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Improved properties of hydroxyapatite–carbon nanotube biocomposite: Mechanical, in vitro bioactivity and biological studies

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Abstract

The present work describes a simple shear mixing technique for developing a hydroxyapatite (HAp)–carbon nanotube (CNT) nanocomposite and the effect of reinforcement on the physical, mechanical, in vitro bioactivity and biological properties of HAp. XRD and FTIR confirmed that the main phase of the composites is HAp. HRTEM images demonstrated the formation of a two-dimensional nanocomposite structure, whereas FESEM images indicated the formation of nanosized HAp grains featuring sporadically distributed CNT molecules. No major phase changes in HAp were observed with up to 5% added CNT. However, adding more than 1% CNTs caused an increase in internal crystal strain and increased substitution of CO_3^{2-} for OH^- and PO_4^{3-} groups in pure HAp. The average crystallite size increased from ~46 nm to ~100 nm with only 0.5% added CNT, remained nearly unaffected up to 2% CNTs thereafter and suddenly decreased at 5% CNTs (~61 nm). The FESEM and HRTEM images clearly showed the attachment of MWCNT chains on HAp grains, which directly affected the samples' fracture toughness and flexural strength. Of the samples, 1% showed maximum values of K_{1C}, whereas 5% showed maximum values of HV and three-point bending flexural strength. The in vitro bioactivity indicated increased apatite formation on the sample surface up to 1% CNTs after 24 weeks. However, adding 2% and 5% CNTs resulted in a manifold increase in apatite formation up to 12 weeks, after which dissolution increased up to 24 weeks, possibly due to increased substitution of CO_3^{2-} for OH^- and PO_4^{3-} groups. This result is confirmed by the FTIR studies. For all added CNT contents, all samples exhibited high haemocompatibility. However, there was a compromise between the observed mechanical properties and in vitro bioactivity studied up to 24 weeks, and care must be taken before selecting any final application of the nanocomposites.

Keywords: Hydroxyapatite; Carbon nanotube; In vitro bioactivity; Fracture toughness; Haemocompatibility

1. Introduction

Since the discovery of carbon nanotubes by Ijima [1], CNTs have emerged as a prospective candidate for materials research due to their special structural features and excellent properties, such as their extremely small dimensions, high aspect ratio (10^3–10^4) and low density, in addition to high tensile strength (~60 GPa), high rigidity (Young's modulus ~1 TPa), high resilience, superb flexibility and excellent electrical ($>105\ Sm^{-1}$) and thermal properties [2–4]. Therefore, CNTs are primarily being used as a reinforcement material in the preparation of nanocomposites, which is expected to produce composites with improved mechanical properties compared to those of single-phase materials. Ruoff et al. determined that when an advancing crack with sufficient stress intensity at its tip encounters multiwalled carbon nanotube (MWCNT) chains, the concentric layers collapse and absorb the associated energy, arresting the propagation of the crack [5]. This mechanism was identified as a toughening mechanism in CNT-based composites. Lupo et al. successfully fabricated ZrO_2/CNT composites by hydrothermal crystallisation [6], and Seeger et al. synthesised MWCNT/SiO_2 composites via partial matrix melting with a Nd:YAG laser [7].

Hydroxyapatite (HAp) has been exclusively used as a bone implant material due to the similarity between its chemical

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composition and crystal structure and that of the inorganic component of bone [8] as well as its excellent ability to bond with living bone tissues. However, the intrinsic brittleness and poor mechanical properties of sintered HAp restricts its employment in major load-bearing applications. Pure HAp has been reported to have low toughness (0.8–1.2 MPa $m^{1/2}$) and poor flexural strength (< 140 MPa) compared to human bone [8]. Therefore, HAp is being predominantly used either as a coating material on various implant materials, such as steel, titanium and titanium alloys [9], or as a scaffold for tissue engineering [10]. To improve the mechanical properties of HAp without sacrificing its biological properties, different reinforcing phases, such as zirconia, glass, silver, carbon fibre, alumina and TiO_2, have been used [11–14]. Some advanced processing techniques (e.g., high pressure and microwave sintering [15]) have also been investigated recently. Researchers are also using carbon nanotubes or nanofibres (CNT/CNF) as a reinforcing material in the HAp matrix to improve its mechanical properties [16–18]. Different techniques, such as the sol–gel process [17], laser surface alloying [19], spark plasma sintering [9] and high-precision plasma spraying [9], have been used to prepare HAp–CNT composites.

