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Fabrication and in vivo evaluation of hydroxyapatite/carbon nanotube electrospun fibers for biomedical/dental application

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ABSTRACT

The aim was to synthesize bioactive electrospun fibers for biomedical and dental application with improved biocompatibility. In situ precipitation of nano-hydroxyapatite (nHA) was performed with various concentrations (0.5%, 1%, 2%, 3%, and 5% wt/wt) of functionalized multi-walled-carbon nanotubes (MWCNTs) by using microwave irradiation technique. The obtained composites were characterized by Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Thermogravimetric Analysis/Differential Scanning Calorimetry (TGA/DSC), and the cylindrical discs were made for mechanical testing. The failure behavior was analyzed by Scanning Electron Microscope (SEM). CNT and HA/CNT were silanized with γ-methacryloxypropyl-trimethoxysilane (MPTS) and mixed with polyvinyl alcohol (10% wt./vol.) and electrospun to fabricate fibers. The biocompatibility of both fibers was accessed by their effects on angiogenesis in a chick chorioallantoic membrane (CAM) assay. The electrospun fibers were analyzed by SEM. FTIR confirmed the structural behavior of pre and post-silanized HA/CNT. XRD showed the phase purity and crystallinity before and after heat treatment. Mechanical properties showed that 3% loaded HA/CNT has higher compressive strength (100.5 ± 5.9 MPa) compared to others and the failure behavior exhibited dispersion of CNT in HA matrix. The HA/CNT electrospun fibers showed significantly more blood vessels formation compared to CNT fibers. These HA/CNT electrospun fibers showed promising results in terms of biocompatibility and with improved mechanical properties of CNT reinforced composites, they can be used in load bearing clinical applications.

1. Introduction

The hard tissues (bone, enamel and dentin) exhibit calcium and phosphate as principal components [1]. Synthetic calcium phosphate (CP) family has been known for hard tissue replacement and reconstruction having remarkable similarity to the biological apatite [2–6]. Hydroxyapatite [HA, $Ca_{10}(PO_4)_6(OH)_2$], tri-calcium phosphate [TCP, $Ca_3(PO_4)_2$] and octacalcium phosphate [OCP, $Ca_8H_2(PO_4)_6$] are prominent candidates [7]. Among these, HA has extensively been used for biomedical and dental application [8–13] and different morphology, stoichiometry and level of crystallinity can be obtained by multiple preparation techniques [14–17], which in turn affects their bioactivity, mechanical properties and dissolution behavior in biological environment [18–20].

Carbon nanotubes (CNTs) are the materials known to have a very unique tubular structure with nanometer scale diameters and large length/diameter ratio [21]. Various attempts have also been made in recent years to further reveal their synthesis processes, properties and exploring their potential applications in nano materials, devices and different composite materials [22,23]. They are known to be very attractive reinforcement for composite materials due to their peculiar mechanical properties such as high elastic modulus, high tensile strength and stiffness [24–29]. It has been demonstrated experimentally that with just 1% (by weight) of CNTs added in a matrix, the stiffness of their resulting composite can increase between 36% and 42% and the tensile strength by 25% [30].

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Electrospinning has proved to be very versatile and resourceful technique with cost effectiveness for the synthesis of variety of continuous nanofibers [31–33]. This technique has been used for fabricating materials for multiple biomedical and dental applications [34–36]. Various polymers [synthetic (poly-L-lactic acid, polyurethane, poly caprolactone, polydimethylsiloxane, poly (lactide-*co*-glycolide), poly(glycerol sebacate), poly(ethylene) glycol, polyvinyl alcohol etc.) and natural (collagen, chitosan, gelatin, silk etc.) and their composite materials have been electrospun to fabricate scaffolds for bone tissue engineering, nerve regeneration, controlled drug release, tooth structure regeneration, guided tissue regeneration, reinforcement of dental composite, bone and cartilage regeneration [34,37–46]. Among these, polyvinyl alcohol (PVA) is most reliable, highly biocompatible, and water soluble synthetic polymers, where the backbone chains are interconnected by hydrogen bonding because of the presence of abundant hydroxyl groups. Considering PVA's low mechanical, and physical properties, the urge to introduce other components (such as graphene, CNT, bi-phasic calcium phosphates, and HA) in the structure with higher reinforcement abilities has been reported [47–52]. Kim et al. [53] synthesized electrospun nanofibrous scaffolds consisting of PVA and HA, which showed dentin regenerative properties. The advantage of electrospun scaffold might be its ability to produce complex geometry of dentin-pulp complex and periodontal regeneration [54,55]. Dental pulp has the ability to regenerate when pulp stem cells are placed with a biodegradable scaffold, where pulp tissue contributes to angiogenesis and vascularity which leads to continuous layer of odontoblast-like cells producing dentin-like tissue along the dentinal wall [56].

Although CNT and HA are proved to be very unique materials with exceptional properties and biomedical and dental applications However, before incorporating such materials into biomedical implants, these need to be established regarding their biocompatibility [29,57,58]. Currently the biocompatibility of biomedical materials is evaluated in vivo usually using mammalian models such as mice, rats, and dogs. In addition to ethical issues, mammalian models are both time and labor intensive as well as expensive. Furthermore, implantation of biomaterials in mammalian models does not allow continuous evaluation of the tissue reactions to the implant [59]. Therefore, there is an alternative in vivo approach for the testing of biomaterials i.e. chorioallantoic membrane (CAM) of a developing chicken embryo that allows continuous visualization of the implant site while providing a rapid, simple, and low-cost screening of tissue reactions to biomaterials. Due to its extensive vascularization and its ease of use, the CAM is a widely utilized research tool [60].

