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Production of tubular porous hydroxyapatite using electrophoretic deposition

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Hydroxyapatite (HA) ceramics have been recognized as bone substitute materials in orthopedic and dental applications because of their chemical and biological similarity to human bone. Porous HA ceramics can be used as scaffold materials, if their microstructure is controlled in terms of pore size and porosity. The objective of the present work was to produce tubular-shaped HA scaffolds for biomedical applications using the cost-effective technique of electrophoretic deposition (EPD) which enables various 3D shapes to be produced. Nano HA powders were produced and mixed with multi-walled carbon nanotubes (MWCNTs) by a hydrothermal process. Calcium acetate ($Ca(CH_3COO)_2$), and phosphoric acid (H_3PO_4) were used as starting materials for the synthesis of the nano HA powders. Porous HA coatings were deposited on carbon rods by EPD at 60 V using a butanol suspension mixture containing nano HA powders and MWCNTs. Nano composite coatings produced were sintered at 1200°C for 60 min to burn-out the carbon rod and MWCNTs. It was shown that MWCNTs provided crack-free coating layers after coating and a porous structure after sintering. The porous HA coatings obtained were coated using different EPD parameters in terms of applied voltage and deposition time. EPD provides a coating thickness of ~310 μm for a deposition time of 480 s. This method enabled the formation of coatings with variable thickness, depending on the duration of coating and applied DC voltage. Hydrothermal mixing of HA/MWCNTs and the mechanism of deposition are also discussed. The methods can produce 3D-shaped HA scaffolds for clinical applications. The results demonstrate that the HA tubes obtained are candidate materials as scaffolds for bone repairing and generation.

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1. Introduction

Hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$] ceramic materials are used as biomaterials in the human body for the repair of bones and teeth.[1)–3)] HA ceramics show excellent biocompatibility and they have the ability to create direct chemical bonds with the hard tissue in the body.[2),4)] HA scaffolds with different shapes can be generated as components for the human skeleton system. Porosity is an important factor for the biological fixation of bioceramics to living bone,[5)] while the pore size and morphology of the scaffolds affects tissue ingrowth to the implanted biomaterials.[6)] Furthermore, porosity plays an important role in the mechanical fixation between biomaterials and hard living tissues. Bioceramics with an interconnected porous structured (~100 μm) show improved osteogenic properties.[7)] Porous HA ceramics are used in many biomedical applications for repairing diseased tissues and organs. For the production of porous HA scaffolds, several methods have been used in literature, including; the polymeric sponge method,[8)] slip casting,[9)] gel casting,[10)] starch consolidation,[11)] electrophoretic deposition (EPD)[12)] and 3D printing.[13)]

EPD is widely used because of its effectiveness in generating coatings and bulk materials. The simplicity of this technique combined with the low cost of the required materials and the ability to form materials with complex shapes makes this method highly advantageous.[14)–16)] The EPD technique also allows the possibility of fabrication porous materials.[17)] EPD relies on the migration of charged particles in a suspension (electrophoresis) and the deposition of particles on the oppositely charged electrode. Despite many advantages, the most critical disadvantage of this technique is crack formation during shaping or post-coating processing. To prevent cracking it is necessary to control the drying conditions carefully or use additives which will prevent crack formation without affecting the material properties.[18),19)]

The purpose of this study was to obtain porous, crack-free HA materials by EPD. Multi-walled carbon nanotubes (MWCNTs) were used to avoid cracking of the deposits. Besides the extraordinary mechanical, electrical and physical properties of MWCNTs, these materials exhibit very low wettability because of the hydrophobicity of their surfaces. In polar solvents, ions in suspension cannot adhere to the nanotubes, so the nanotubes have a very low surface charge which manifests itself as sedimentation of nanotubes in suspensions. Therefore, the surfaces of the nanotubes must be modified by the use of functional groups such as carboxyl (–COOH), carbonyl (–C=O) and hydroxyl (–OH) groups to render them hydrophilic. In this research, mixture of HA and CNTs were obtained by means of a hydrothermal process. Reaction with strong acids (3:1 volume H_2SO_4–HNO_3) is the conventional technique for the functionalization of CNTs, but this technique can damage CNTs disrupting their crystallinity and introducing imperfections. In the present

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study, the hydrothermal process was used to functionalize the CNTs. Unlike acid treatment, the hydrothermal process requires few steps and is also an economical and environmentally benign method.

