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Preparation, characterization and properties of nano-hydroxyapatite/polypropylene carbonate biocomposite

Jianguo Liao [a,*], Yanqun Li [a], Qin Zou [b], Xingze Duan [a], Zhengpeng Yang [a], Yufen Xie [a], Haohuai Liu [c]

[a] School of Materials Science and Engineering, Henan Polytechnic University, Jiaozuo 454000, China
[b] Analytical & Testing Center, Sichuan University, Chengdu 610064, China
[c] School of Chemistry and Chemical Engineering, Analytical and Testing Center, Guangzhou University, Guangzhou 510006, China

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ABSTRACT

The combination of nano-hydroxyapatite (n-HA) and polypropylene carbonate (PPC) was used to make a composite materials by a coprecipitation method. The physical and chemical properties of the composite were tested. Scanning electron microscope (SEM) observation indicated that the biomimetic n-HA crystals were uniformly distributed in the polymer matrix. As the n-HA content increased in the composite, the fracture mechanism of the composites changes from gliding fracture to gliding and brittle fracture. Furthermore, the chemical interaction between inorganic n-HA and polypropylene carbonate was also investigated and discussed in detail. The hydrogen bonds might be formed between $-OH/CO_3^{2-}$ of n-HA crystal and the ester group (–COO–) of PPC. The tensile strength of n-HA/PPC (40/60) was similar to that of the cancellous bone, and reached ca 58 MPa. The osteoblasts were cultured for up to 7 days, and then the adhesion and proliferation of osteoblasts were measured by Methyl thiazolyl tetrazolium (MTT) colorimetry assay and SEM. The cells proliferated, grew normally in fusiform shape and well attached. The in vitro test confirmed that the n-HA/PPC composites were biocompatible and showed undetectable negative effect on osteoblasts. In vivo implantation of the composite in New Zealand white rabbits was performed. It can stimulate the growth of a new bone, and at the same time the material begins to degrade. These results suggested that the composite may be suitable for the reparation or replacement of bone defects and possessed the potential for clinical applications.

1. Introduction

Hydroxyapatite (HA), the main inorganic component of human bone tissues, possesses good biocompatibility and high bioactivity [1,2], and has been widely used to repair bone defects [3–5]. However, it is not suitable to be used for load-bearing applications for bone repair due to its brittleness, fatigue failure and slow degradation rate in vivo [6]. Since natural bone is a nano-hydroxyapatite/collagen composite at the microscopic scale, a polymer matrix composed of particulate and bioactive components provides a suitable material for substitute of the cortical bone [7,8]. The bioactivity of the composite, which is rendered by the bioactive component in the composite, can promote the ingrowth of the tissues adjacent to the implant which leads to the formation of a strong bond between bone tissues and the implant after implantation. Therefore, by simulating the composition of natural bones, n-HA/natural polymer [9–14] and n-HA/synthetic polymer [15–19] composites are widely studied for the applications in hard tissue repair materials.

PPC, the copolymer of carbon dioxide (CO_2) and propylene oxide (PO), is a class of biodegradable aliphatic polycarbonate polymers [20,21], which has flexibility with elongation of 200–1000% at break. Studies have shown that PPC can be degraded in acid and alkali solution, and does not cause inflammation in vivo [22]. Low molecular weight PPC can degrade even faster compared to high molecular weight polymers [23]. Besides, PPC has shown desirable biocompatibility, which can be applied in biomedical area. Thus, it can make a great contribution to this novel inorganic/organic composite system.

In this study, n-HA and polypropylene carbonate (PPC) were used to form a composite biomaterial by solution blending and microwave heating techniques. Similar composites have been studied previously, however, the conventional preparation methods for such composites have some drawbacks, such as i) the aggregation of the nanoparticles in the continuous polymeric phase leading to non-uniform dispersion and consequently decrease composite's properties, and ii) the preparation technology of in-situ synthesis is complex [24–26]. The process of coprecipitation can precipitate n-HA and PPC simultaneously and form

* Corresponding author.
E-mail address: liaojianguo10@hpu.edu.cn (J. Liao).

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n-HA/PPC composites in which the n-HA is homogeneously distributed in the PPC matrix. Microwave heating has the advantages of immediacy, integrity, efficiency utilization of energy, etc. Therefore, we choose coprecipitation method — microwave method.

2. Materials and methods

2.1. Materials

The slurry of nano-hydroxyapatite (n-HA) used for composites was prepared by our laboratory according to Ref. [27]. Calcium nitrate and sodium phosphate were separately dissolved in deionized water. Sodium phosphate solution was dropped slowly into the calcium nitrate solution with stirring and was heated to 70 °C–80 °C. The pH value of the solution was kept between 10 and 12 by adding sodium hydrate aqueous solution. When the reaction ended, HA precipitate was obtained after aging 48 h, and after being fully washed with deionized water, the nanoscale needle-like HA crystal (n-HA) slurry was obtained.

Polypropylene carbonate (PPC) was supplied by Henan Tianguan Group Co., Ltd., China. PEG6000 was from Chengdu Chemical Agent Co., Ltd., China. The ethanol was purchased from Chengdu Chemical Agent Co., Ltd., China, AR grade.