Most of the above-mentioned processes require the functionalisation of ceramics, which further requires the use of precision techniques. In the present work, we adopted an alternative and cost-effective shear mixing technique to prepare CNT–HAp composites with different proportions of pristine CNT molecules and investigated the effect of the reinforcement on the physical, mechanical, biological and in vitro bioactivity properties of HAp. The CNT–HAp composites were prepared by adding different weight percentages of CNT, and a comparative analysis of the various properties is reported.

2. Experimental

2.1. Materials

Analytical grade ortho-phosphoric acid (H_3PO_4) and calcium hydroxide ($Ca(OH)_2$) (S.D. Fine-Chem Ltd., India) were used as starting reagents for the synthesis of pure hydroxyapatite (HAp) powder via the wet chemical method. The detailed methods used for this synthesis and characterisation have been described elsewhere [20]. MWCNTs (> 95% purity, 10–30-nm diameter and 1–10 μm average length) were procured from Nanoshel LLC, USA.

2.2. HAp–CNT composite preparation

Calcined (800 °C) HAp and CNT powders (with four different CNT contents of 0.5%, 1%, 2% and 5%) were mixed counterclockwise in a high-energy planetary ball mill (Fritsche Pulverissette 5, Germany) at 300 rpm for 5 h in acetone. To minimise contamination during milling, a zirconia pot and balls with a ball-to-powder weight ratio of 10:1 were used. The milled powders were sieved and used to prepare pellet samples (12 mm diameter × 4 mm thick) in a uniaxial press (PEECO, India) at 150 MPa for 2 min. Sintering was performed in two steps in argon. In the first step, the temperature was increased to 700 °C at a heating rate of 3 °C/min. Then, the temperature was increased to 1250 °C at a heating rate of 6 °C/min (1 h dwelling at the highest temperature).

2.3. Characterisation

The apparent porosity (A.P.) and bulk density (B.D.) of the sintered specimens were estimated by Archimedes' water displacement method using the formulae [B.D. $= D/(W-S)$ g/cm^3] and [A.P. $= (W-D)/(W-S)100\%$], where D is the dry weight, W is the soaked weight and S is the suspended weight, and the relative density was calculated from the theoretical density.

X-ray diffraction (XRD, Ultima III Rigaku, Japan) and Fourier transform infrared spectroscopy in the mid-IR range (FTIR, IR Prestige 21, 200VCE, Schimadzu, Japan) were used to characterise the prepared phases. The microstructure and morphology of the nanocomposites were studied using field emission scanning electron microscopy (FESEM, Hitachi, S4800, Japan) and high-resolution transmission electron microscopy (HRTEM, JEOL, JEM 2100, Japan).

The hardness and fracture toughness (K_{1C}) of the polished sintered samples (< 0.02 μm R_a) were evaluated using an automated Vickers hardness testing machine (LECO, LV-700AT, MI) via the indentation method (ASTM C1327). Four different indentation loads of 0.3, 1, 3 and 5 kg were applied to each sample with a holding time of 10 s. A minimum of six measurements were obtained for each sample, and the average was used to calculate VH. K_{1C} was calculated using the equation for a radial-median crack: $K_{1C} = 0.016(E/H)^{0.5}(P/C^{1.5})$, where E is Young's modulus (obtained from the nano-indentation method, not discussed here), H is the Vickers hardness, P is the indentation load and C is the half-radial crack length. This equation was used because the c/a value was greater than 2.5, where c is the average crack length and a is the average crack diagonal [21,22].

The flexural strength of the nanocomposites was evaluated using a universal testing machine (Instron 4204) by three-point bending following ASTM C1674, and fractography images were obtained using an FESEM. For the flexural strength measurements, bar samples were prepared, cold-isostatically pressed at 150 MPa and sintered under an argon atmosphere at 1250 °C. The flexural strength was calculated by using the equation $\sigma = 3Fl/2bd^2$, where l is the specimen length, F (N) is the total force applied to the specimen by the loading pin and b and d are the specimen width and thickness, respectively.

The haemocompatibility of the nanocomposite samples was tested with the samples in contact with mammalian blood to verify their cytotoxicity for their use in biomedical implants (following the guidelines of ASTM F756 specifications), where the physiological tolerance of the implant material was evaluated by calculating the % haemolysis in blood. In this process, goat blood, which was pre-treated with sodium citrate (anti-coagulant), was collected and diluted with normal saline to a ratio of 8:10. The samples, which were in the form of round pellets (with no sharp edges), were placed in standard