2. Experimental

2.1 Preparation of HA/MWCNTs

Quantities of calcium acetate ($Ca(CH_3COO)_2$) and, phosphoric acid (H_3PO_4) were selected to achieve a Ca/P ratio of 1.67. Calcium acetate (≥99%, Sigma-Aldrich Co. LLC.) (30 g) was dissolved in distilled water (300 cm^3) and ammonia solution (28%, Merck Co. Darmstadt, Germany) was added to adjust the pH to above 10, and then phosphoric acid (85%, Merck Co. Darmstadt, Germany) (12.99 g) was added drop wise at 25°C under magnetic stirring. To maintain the pH of the mixture above 10, addition of ammonia was maintained. To obtain a MWCNTs/HA weight ratio of 2/98, MWCNTs with a diameter in the range 10–30 nm and a length up to 30 μm (Shenzen Nanotechnologies Co Ltd., China) (0.03655 g) were added to the reacted mixture and ultrasonicated for 5 min. The resulting mixture was then placed in a Teflon® lined autoclave for hydrothermal treatment at 200°C for 2 h. Centrifuging followed by washing with distilled water was used remove unwanted impurities from the solution. The washed HA/MWCNTs precipitates were dried at 100°C for 24 h.

2.2 Electrophoretic deposition

The stability of the suspensions, in other words how long the particles are suspended, is the key parameter for the production of homogeneous mass materials by the colloidal processes. To maintain the repulsion between the particles in the suspension surface agents are used. Stable suspensions were obtained using butanol (Merck Co. Darmstadt, Germany) as a solvent and triethanolamine (TEA) (Merck Co. Darmstadt, Germany) as a dispersant. The mass percent of the particles was 2% and the addition of TEA to the suspension was fixed at 0.3 mass % of the solid loadings. The stirring process was performed using two different sets of equipment. In the first step, mixing was performed in an orbital shaker for 2 h and the second step involved ultrasonication for 2 h. For the electrophoretic deposition process, a customized setup was used. The deposition electrodes were cylindrical carbon rods and the counter electrodes were 316L stainless steel. By tuning the deposition times, porous tubes exhibiting different characteristic properties were obtained. The deposition cell designed for the fabrication of the tubes is shown in **Fig. 1**. The stainless steel counter electrode surrounds the rod-shaped carbon deposition electrode. EPD experiments were performed at 60 V and for different periods of time (up to 480 s by 60 s increments).

The electrophoretically shaped HA tubes on the carbon rods were dried at room temperature for 48 h. This process was implemented to avoid cracking. The deposit obtained was heat treated from room temperature to 1200°C at a heating rate of 2°C/min and maintained at 1200°C for 60 min to produce porous HA scaffolds. A low heating rate was used to avoid cracking during the burning-out of the carbon rods during heat treatment.[12)]

2.3 Characterization

Hydrothermally mixed HA/MWCNTs powders were characterized by transmission electron microscopy (TEM, JEOL 2100 HRTEM) to determine the interaction mechanism between HA and MWCNTs. Thermo gravimetric (TG) and differential thermal analysis (DTA) was carried out using (Rigaku Thermo Plus TG8120) at a heating rate of 10°C/min to determine the burning-out of carbon rods and MWCNTs. A microstructural evaluation of porous HA was conducted by scanning electron microscopy (SEM, JEOL JSM-5600).

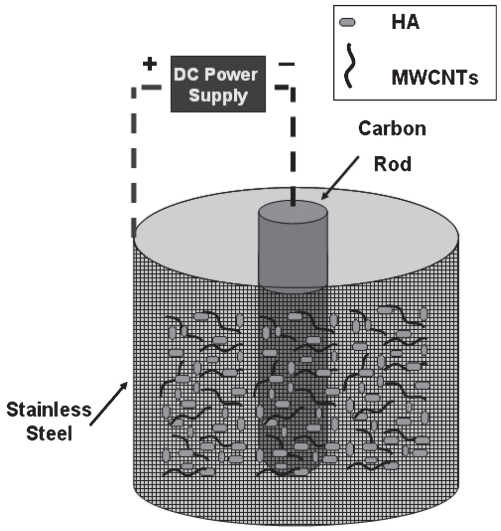


Fig. 1. Schematic illustration of the EPD cell.

