were obtained after washing with D.I. water at least three times to neutral pH. Finally, FHA precipitation was treated hydrothermally with stirring at 140 °C (heating rate 10 °C/min) under 0.3 MPa for 24 h in an autoclave. After hydrothermal treatment, nano-FHA particles were rinsed with D.I. water and dried in an oven at 60 °C for 12 h before use and characterization.

2.3. Preparation of PEEK/nano-FHA biocomposite

Polyetheretherketone/nano-fluorohydroxyapatite composite (PEEK/nano-FHA) was fabricated containing 40 wt% nano-FHA (approximately 29.6 vol% reinforcement level) by compression molding methods. In brief, 40 wt% nano-FHA powder and PEEK powder were dispersed in alcohol using an electronic blender to obtain a homogeneous powder mixture. After well dispersed, the mixture was dried in a forced convection oven at 90 °C to remove the excess alcohol. The resulting powder mixture was placed in two specially designed molds, i.e. disks (15.0 mm diameter and 2.0 mm thick) for physical and chemical characterization and *in vitro* testing, and cylindrical implants (4.0 mm diameter and 7.0 mm length) for *in vivo* measurement (Fig. 1). The molds and powder mixtures were preheated to 150 °C under a load of 35 MPa, and the temperature was increased to 375 °C under a load of 15 MPa. After reaching the target temperature, the temperature and pressure were held for 10 min. Then the die and samples were air cooled to 150 °C, and the samples were removed from the molds.

Samples in disc shape and cylindrical implant were divided into two groups. Disk samples of PEEK/nano-FHA biocomposite were polished with a series of increasing SiC abrasive papers (400, 1000, 1500, 2000 grit), cleaned ultrasonically for 20 min in baths of acetone, anhydrous ethanol and D.I. water respectively, and dried at 50 °C overnight and then tested by a mechanical profilometer (Dektek8 stylus profiler, Veeco, Plainview, USA). Disk samples with roughness average (Ra) below 0.2 μm were collected and designated as smooth groups. Some disk samples from the smooth groups were blasted by TiO_2 particles ($\Phi = 180–212$ μm) and disks with Ra = 2.0–3.0 μm were collected and designated as rough groups. In order to remove any potential free TiO_2 nanoparticles, all samples were cleaned with D.I. water using an ultrasonic cleaner for 8 h. The D.I. water was changed every 20 min during the ultrasonic cleaning process. The bare PEEK samples were also prepared according to the same process and cut into the same shapes as control group.

2.4. Chemical and morphological characterization

Fourier transform infrared spectrometry (FT-IR, Magna-IR 750, Nicolet, USA) was used to identify the functional groups of the as-prepared FHA powders and PEEK/nano-FHA composites. The spectra were recorded from 4000 cm^{-1} to 400 cm^{-1}.

Please cite this article in press as: Wang L, et al., Polyetheretherketone/nano-fluorohydroxyapatite composite with antimicrobial activity and osseointegration properties, Biomaterials (2014), http://dx.doi.org/10.1016/j.biomaterials.2014.04.085