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Characterization of nickel-doped biphasic calcium phosphate/graphene nanoplatelet composites for biomedical application

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ABSTRACT

The effect of the addition of an ionic dopant to calcium phosphates for biomedical applications requires specific research due to the essential roles played in such processes. In the present study, the mechanical and biological properties of Ni-doped hydroxyapatite (HA) and Ni-doped HA mixed with graphene nanoplatelets (GNPs) were evaluated. Ni (3 wt.% and 6 wt.%)-doped HA was synthesized using a continuous precipitation method and calcined at 900 °C for 1 h. The GNP (0.5–2 wt.%)-reinforced 6% Ni-doped HA (Ni6) composite was prepared using rotary ball milling for 15 h. The sintering process was performed using hot isostatic pressing at processing conditions of 1150 °C and 160 MPa with a 1-h holding time. The results indicated that the phase compositions and structural features of the products were noticeably affected by the Ni and GNPs. The mechanical properties of Ni6 and 1.5Ni6 were increased by 55% and 75% in hardness, 59% and 163% in fracture toughness and 120% and 85% in elastic modulus compared with monolithic HA, respectively. The in-vitro biological behavior was investigated using h-FOB osteoblast cells in 1, 3 and 5 days of culture. Based on the osteoblast results, the cytotoxicity of the products was indeed affected by the Ni doping. In addition, the effect of GNPs on the growth and proliferation of osteoblast cells was investigated in Ni6 composites containing different ratios of GNPs, where 1.5 wt.% was the optimum value.

1. Introduction

One of the most exciting areas in engineering disciplines involves the development of various devices for health care. Calcium phosphate (CP)-based biomaterials have attracted great attention as bone replacements due to their similarity in composition and crystal structure to bone minerals, excellent biocompatibility, bioactivity and biodegradability. However, it is imperative to understand the mechanical responses of artificially grown calcium phosphate ceramics [1]. Some of the CPs are implantable in applications such as fracture fixation plates, nails and screws in orthopedics, orthodontic wires, total joint replacement prostheses, etc. [2,3]. Among these bioceramics, the most well-known and extensively used compounds are hydroxyapatite (HA) and tricalcium phosphate (TCP) for medical implants, bone defect fillers and bone tissue engineering [4,5]. However, many reports have mentioned the limitation of using HA and TCP due to the resulting high insolubility and poor mechanical properties, such as low ductility and brittleness, which in turn cause implant loosening and subsequent implant failure [6]. Therefore, biomaterial engineers have focused on improving the composite properties using different types of materials and synthetic techniques. Crystal structure modification of HA via ionic substitution to enhance the solubility and biological properties has attracted considerable attention. In the past two decades, there have been several studies on the substitution of calcium in apatite compounds with various metal ions such as monovalent (Ag^{+} and Na^{+}) [7,8], divalent (Mg^{2+}, Mn^{2+}, Sr^{2+}, Cd^{2+} and Pb^{2+}) [9–13] and trivalent (La^{3+}, Y^{3+}, In^{3+}, Ga^{3+} and Eu^{3+}) ions [14–18]. The focus of the research objectives is the improvement of the bioactivity and osteoinductivity compared with pure HA; however, only a few studies have investigated the effect of such substitution on the mechanical properties of HA [19,20]. Nickel, a first-row transition element, is rarely investigated as a dopant in HA. Nickel possess good mechanical and

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anti-corrosion properties and has attracted much attention as a biomaterial for medical applications [21,22]. However, previous results concerning Ni-doped apatite structures have been rather inconsistent, as outlined below.

Lopez et al. [23] studied HA synthesized in the presence of Ni and its effect on calcium phosphate formation from aqueous solution. Their results indicated that the presence of Ni in the solution inhibits calcium hydroxyapatite and octacalcium phosphate formation and may modify the precipitation of octacalcium phosphate. Lopez et al. [24] also investigated the effect of Ni on the crystallinity and thermal stability of Ni-doped HA solid solutions. The results indicated that Ni decreases the degree of crystallinity and thermal stability of the synthesized materials. In addition, the results indicated that the effect of Ni on HA crystallization is similar to that of Mg and Zn. Mabilleau et al. [25] investigated the effects of Ni^{2+} on the HA growth in vitro. These researchers demonstrated that Ni^{2+} affected the Ca/P ratio, crystal size and crystal lattice of HA. Although Ni^{2+} is a cytotoxic and carcinogenic ion, many medical applications still use Ni-based alloys, such as Ti–Ni super-elastic alloy and Ni–Cr-based alloys, for various applications such as orthodontic wires, orthopedic implants for osteosynthesis, stents for various applications, and bone substitution materials. It should be noted that a high Ni content (approximately 50%) is of great health concern and is associated with Ni^{2+} in vitro and in vivo biocompatibility studies. Taira et al. [21] investigated the cytotoxicity of Ni^{2+} on three fibroblasts, L929, Balb/3T3clone A31 and MC3T3-E1. The results indicated that Ni^{2+} exhibits dose-dependent cytotoxicity.

In the past two decades, nanotechnology has played a significant role in the advancement of medicine and material science. For instance, carbon-based nanomaterials, especially GNPs, have generated significant interest in the scientific community due to their excellent mechanical and biological properties [26–30]. Recently, several studies on GNPs/HA composites with enhanced mechanical and biological properties due to the presence of GNPs have been reported [6,31–41]. To the best of our knowledge, the effect of graphene on the cellular toxicity and physiochemical properties of metallic ion (especially Ni^{2+})-doped HA is largely unexplored. Thus, the present work assumes significance not only for understanding the biological properties of Ni-doped HA and GNPs composites but also for providing insight into the development of HA/β-TCP biphasic (BCP) composites with enhanced mechanical properties. Several experimental techniques, including physical, mechanical and biological testing, were employed to investigate the effect of GNP addition on Ni-doped BCP synthesis. Advances in the general understanding of the phenomena that occur when Ni and GNPs are added to HA are described.

