Materials Science and Engineering C 61 (2016) 526–533

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Strontium-doped calcium polyphosphate/ultrahigh molecular weight polyethylene composites: A new class of artificial joint components with enhanced biological efficacy to aseptic loosening

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ARTICLE INFO

Article history:
Received 22 October 2015
Received in revised form 10 December 2015
Accepted 28 December 2015
Available online 30 December 2015

Keywords:
SCPP/UHMWPE composites
Aseptic loosening
Biological efficacy

ABSTRACT

To enhance implant stability and prolong the service life of artificial joint component, a new approach was proposed to improve the wear resistance of artificial joint component and endow artificial joint component with the biological efficacy of resistance to aseptic loosening. Strontium calcium polyphosphate (SCPP) were interfused in ultrahigh molecular weight polyethylene (UHMWPE) by a combination of liquid nitrogen ball-milling and flat-panel curing process to prepare the SCPP/UHMWPE composites. The micro-structure, mechanical characterization, tribological characterization and bioactivities of various SCPP/UHMWPE composites were investigated. The results suggested that this method could statistically improve the wear resistance of UHMWPE resulting from a good SCPP particle dispersion. Moreover, it is also observed that the SCPP/UHMWPE composites-wear particles could promote the production of OPG by osteoblasts and decrease the production of RANKL by osteoblasts, and then increase the OPG/RANKL ratio. This indicated that the SCPP/UHMWPE composites had potential efficacy to prevent and treat aseptic loosening. Above all, the SCPP/UHMWPE composites with a suitable SCPP content would be the promising materials for fabricating artificial joint component with ability to resist aseptic loosening.

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1. Introduction

Total joint replacement (TJR) is a common operation performed to treat severe arthritis affecting loading bearing joints for its effectiveness in reducing pain and improving joint function [1–4]. UHMWPE, as a bearing material in artificial joints, has been used in orthopedics due to its notable properties such as chemical inertness, lubricity, impact resistance, and abrasion resistance [5,6]. However, billions of wear particles with submicrometer size generated from UHMWPE components due to their poor wear resistance could cause adverse pathological reaction in the surrounding tissues [7,8]. This pathological reaction could cause osteolysis and joint loosening through inhibiting bone formation and promoting bone resorption so that it could not ideally promote the healing of the joint. This pathological phenomenon is referred as the aseptic loosening of artificial joints limiting the long-term success of artificial joints after TJR [9,10].

The recent studies indicate that UHMWPE can be modified to reduce the generation of wear particles for different purposes. For example, the wear tests showed that the wear behavior of UHMWPE could be improved by gamma irradiation crosslinking. However, gamma radiation does not bring any biological properties for reducing pathological reaction caused by wear particles but leaves behind long-lived residual free radicals [11], which could cause oxidation in the long term and also detrimentally affect mechanical properties [12–17]. The addition of antoxidant such as Vitamin E could increase oxidation stability but it could not yet make up for the loss of mechanical properties [18–20]. On the other hand, there are some reports about the improvement in wear resistance of fillers-modified-UHMWPE in the construction of artificial joints. A kind of blend with desirable fluidity by melt mixing UHMWPE and low molecular weight polyethylene was obtained by Ling xu et al. and showed good comprehensive physicochemical properties. However, the biological function also is the limitation [21]. Hydroxyapatite is an ideal filler for reinforcing UHMWPE due to the chemical inertness, corrosion resistance and antiallergenic properties. However, due to its mechanical design, the modified components of an artificial joint are inevitably subjected to a wear process that generates particles and cause aseptic loosing [22]. Therefore, the current challenge is how to enhance biological efficacy (i.e. the ability of resistance to aseptic loosening) of UHMWPE implants without sacrifice of wear resistance and oxidation stability.

As one class of calcium orthophosphate ceramics, calcium polyphosphate (CPP) is widely investigated due to its advantages of controlled biodegradable property, biocompatibility and bioactivity

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http://dx.doi.org/10.1016/j.msec.2015.12.077
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[23,24]. In our previous studies, we introduced strontium (Sr) into CPP to synthesize Sr-doped calcium polyphosphate (SCPP) to develop a new kind of inorganic Ca–P polymer used as bone repair materials. It was found that SCPP represented a promising bone graft because of its better biocompatibility, mechanical strength, and stimulation to growth factor secretion [25–28]. Moreover, we also researched the effects of SCPP particles on cytokines from macrophages and osteoblasts leading to aseptic loosening. The results confirmed that SCPP particles could inhibit the expression of TNF-α and RANKL and promote the expression of OPG, thus inhibit bone resorption and promote bone formation [29]. Above all, we believe that the introduction of SCPP particles (as reinforcing filler) into UHMWPE might be able to develop a new artificial joint component that not only possesses the enhanced wear-resistance, but also has the biological efficacy of resisting aseptic loosening.

Base on these studies, the solid-state mixing method by using liquid nitrogen ball-milling and flat-panel curing is adopted to prepare SCPP/UHMWPE composites since the ball-milling is helpful to provide a shear stress ensuring uniform dispersion of SCPP particles. Then the mechanical properties and bioactivities of this SCPP/UHMWPE composite were analyzed. The hardness, impact property and tribological properties of SCPP/UHMWPE composites were respectively analyzed by stiffness test, impact test and the frictional experiment. The in vitro cellular behaviors and effect of the SCPP/UHMWPE wear particles on OPG and RANKL mRNA and protein expression of osteoblasts were examined. The results would provide a promising wear-resistant material with the novel biological efficacy for fabricating acetabular cup.

