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Development of multi-walled carbon nanotubes reinforced monetite bionanocomposite cements for orthopedic applications

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ABSTRACT

In this study, we present results of our research on biodegradable monetite (DCPA, $CaHPO_4$) cement with surface-modified multi-walled carbon nanotubes (mMWCNTs) as potential bone defect repair material. The cement pastes showed desirable handling properties and possessed a suitable setting time for use in surgical setting. The incorporation of mMWCNTs shortened the setting time of DCPA and increased the compressive strength of DCPA cement from 11.09 ± 1.85 MPa to 21.56 ± 2.47 MPa. The cytocompatibility of the materials was investigated *in vitro* using the preosteoblast cell line MC3T3-E1. An increase of cell numbers was observed on both DCPA and DCPA-mMWCNTs. Scanning electron microscopy (SEM) results also revealed an obvious cell growth on the surface of the cements. Based on these results, DCPA-mMWCNTs composite cements can be considered as potential bone defect repair materials.

1. Introduction

This work represents the results of our work in the general field of composite bone cement for orthopedic applications [1]. Calcium phosphate cement (CPC) was chosen to be the matrix phase, with its well proven capability to repair bone defects and stabilization of implants in surgery [2–4]. Surface-modified multi-walled carbon nanotubes (MWCNTs) were introduced as fillers with a view to improving the mechanical properties of CPCs. As compared to single-walled carbon nanotubes (SWCNTs), MWCNTs are more amenable to chemical surface modification. In the case of SWCNTs, the surface modification may break some C = C double bonds, leaving "holes" in the structure on the nanotube and thus modifying its mechanical properties [5]. In contrast, for MWCNTs, only the outer wall is modified.

In general, there are two major groups of CPCs based on their end phases: apatite, mainly hydroxyapatite (HA, $Ca_{10}(PO_{4)6}(OH)_2$) based, and brushite (DCPD, $CaHPO_4.2H_2O$) based [6,7]. Apatite-based cements are more frequently studied because they are stable at a pH value closer to the physiological environment. The disadvantage of apatite cement is its limited resorbability. DCPD-based cements exhibit faster resorbability than apatite-based cements under physiological conditions. This is attributed to the fact that DCPD is a metastable calcium phosphate phase [8,9]. However, it was observed after initial fast degradation of DCPD *in vivo*, the remaining DCPD was converted into stable apatite, resulting in no dissolution [10,11].

Monetite (DCPA, $CaHPO_4$) exhibits chemical composition and solubility close to DCPD [12], thus making it a potential biodegradable cement candidate. Its application as bone regeneration material has been well demonstrated [9,13,14]. The driving force behind using DCPA as an alternative to DCPD is that DCPA does not reprecipitate into apatite *in vivo*, providing greater degradation possibility for bone regeneration [13]. However, due to the brittle nature of ceramics, the applications of the DCPA and DCPD cement in various stress-bearing situations are expected to be limited. Because of this issue, adding a small amount of reinforcement such as fibers to cement is considered as a potential solution [15,16]. The main objective of this paper is to evaluate MWCNTs as a class of potential reinforcement to DCPA cement composition to enhance the mechanical strength without sacrificing any other important property such as injectability, hardenability, washout resistance, and biocompatibility.

The incorporation of carbon nanotubes (CNTs) in both single-walled and multi-walled configurations as reinforcements in construction cements has been known in the literature [17,18]. As compared to conventional fibers, CNTs exhibit several distinct advantages. First, CNTs have significantly greater strengths than other fibers, which should improve overall mechanical behavior [18]. Second, CNTs have much higher aspect ratios, requiring significantly higher energies for crack propagation around CNTs as opposed to fibers with shorter aspect ratios [18]. Third, the smaller diameters of CNTs enable uniform distribution at high concentrations in the cement matrix [18]. Finally, CNTs can be functionalized, enabling them to chemically react with cement components, providing routes for other forms of interaction and more enhancement of mechanical properties of the cement matrix [18]. In biomedical CPCs, CNTs were also introduced. The composite cements were prepared by simply mixing hydroxylated and carboxylated MWCNTs to apatite CPCs, resulting great mechanical strength improvement combined with desirable biocompatibility [19,20]. It was important

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to note that as compared to functionalized MWCNTs, regular MWCNTs are expected to have low chemical wettability for their dispersion in CPC matrices and poor bonding to CPC for required mechanical improvement.

