



Carbon nanotube-based bioceramic grafts for electrotherapy of bone

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ABSTRACT

Bone complexity demands the engineering of new scaffolding solutions for its reconstructive surgery. Emerging bone grafts should offer not only mechanical support but also functional properties to explore innovative bone therapies. Following this, ceramic bone grafts of Glass/hydroxyapatite (HA) reinforced with conductive carbon nanotubes (CNTs) – CNT/Glass/HA – were prepared for bone electrotherapy purposes.

Computer-aided 3D microstructural reconstructions and TEM analysis of CNT/Glass/HA composites provided details on the CNT 3D network and further correlation to their functional properties. CNTs are arranged as sub-micrometric sized ropes bridging homogeneously distributed ellipsoid-shaped agglomerates. This arrangement yielded composites with a percolation threshold of $p_c = 1.5$ vol.%. At 4.4 vol.% of CNTs, thermal and electrical conductivities of $1.5 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}$ and $55 \text{ S} \cdot \text{m}^{-1}$, respectively, were obtained, matching relevant requisites in electrical stimulation protocols. While the former avoids bone damaging from Joule's heat generation, the latter might allow the confinement of external electrical fields through the conductive material if used for in vivo electrical stimulation. Moreover, the electrically conductive bone grafts have better mechanical properties than those of the natural cortical bone.

Overall, these highly conductive materials with controlled size CNT agglomerates might accelerate bone bonding and maximize the delivery of electrical stimulation during electrotherapy practices.

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1. Introduction

Earlier studies have shown that healthy bone tissue has the ability to generate endogenous electric signals that affect its regeneration mechanism [1]. This is achieved by activating voltage-gated Ca^{2+} channels in the plasma membrane of “bone-forming” cells [2,3]. When bone is ill or fractured these endogenous signals are compromised [4].

Clinical studies have reported that exogenous electrical stimulus delivered to the fractured bone site accelerates the regeneration process by compensating the mislaid endogenous electrical signals found in the healthy bone tissue [4]. Recent findings gave evidences that such process can be regulated in situ with preferential stimulation by new “smart” electrical conductive bone grafts. These are able to confine exogenous electrical fields on their surface and deliver it locally to bone cells [5–7]. To guarantee this under electrical stimulation routines, bone grafts should present the following combined requisites: (1) mechanical strength, to keep its structural integrity during the first stages of bone regeneration and, thus, to preserve the electrical percolation and the interconnected porous network [8]; (2) higher electrical conductivity than the surrounding biological environment, at the damaged bone site, forcing electric currents to travel along the easier pathway,

the highly conductive graft material [9]; (3) and thermal conductivity, to avoid cellular and tissue damage from heat generation due to Joule's effect [10].

Nonetheless, the clinical availability of bone grafts conjugating the above requisites is limited. Some material processing strategies have been applied including the reinforcement of matrices with conductive metallic fillers (e.g. stainless steel fibers [11]). Though, conductive carbon-based fillers such as nanotubes (CNTs) are much more promising candidates than the metallic ones due to the strong depression of corrosion-related toxicological risks under electrical stimulation routines. Also, CNT fillers having high aspect-ratio yield conductive matrices, typically of $100 \text{ S} \cdot \text{m}^{-1}$, at percolation values as low as 0.6–3.5 vol.% [12,13]. Furthermore, at this small CNT loading the grafting qualities of the matrices are advantageously preserved [14].

An example of CNT composites that have been applied in bone stimulation is the polymeric based ones [5]. Yet, they have lower bone grafting qualities than well known bioceramics (e.g. calcium phosphates – CaPs). Despite sharing the same dielectric characteristic, research exploring the CNT–CaPs conductivity has been neglected [15]. By combining the biological profile and mechanical strength of the Glass/Hydroxyapatite (HA) ceramic [16,17], with the morphology and electrical conductive of the CNTs [18], a superior multi-functional bone graft is proposed here – CNT/Glass/HA – inspired in the respective apatite-like phase and the collagen type I fibers of the natural bone [19].

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The present work focuses on the study of the effects of the CNT loading in mechanical, electrical and thermal behaviors of hot-pressed CNT/Glass/HA composites. The CNT percolation threshold and properties of the composites were correlated with the 2D and 3D microstructures developed during the confined sintering.

2. Materials and methods

2.1. Sample preparation

Commercially available CNTs (NC7000, Nanocyl, Belgium) were used in this work for preparing the composites. Due to the presence of impurities (~12 wt.%) from the growth technique used, the CNTs were purified following a non-destructive procedure that involved an annealing at 1900 °C for 8 h in a flowing Ar atmosphere. The purity of the samples was assessed by energy dispersive spectroscopy (EDS, Bruker Quantax 400) and quantitatively by thermogravimetric analyses (TGA, SETSYS Setaram working under an O₂ flow of 200 sccm at 10 °C·min⁻¹).

Hydroxyapatite (HA) powder was planetary co-milled with P₂O₅-glass (65 P₂O₅, 15 CaO, 10 CaF₂, 10 Na₂O mol%) in 97.5/2.5 proportion in weight, yielding to a final particle diameter size of D_{0.5} = 1.8 ± 1.4 μm. CNT/Glass/HA composite powder suspensions were mixed by a two-step process with different volume fractions of CNTs (0, 0.4, 0.9, 1.8, 3, 4.4 and 7.8 vol.%) in isopropyl alcohol (≥99.8%, Sigma-Aldrich). This process contemplates a mechanical approach, not used excessively to avoid damage to CNTs: (1) a high-speed shearing (HSS) for 15 min (IKA T25-Ultra-Turrax, working at 20,500 rpm) followed by (2) a 60 min sonication step (Selecta, working at 60 kHz, 200 W). Afterwards, the composite powders were dried, also by a two-step method to avoid phase separation: (1) fast evaporation, by combining heating at 80 °C and vacuum under a magnetic shearing until a high viscosity slurry is obtained and (2) slow evaporation, in an oven at 60 °C for 24 h. Once dried, the powders were crushed in an agate mortar and sieved to less than 75 μm.