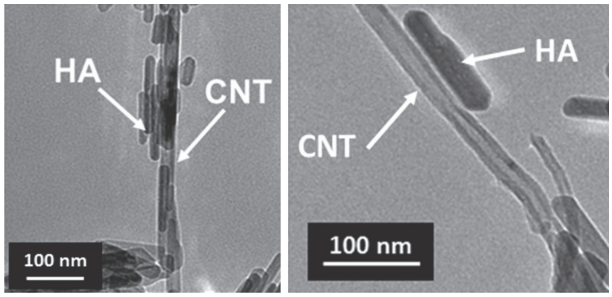


Fig. 2. TEM micrographs of HA/MWCNTs after hydrothermal process.

3. Results and discussion

TEM images of the HA/MWCNTs powders synthesized by the hydrothermal process are shown in **Fig. 2**. The powders synthesized are nanosized. The synthesized nano powders have rod-like shapes. The accomplishment of interactions between the HA and CNTs by means of a hydrothermal process was confirmed by TEM observations. The HA *c* face has negative (−) sites and the *a* face has positive (+) sites because they are composed PO_4^{3-} and Ca^{2+}, respectively.[20)] The HA nano particles become attached to the MWCNT walls by the *c* axis of the HA crystals parallel to MWCNT lengthwise because of the positive (+) sites of the HA crystals under hydrothermal conditions (Fig. 2).

The suspensions obtained with HA nano powders are shown in **Fig. 3**. Visual inspection of the suspensions during the sedimentation tests revealed their stability. The stability of the suspension is an indication of the suitability of the suspension for the EPD process. As is widely known, alcohols donate a proton, and then adsorption of protons or hydroxyls occurs on the particle surface. HA/MWCNT powders were added to butanol for the EPD process. The use of butanol as the medium might help positively charge the HA particles because of the protonation phenomena.[21)]



Fig. 3. HA and HA/MWCNT suspension photograph.

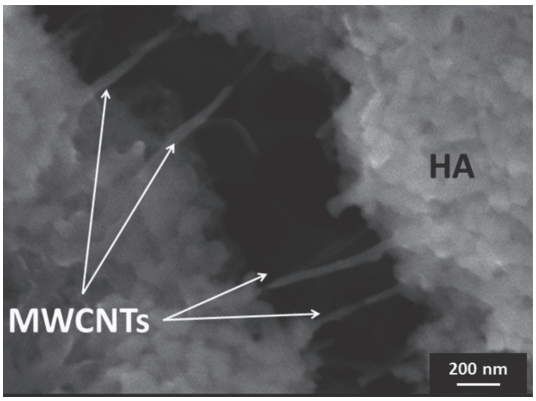


Fig. 4. SEM micrograph of the surface of HA/MWCNTs deposit layer after drying.

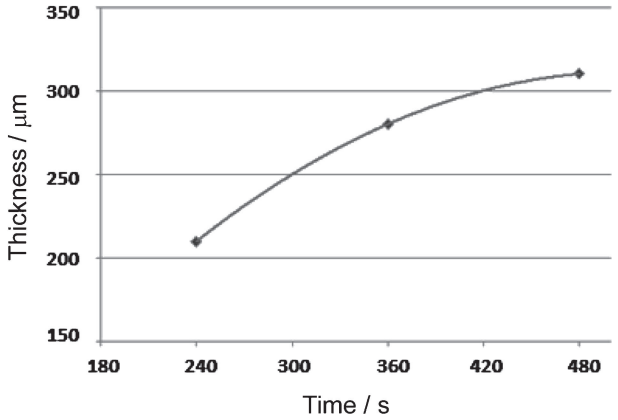


Fig. 5. EPD shaped HA porous tube thickness after sintering as a function of time.

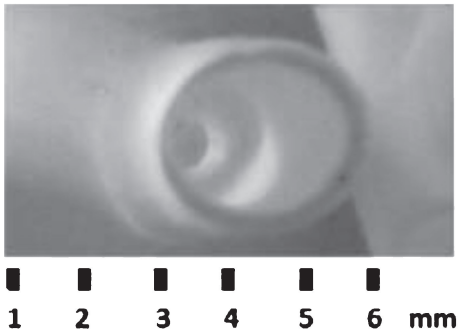


Fig. 6. EPD shaped HA porous tube ceramics after sintering (at 60 V for 480 s).