2.2. Preparation of n-HA/PPC composites

The n-HA slurry (36%) and DMAC (N, N-dimethylacetamide, 400 mL) were mixed in a three-neck flask with continuous stirring at 120 °C. After water was completely evaporated, n-HA/DMAC slurry was obtained. PEG was used to modify n-HA crystals and improve their dispersion in the solution. At 140 °C, PEG (the ratio of PEG dose and n-HA dose was 8:100, in wt.%) and PPC (from 20 to 60 wt.% separately) were added into the above n-HA/DMAC solution and stirred for 3 h followed by full washing with anhydrous ethanol and dried in a vacuum oven at 40 °C for 48 h, thus n-HA/PPC composite powder was obtained. The n-HA: PPC ratios (wt.%) of the composites range from 10:90 to 60:40. Samples with standard shape, according to GB/T 1447-2005 standard, were made for mechanical testing by microwave heating at 40–50 °C. The dimensions of testing specimens were carefully machined to be 150 mm × 10 mm × 4 mm, and the gauge length is 50 mm. All tests were performed at an ambient temperature (25 °C), and five specimens were used in each test to obtain the average value.

2.3. Characterization and analysis of n-HA/PPC composites

The samples for X-ray diffraction (XRD, D8 ADVANCE, Bruker, Germany), Fourier transform infrared absorption spectra (FT-IR, Netzsch, STA409PC/4/H-TENSOR27, Germany) and Raman spectra (RS, inVia, Renishaw, England) analysis were ground to fine powders and dried in a vacuum oven at 40 °C for 24 h before testing. XRD from a Cu X-ray tube was used to detect the phase composition and crystallinity. The 2θ of the measured samples ranged from 10° to 65°. FT-IR and RS were used to determine the bonding between inorganic phase and polymer phase. The morphology of the n-HA/PPC composites was observed by field emission gun scanning electron microscopy (FEG-SEM, FEI Quanta 250) and an X-Max 30 mm^2 detector energy dispersive X-ray spectrometer (Bruker Quantax 200 Xflash 6|30 EDS). Mechanical properties were evaluated using a WDW-2C electronic universal testing machine (Jinan Kehui Test Equipment Co., Ltd., China). The average values of 5 + tests were reported.

2.4. Biocompatibility test

2.4.1. Cell culture

Osteoblast MG-63 was isolated via a sequential collagenase digestion from neonatal rat calvaria according to an established protocol. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% bovine serum (FBS, Gibco) under 5% CO_2 atmosphere at 37 °C. The medium was changed every two days. After confluence, the monolayer cell was washed twice with phosphate buffered saline (PBS) and incubated with a trypsin–EDTA solution (0.25% trypsin, 1 mm EDTA, Gibco) to detach the cells. The effect of trypsin was then inhibited for the complete medium joining at room temperature. Thereafter, the cells were re-suspended in a complete medium for re-seeding and growing in new culture flasks. Osteoblasts at the third passage were used for cell experiments. The initial cell seeding density was 1×10^4 cells $well^{-1}$ (24-well plate) in this study.

2.4.2. Cell viability

Each specimen (Ø8.0 mm × 1.0 mm) was sterilized by ethylene oxide gas, immersed in a well with 2 mL of fresh medium (without cells), and extracted overnight in an incubator. MG63 cells cultured in media for 3 days were seeded on the top of pre-wetted specimens (1×10^4 cells/specimen). The specimens were then placed in the wells of plastic dishes (24-well cell culture plates, Corning, USA) and left undisturbed in an incubator for 3 h to allow the cells to attach and an additional 1 mL culture medium was added into each well. The cell/composite constructs were cultured in a humidified incubator at 37 °C with 95% air and 5% CO_2 for 1, 4, and 7 day(s). The media were changed every 2 days. The morphology of MG63 cells cultured with the cement and plastic (as a control) was observed by an inverted phase contrast microscope (Nikon TE300, Japan). Cell proliferation was measured at 1, 4, and 7 day(s) using MTT assay. MTT reagent (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) is enzymatically converted by living cells into a blue/purple formazan product. The intensity of the colored formazan is directly related to the number of viable cells and thus to their proliferation in vitro. MTT reagent was added to each sample and incubated at 37 °C for 4 h. The colored formazan product was solubilized into a solution with dimethyl sulfoxide (DMSO) and the solution of each sample was removed out for assay in a 96-well plate.

The suspension was centrifuged and the absorbance of the supernatant was measured by a microplate reader (Bio-Rad 680) at the wavelength of 490 nm. Each treatment was performed five times and the average value was used as the final result.

2.4.3. Statistical analysis and SEM observation

Statistical analysis was assessed using software SPSS (v10.0). The least significant difference method (assuming equal variances) was performed to measure the statistical significance between experimental groups. A value of $^{**}p < 0.01$ was considered to be an outstanding statistical significance.

The scanning electron microscope (JEOL, JEM-100CX, Japan) was used to observe the specimens. Cells cultured for 2 and 4 day(s) on cements were rinsed with PBS, fixed with 1 vol.% glutaraldehyde, subjected to graded alcohol dehydration, rinsed with isoamyl acetate, and sputter coated with gold.

2.4.4. Biocompatibility in vivo

The n-HA/PPC (40/60) composite was implanted into eight healthy New Zealand white rabbits with the weight of about 2.0 kg each. The rabbits were anesthetized with pentobarbital sodium. A 3-cm parallel incision was made on the femur of the rabbit. The periosteum was retracted and the femur was exposed. A defect (Ø2 mm × 5 mm) was made by a drill bit. A composite construct (Ø2 mm × 5 mm) was inserted into the defect and the gap was sutured by suture line. Rabbits were sacrificed at 4 and 12 weeks after implantation. The composite was excised together with surrounding tissue fixed in 10% neutral buffered formalin, decalcified and embedded in paraffin. Tissue blocks were sectioned at 5 μm in thickness and stained with hematoxylin and eosin (H&E), then observed by an optical microscope (Olympus, Japan). Moreover, X-ray microradiography was employed to monitor the process of ectopic bone formation.