2. Materials and methods

2.1. Materials

Calcium nitrate tetrahydrate $Ca(NO_3)\cdot 4H_2O$, monobasic ammonium dihydrogen-phosphate $(NH_4)H_2PO_4$, nickel (II) nitrate hexahydrate $Ni(NO_3)_2\cdot 6H_2O$ and ammonium hydroxide solution (NH_4OH) were purchased from Sigma Aldrich. Cetyl trimethyl ammonium bromide (CTAB) was selected as the dispersion media. Graphene nanoplatelets (GNPs) were obtained from XG Sciences, Lansing, MI, USA. All the chemicals were of analytical grade and used without further purification.

2.2. Powders and composite bulk preparation

Ni-doped HA (3 and 6 wt.%) was synthesized at room temperature using the continuous precipitation method. The desired amounts of $Ca(NO_3).4H_2O$ and $Ni(NO_3)_2\cdot 6H_2O$ were mixed to produce the nitrate solution. This step was followed by the drop-wise addition of $(NH_4)H_2PO_4$ solution to the nitrate solution under vigorous magnetic stirring. The pH of the solutions was adjusted with the addition of NH_4OH to 10 and 11 for $(NH_4)H_2PO_4$ and nitrate solution, respectively. The suspension was allowed to settle for 24 h to remove the supernatant. The precipitate was filtered and washed six times with doubly deionized water (DDI). The obtained powder was dried at 100 °C for 24 h and calcined at 900 °C for 1 h. Finally, the powder was ball-milled at 300 rpm in a planetary ball mill (PM 100, Retsch, UK) for 2 h to produce the fine powder. The respective compounds were labeled HA, Ni3 and Ni6.

The appropriate quantities of Ni6 powders and GNPs were separately dispersed in DDI with 1 wt.% CTAB and 1-h sonication. To fabricate the composites, dispersions of GNPs and powders with concentrations of 0.5 wt.%, 1 wt.%, 1.5 wt.% and 2 wt.% GNPs were prepared by sonication for 1 h followed by planetary ball milling with a zirconia ball and a rotational speed of 400 rpm for 15 h to obtain a good degree of mixing. The milled slurry mixture was dried in an oven at 90 °C for 24 h. The dried powders were then pressed at 250 MPa using an uniaxial press to form discs (5 mm in diameter × 2 mm in height) and sintered at 1150 °C with a 1-h holding time by hot isostatic pressing (HIP) in a high purity argon gas atmosphere at 160 MPa. The heating and cooling rates did not exceed 5 °C min^{-1}.

2.3. Sample characterization

2.3.1. Physical and chemical characterization

The relative density for each composition was measured using Archimedean's method using 3.16 g cm^{-3}, 3.07 g cm^{-3} and 2.2 g cm^{-3} for HA, β-TCP and GNPs, respectively. The composites were molded with epoxy and polished in a single direction with 600, 1200 and 2000 grit SiC paper. The final polishing was performed with 9, 3, and 0.5 μm polishing compounds to obtain a consistent surface roughness for all of the samples. The surface morphologies of the disc compacts were characterized using field-emission scanning electron microscopy (FESEM, FEI Quanta 200 F). Energy dispersive X-ray spectroscopy (EDS) with an EDS system attached to the FESEM instrument was used to investigate the elemental composition of the samples. Fourier-transform infrared spectroscopy (FTIR) was performed using a Perkin Elmer System series 2000 spectrophotometer (USA) with a frequency range of 400–4000 cm^{-1}. An X-ray diffractometer (PANalytical Empyrean) with *CuKα* (λ = 1.54178 Å) radiation was used for the phase analysis of the samples. Raman spectroscopy (Renishaw inVia Raman Microscope) was performed to characterize the GNP samples using 514-nm laser excitation, 0.8-mW laser power and 20-μm spot sizes.

2.3.2. Characterization of mechanical properties

Micro-indentation investigations of the hardness and fracture toughness were performed using a Vickers hardness tester. The elastic modulus measurements from the nanoindentation test were used to measure the fracture toughness. The fracture toughness values were based on three samples with five indents per sample. The indentation fracture toughness was calculated using the Antis equation.

2.3.3. Mineralization in simulated body fluid (SBF)

The bioactivity of the sintered samples (with a thickness of 3 mm and a diameter of 5 mm) was evaluated by examining the formation of bone-like apatite on the samples in simulated body fluid (SBF) solution. The sintered samples (HA, Ni6 and 1.5Ni6) were soaked in SBF with pH (7.4) and ion concentrations (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, $HPO^{2-}{}_4$ 1.0, $SO^{2-}{}_4$ 0.5 mM) nearly identical to those in human blood plasma [42]. As brief, the SBF was prepared by dissolving reagent-grade mixtures of $CaCl_2$, $K_2HPO_4.3H_2O$, KCl, NaCl, $MgCl_2.H_2O$, $NaHCO_3$ and Na_2SO_4 in distilled water and buffering at pH 7.4 with tris(hydroxymethyl)aminomethane and hydrochloric acid (HCl). The as sintered samples were soaked in SBF at 37 °C in a humidified atmosphere containing 5% CO2 for 7 days at a surface-area-to volume ratio of 0.1 cm^2/mL. The SBF solution was renewed once in 2 days. After 7 days, they were removed from SBF, gently rinsed with