2. Materials and methods

2.1. Materials and preparation of SCPP/UHMWPE composites

UHMWPE was kindly provided by Beijing Second Subsidiary Additive Factory with an average viscosity molecular weight of 2.5×10 [6]. β-SCPP powders with an average size of 10 μm were prepared by the previous method [26,27,30,31]. Four SCPP contents against UHMWPE were chosen to be 0%, 5%, 10%, 20%, and 30% (*w*/*w*) in preparing the homogeneous solid-state mixtures. UHMWPE and SCPP were mixed at 25 Hz for more than 12 h by a Mixer Mill (Retsch, MM400, Germany) after pre-cooled in liquid nitrogen discontinuously. The resultant SCPP/UHMWPE agglomerates were added into the mold to prepare the composite specimens a temperature of 200 °C without pressure for 15 min for deaeration in the hot press instrument. After the deaeration, the agglomerates were pressed under the pressure of 10 MPa for 30 min and then cooled at room temperature. The thickness of each composite was 4 mm for various measurements.

2.2. Microstructural analysis

Scanning electron microscopy (SEM, JSM-5900LV, JEOL) observation was carried out on the agglomerates (before the molding), the surface and the cross section of SCPP/UHMWPE composites. Energy dispersion X-ray spectrometry (EDS) was also used to analyze atomic elements of composites section under an observation of SEM at 20 kV.

2.3. Mechanical characterization

Hardness testing of SCPP/UHMWPE specimens was conducted using a Durometer (Shore-D DIN 53505, Hiroshima). Specimens of 4 mm thickness were placed on the platform of the testing instrument. The readings were taken thrice at the three different spots at a distance of 5 mm from each other. For the Charpy impact test, an instrumented drop weight impact tester (China) was used. The specimen dimensions were $40 \times 10 \times 4$ mm^3, and a 2-mm deep notch was done in each specimen. SEM observation was done in order to observe the structure at rupture.

2.4. Fatigue crack propagation testing

Fatigue crack propagation testing of each specimens was done following ASTM E-647 [32]. Specimens were tested at a frequency of 5 Hz and a stress ratio of 0.1 in tension. Crack length was monitored optically every 20,000 cycles. Tests were performed at room temperature.

Stress intensity factor ranges at crack inception (ΔK) were reported at a threshold crack growth rate of 10–6 mm/cycle. All testing was done in an aqueous bath at 40 °C to simulate the physiologic temperature of the joint. At least 3 specimens were tested for each specimen.

2.5. Tribological characterization

The friction behavior of the specimens was measured by using an MM-200 friction and wear tester under dry station [33]. The test specimen ($40 \times 10 \times 4$ mm^3 in size) was used for sliding. Sliding was performed under ambient conditions of a 200 r/mm rate, a 100 N loading, 120 min test duration and all the tests were repeated for four times. The wear ratio of the testing specimens was calculated by volume loss of per sliding distance as follows: $\omega = \Delta W/2\pi\,\rho RN$ [ω represents the wear rate (mm^3/m), ΔW represents the mass loss (g), ρ represents the density of the sample (g/mm^3), R represents the radius of the counterpart (steel ring) (m), and N represents the number of rotations].

In order to investigate the mechanism for the improvement of the tribological properties of specimens by SCPP fillers, the micrographs of the worn surfaces of specimens were obtained with SEM.

2.6. Wear particles of SCPP/UHMWPE composites and their effect on the proliferation of MG63 cells

Wear particles of each specimen were obtained from the tribological measurement and were divided into six groups (0%SCPP/UHMWPE, 2.5%SCPP/UHMWPE, 5%SCPP/UHMWPE, 7.5%SCPP/UHMWPE, 10%SCPP/UHMWPE and SCPP). Then wear particles with the size of <10 μm was obtained by filtrating and sterilized by ethylene oxide vapor. MG63 (purchased from West China Hospital, Sichuan University) cell lines were used in this study to ensure reproductive results. MG63 cells were cultured in RPMI-1640 medium, supplemented with 10% fetal calf serum and 1% penicillin/streptomycin and incubated at 37 °C in 5% CO_2 humidified atmosphere. Cells from passage 3 were used for being challenged with various wear particles. Cells were seeded in 24-well plates at a density of 1×10^5 cells/ml and were incubated for 4 h. The medium was removed and new medium containing various wear particles (0.1 mg/ml, i.e., 1×10^7/ml for each type of particle. Our preliminary experiments had shown that this concentration did not affect the activation of cells seriously) were added. For a control, cells were plated in the same fashion and cultured in fresh medium only. Due to the low specific gravity of UHMWPE, inverted culture method was used for it. MG63 cells were challenged with each wear particles separately for 1, 2 and 4 days to measure the effect of particles on cells proliferation by MTT assay. Briefly, each plate was taken out on the first, second, and fourth day, respectively. Wear particles were removed and 60 ml per well of MTT solution (5 mg/ml in phosphate buffered saline) was added, then the plate was incubated at 37 °C for 4 h to allow the formation of formazan crystals. The MTT solution was then removed and replaced by 150 ml DMSO, then the plates were shaken for 10 min. The optical density (OD) was measured at 492 nm with a Microplate Reader (Model550, Bio Rad Corp.).

2.7. Measurement of OPG/RANKL released from MG63 cells by ELISA

Commercially available ELISA reagents were employed for the immunoenzymatic determination of OPG and RANKL. All of the procedures were performed following the manufacturer's instructions (R&D Corp). A standard curve was plotted to determine the concentration of OPG/RANKL released from MG63 cells.

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