The purpose to use PVA in this study is that it is hydrophilic and can strongly interact with the hydroxyl group present on surface of HA and thus it is convenient to electrospun from an aqueous medium. In this experimental procedure, attempts have been made to optimize conditions including exposure time and power input for the microwave assisted wet synthesis of HA in CNT with emphasis to synthesize electrospun (PVA/HA/CNT) fibers with potential application for pulp-dentin and periodontal regeneration.

2. Material and methods

2.1. Functionalization of carbon nanotube

Purified short-multi-walled carbon nanotubes [(MWCNT) Purity >95%, outside diameter:10–20 nm, inside diameter:5–10 nm] were purchased from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences, China. All chemicals used in this study were analytical grade. The as received MWCNTs were heat treated at 500 °C with the heating ramp of 5 °C·min^{-1} in vacuum furnace for considering hold time of 2 h as a purpose to remove amorphous MWCNT. Then 4 M nitric acid (HNO_3, BDH AnalaR Laboratory, UK) was added in heat treated MWCNT and this mixture was refluxed in microwave oven (Samsung MW101P-K) for 10 min with 30s ON: OFF intervals. After that the refluxed mixture was filtered by using filter paper and washed out with concentrated hydrochloric acid (HCl, BDH AnalaR Laboratory, UK) and deionized water until it approaches to pH ~ 5 and then drying operation was performed in hot air oven (WiseVen WOF-155, South Korea) at 80 °C for 24 h. For further oxidative treatment 30% hydrogen peroxide (H_2O_2, Merck, Germany) solution was mixed in with HNO_3 treated MWCNT sample by simple stirring. In order to minimize the tube damages, the mixture was sonicated in a conventional ultra-sonication bath for only 2 h utilizing the idea of low power sonicating bath and a relatively low acid exposure time.

2.2. In situ synthesis of HA/MWCNT composite

HA was synthesized by microwave irradiation technique as previously described by our group [12]. The calcium/phosphorus (Ca/P) ratio was 1.67. In situ precipitation of HA was performed with functionalized MWCNTs (f-CNT) by using following method. 0.5 M calcium nitrate [$CaNO_3$ (Fischer Scientific UK)] and 0.29 M diammonium hydroghen phosphate [DAHP (BDH AnalaR Laboratory, UK)] solutions were prepared in deionized water as solvent. The pH of both solutions were maintained at 10 by adding ammonium hydroxide [NH_4OH (BDH AnalaR Laboratory, UK)] solution in intervals. The f-CNT samples with 0.5%, 1%, 2%, 3% and 5% by weight were mixed in $CaNO_3$ solution along with stirring followed by sonication of mixture for 30 min. The solution was refluxed in microwave oven for 10 min with 15 s ON: OFF intervals. The solution was filtered and dried in oven at 80 °C for 24 h later heat treated in vacuum furnace at 1100 °C with the heating ramp of 5 °C·min^{-1} with 1 h stay time and ball milled for 24 h.

2.3. Silanization of HA/CNT composite

The silane treatment of f-CNT and HA/CNT composite was prepared by the modified method [12]. 1.0% vol. of γ-methacryloxypropyltrimethoxysilane (MPTS, Sigma Aldrich, St. Louis, USA) solution was prepared using solvent mixture of 90% vol. ethanol and 10% vol. deionized water. The pH of the solvent mixture was adjusted to 4 by 3.0 M acetic acid. The silane solution was stirred and allowed to hydrolyze (activate) for 1 h. The powders were added and dispersed by ultra-sonication for 15 min. Then, the mixture was stirred for 24 h at room temperature. After the silane grafting process, the reaction mixture was filtered and rinsed with absolute ethanol to remove physically adsorbed silanes. The powder was dried over night at room temperature and then dried at 60 °C in an oven for 72 h to enhance the condensation of surface silanol molecules and to remove any remaining solvent.

2.4. Characterizations

2.4.1. Fourier Transform Infrared Spectroscopy (FTIR)

Characteristic functional groups of powder (non-silanized and silanized f-CNT, HA, and HA/CNT) samples were identified using Fourier Transform Infrared (FTIR, Nicolet 6700, Thermo Scientific, USA) spectroscopy. Photo acoustic cell was used as detector. Spectra were collected over the region 4000–400 cm^{-1} at resolution of 8 cm^{-1} averaging 256 scans.

2.4.2. X-ray diffraction (XRD)

X-ray diffraction (XRD) technique was used to evaluate phase transformation of as-synthesized and heat treated HA and HA/CNT powdered samples. Analysis was carried out on diffractometer system PERT-PRO using Goniometer geometry (PW3050/60) at room temperature with Cu K-α radiation. XRD pattern was recorded continuously with 2Θ from 20° to 80° with a step size of 0.02.

2.4.3. Thermogravimetric analysis/Differential Scanning Calorimetry (TGA/DSC)

Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) of f-CNT, HA and HA/CNT samples were performed on