CNT/Glass/HA composite powders were consolidated by hot-pressing at a fixed pressure of 30 MPa for 60 min at 1100 °C, under vacuum conditions. Cylindrical-shaped samples with 20 mm diameter and 15 ± 1 mm in height were produced and then cut into (1) thinner slices of 1 ± 0.05 mm for bending tests and (2) parallelepipeds of 2.5 × 2.5 × 4.5 mm³ for compression tests and electrical measurements.

2.2. Phases, 2D and 3D microstructures

The phase composition of sintered composites was determined by the Gazzara and Messier method [20]. The X-ray diffraction (XRD) patterns (X'Pert-MPD, Philips, Netherlands) were collected with a CuKα₁ radiation (λ = 0.154056 nm) in 0.02° steps in the interval 2θ 10–80°. Before microstructural characterization, the surfaces were ground, lapped and polished down to 50 nm colloidal silica. Selected samples of all materials were observed by optical microscopy (Microphot, Nikon, Japan), scanning electron microscopy, SEM (SU-70, Hitachi, Japan) and by transmission electron microscopy, TEM (H9000-NA, Hitachi, Japan). In order to reveal the distribution of the β-tricalcium phosphate (β-TCP) phase in the microstructure of the ceramic composite, a selective chemical etching was performed with 0.56 M citric acid for 4 min at room temperature, as previously adopted [21].

Of particular interest concerning the percolation threshold are the size, shape, aspect ratio, volume fraction and distribution of CNTs and CNT agglomerates. These data were obtained from plane sections for all composite and from three-dimensional analysis through in-depth serial sectioning for the 4.4 vol.% CNT composite. The experimental procedure and 3-D microstructure reconstruction studies from these consecutive planes were carried out according to the following: (1) controlled polishing of the composite surface with a 50 nm sized silica

suspension and a smooth polishing paper, under an applied load of 10 N for 10 min; (2) digital image acquisition of the microstructure; (3) depth marking with a Vickers indenter using a 3 N load; (4) repetition of steps (1) to (3); (5) alignment and stacking of the digital images; and (6) rendering for visualization and analysis of CNT agglomerates. The microstructure of the 4.4 vol.% CNTs composite was characterized after stacking and aligning one hundred consecutive 2-D planar sections using a 3D reconstruction plugin (3D viewer) and the freeware ImageJ [22]. Thus, the reconstructed volume for the sample is approximately 100 × 100 × 50 μm³. The depth of material (Δh) removed after each polishing cycle was about 0.5 μm and measured using the average size of the diagonals (before – d₁ – and after – d₂ – polishing) of the square shaped indentation mark and the pyramid angle φ = 136° [23], according to

$$\Delta h = \frac{d_1 - d_2}{2 \tan\left(\frac{\phi}{2}\right)} \quad (1)$$

2.3. Mechanical, electrical and thermal properties

The modulus of rupture was measured using the circular plate geometry with the Ø20 × 1 mm disks using a load cell of 2.5 kN under a constant displacement rate of 1.0 mm·min⁻¹ (Z020, Zwick/Roell, Germany). The biaxial flexural strength values were calculated according to an international standard method, ASTM F394-76: the specimen is supported by 3 ball bearings spaced 120° apart on a 13 mm diameter circle. Loading was applied by a cylinder with a flat loading face with 1.0 mm diameter. For the compressive strength, a cross head speed of 1.0 mm·min⁻¹ was used for the 2.5 × 2.5 × 4.5 mm³ samples.

Direct current (DC) conductivity measurements of the Glass/HA/CNT composites and Glass/HA matrix were performed on samples of similar dimensions, by fixing copper wires to the smaller faces of the 2.5 × 2.5 × 4.5 mm³ parallelepipeds. The characterization was performed at room temperature in a programmable electrometer (617, Keithley, USA) with voltage applied in 0.5 V step in the range of 0–100 V for dielectric samples. For the conductive samples a programmable power supply (IPS-603, ISO-TECH, UK) was used by applying a voltage in the range 0–1 V in 0.1 V steps.

The impedance spectroscopy measurements were carried out as a function of the temperature (80–360 K), in the frequency range of 40 Hz–2 MHz using an impedance analyzer (4292A, Agilent, USA).

Thermal diffusivity (α) was determined in Ar atmosphere as a function of temperature, from 298 to 673 K, on 8.8 mm × 8.8 mm² specimens of ~1 mm in thickness by the laser-flash method (Thermoflash 2200, Holometrix NETZSCH, USA). The specific heat (C_p) as a function of temperature was estimated by the rule of mixtures from the chemical composition and the heat capacity data of each phase computed using the HSC Outokumpu code [24]. Thermal conductivity (k) was then calculated from the thermal diffusivity, the density, and the specific heat, using the following expression: k = α · ρ · C_p.

3. Results and discussion

3.1. Purification of CNTs

CNTs were placed inside a graphite crucible that allows the exhaust of vapor contaminants while the liquefied ones will deposit at the bottom of the crucible and infiltrate into the porous walls, as is schematically described in Fig. 1a. Different reactions may occur as a function of position, a trend also observed by Tsuchida et al. in a different system [25]. So, according to the reactions/impurity phases, four main regions were identified: (R1) outside the crucible; (R2) top region, inside the crucible; (R3) bottom region, inside the crucible; and (R4) bottom region, incorporated into the graphite. SEM micrographs and EDS spectra of the representative solid impurities of each region are shown in Fig. 1b

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