The stability of the suspensions is an indication of the identical charges generated on the surfaces of individual particles, and also implies the degree of the repulsion between particles. A higher repulsion between the particles means the suspensions are more stable, and as a consequence the particle movement during the EPD process accelerates. TEA was added to improve the stability of the suspension. TEA is an organic-based dispersion agent which has a low molecular weight. TEA can help the removal of a proton from an alcohol because of autoprotolysis reaction. This allows the surface of particles to become positively charged, which increases the suspension stability because of the electrostatic stabilization of the particles in the suspension.[22)]

The porous tubes were obtained on the carbon rods under an applied voltage of 60 V at different deposition times from the suspensions involving 2 mass % nano powders. According to Wang et al., during mass material production, the thickness of the bulk must not exceed 50 μm, as cracking occurs above this value. Because of this, the deposition should be performed incrementally to achieve a greater thickness in the materials.[12),23)] The porous tubes reinforced with carbon nanotubes do not have to be deposited gradually. Therefore, the shaping process was performed economically within short durations in a single step. However, the drying conditions and solvent are important factors in obtaining crack-free coatings. During the drying process, a rapid solvent evaporation results in the formation of many cracks because the particles rapidly move apart from each other. Therefore, evaporation rates of solvents can affect coating quality. The evaporation rates of alcohols differ with methanol, ethanol, and isopropyl alcohol evaporating more readily than butanol. A butanol suspension can therefore help prevent cracking of coating layers in comparison with other alcohols. **Figure 4** shows crack inhibition by the bridging of MWCNTs on the coating layer.

In the EPD process, the thickness and mass of the deposits increases with applied voltage and time.[24)] **Figure 5** shows the thickness of sintered (1200°C, for 60 min) porous tubes with deposition time. Porous HA tubes with a thickness less than ~200 μm could not be observed as they were damaged during the handling process because of their insufficient mechanical strength. The maximum thickness of the porous HA tubes obtained after a sintering process was ~310 μm for 480 s coating time (**Fig. 6**). This shows that saturation of the coating was occurred around 480 s.

The thermal expansion values of the coatings and substrates need to be as close as possible, to prevent micro-cracking because of thermal strain during the sintering process. The sintering of the HA tubes was performed at a slow heating rate to decrease thermal strain effect between the coating and substrate. This is because of the inhibition of crack formation on the deposited materials and also to maintain the gradual burning out of the carbon deposition rods to inhibit any damage on the tubes.[12),23)] The MWCNTs incorporated into the HA to inhibit crack evolution were burned out during sintering. This phenomenon is shown in **Fig. 7**. TG observations show that the carbon rod and MWCNTs were burned-out at 926 and 724°C respectively. According to DTA observation of HA/MWCNTs powders, an exothermic peak was observed at approximately 665°C because of the burning-out of MWCNTs.

The MWCNTs incorporated in the 2 mass % of the HA nano powders is believed to give rise to 3–4% pore formation because of the lower density of the MWCNTs compared with HA. The burn-out temperature of CNTs is approximately 600°C, and is therefore ideal in an HA matrix for obtaining crack-free materials. Therefore, MWCNTs can play an important role not only in the coating and drying process but also in the sintering process because of their thermal stability in comparison with polymeric substitute materials. SEM investigations conducted after the sintering process show that there is no evidence for the presence of cracking (**Fig. 8**). In addition, the tubes are porous and pores are interconnected to each other (**Fig. 9**). The pore morphology of HA ceramics is very important particularly in biomaterial applications. The connection between the pores is very important for osteoconductive and osteintegration.[2),6),7)] Cell infiltration is maintained by means of the movement of bodily liquids through the pores, and this enables bone formation.[2),7)] The obtained tubular structure enables bone ingrowth into the cavity of the materials. Moreover, the tube wall have a porous structure and a pore size is <20 µm. Therefore, it is deduced that the pores occur in an interconnected way that can offer improved bone growth into the pore site by appropriate surface area.[25)] Furthermore, pores of this type provide an attachment point for osteoblast adhesion and differentiation.[26)–30)] The methods can produce 3D-shaped HA scaffolds for clinical applications. By selecting of template, porous HA ceramics can be prepared with various shape and porous structure.

4. Conclusion

The production of porous tubes was performed by means of EPD for use in biomedical applications. The greatest drawback of crack formation for the production of the mass and complex shaped materials with the EPD process is inhibited by using MWCNTs. By means of the hydrothermal process, the mixing of CNTs and HA was obtained. The desired interaction of the carbon nanotubes and HA nanoparticles were confirmed with observations of TEM achieved by the hydrothermal process. The addition of 2 mass % MWCNTs to HA-based coatings had a positive effect on the inhibition of micro-crack formation on the coated layers and peeling off the coating. The HA porous tubes processed with EPD (DC 60 V, 480 s) had a thickness of 310 µm after sintering (1200°C, for 60 min). After the sintering process, porous crack free HA micro tubes were obtained. The experiments conducted reveal that the HA-MWCNT combination enables the production of complex-shaped ceramics using the EPD process.

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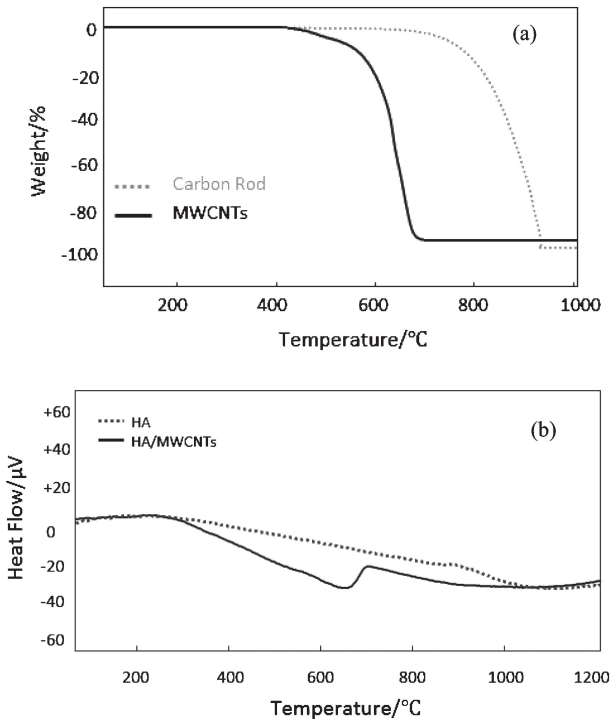


Fig. 7. Thermal analysis experiment: (a) TG results of carbon rod and MWCNTs, (b) DTA results of HA and HA/MWCNTs.

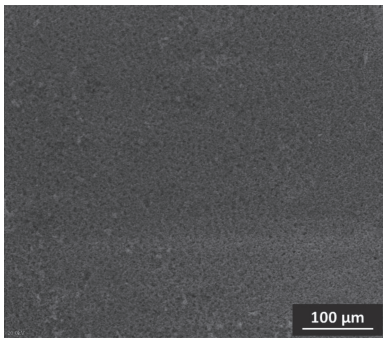


Fig. 8. SEM micrograph of the surface of the porous HA tube after sintering.

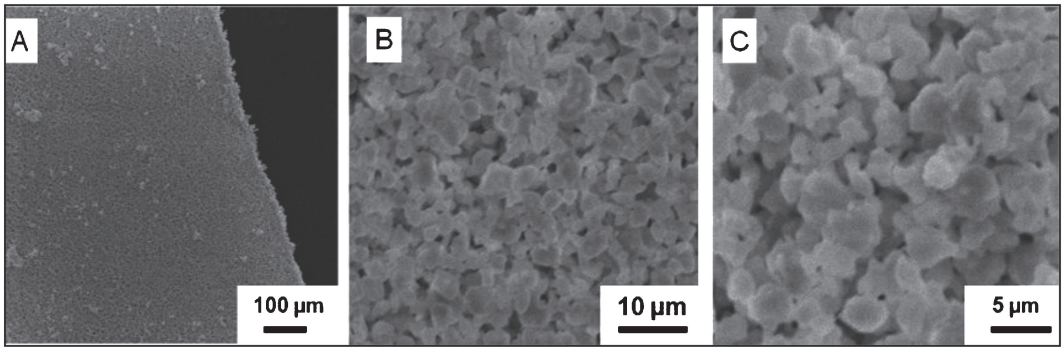


Fig. 9. SEM micrograph of the porous HA tube after sintering. (A 100×, B 2000×, C 3500×).

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