This paper is the first attempt to combine MWCNTs into a DCPA cement. Both surface-modified and untreated MWCNTs were added to DCPA cements. The effects of MWCNTs content on the self-setting property, structure, mechanical performance, and cytocompatibility of DCPA cements were evaluated using various techniques. These include the uses of Gillmore needle, a universal mechanical testing machine, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM) and in vitro evaluation using preosteoblast cells, respectively.

2. Experimental details

2.1. Materials

All chemical reagents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The powder component of DCPA cement is calcium hydroxide ($Ca(OH)_2$, >95%) without any further treatments. Setting solution was prepared by mixing 6 g sodium bicarbonate ($NaHCO_3$, >99.7%), 12.95 ml phosphoric acid (H_3PO_4, 85%) solution and 2.05 ml deionized (DI) water. It is important to add the H_3PO_4 by titrating droplets because the solution is highly reactive. Setting solutions were stored in tightly-capped glass bottles.

The multi-walled carbon nanotubes (95% purity, black) purchased from Sun Innovation, Inc. (Fremont, CA, USA) were used in this study. In the first step for surface modification of MWCNTs, impurities were removed by treating MWCNTs with solution of concentrated sulfuric acid (H_2SO_4, 98%) and nitric acid (HNO_3, 70%) volume 3:1. The mixture was subsequently ultrasonicated (power of 100 W and nominal frequency of 50 kHz at ambient temperature) for 2 h and then magnetically stirred at 70 °C for 3 h. After the mixture was cooled to room temperature, it was diluted with deionized water and then poured in centrifuge tubes and centrifuged with centrifuge machine (3000 rpm for 3 min), and washed with distilled water until the pH value of the filtrate was around seven. Sediment was dried in the furnace at 70 °C, giving the CNT-COOH functional surface of MWCNTs (mMWCNTs) [21].

2.2. Cement synthesis

Cement pastes were prepared by manually mixing $Ca(OH)_2$ with setting solution, DI water and surface-modified MWCNTs in an agate mortar by using an agate pestle. Untreated MWCNTs were also applied as control groups. Initially, 1.235 g of $Ca(OH)_2$ mixed at least 2 min with 1.6 ml of DI water to form paste with $Ca(OH)_2$ uniformly dispersed in the water. After the dispersion step, different amounts of mMWCNTs [0, 0.5, 1, 2 wt% of $Ca(OH)_2$] were added to the sample and again mixed with an agate pestle to completely disperse all of the mMWCNTs particles in the solution. These compositions were chosen based on our preliminary experiments. The color of the solution after adding mMWCNTs gradually changed from white to grey and finally turned to dark grey. Finally, 1.5 ml of setting solution was added to the materials above and shaped in the mold to make it a disk sample for compression test and cell culture. The mold cavity has an inner diameter of 1.27 cm and was fitted with a plunger, which fits tightly into the mold cavity. The plunger has a weight of 53.2 g to provide gentle pressure for cement shaping. The setting of cement occurred in 37 °C incubator with 100% humidity. DCPA cements with 1 wt% untreated MWCNTs were also prepared as blank control.

2.3. Injectability testing

The as-mixed paste was loaded into a syringe (BD Biosciences, San Jose, CA, USA) and subsequently injected into a petri dish to evaluate the injectability. The output diameter of tested syringe (1.36 mm) is between gauge 15 (1.449 mm) and gauge 16 (1.291 mm), smaller than the needle size used in clinical cement injection [22]. The sample was considered to have a suitable injectability if it was not stuck in the syringe during injection.

2.4. Washout resistance testing

Washout resistance evaluation of DCPA formed in the presence and absence of MWCNTs was tested using saliva like solution (SLS) [23]. It contained 1.2 mmol/L $CaCl_2$, 0.72 mmol/L KH_2PO_4, 30 mmol/L KCl and 50 mmol/L HEPES buffer (*N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid), and its pH level was adjusted to 7 using 0.1 mol/L NaOH. All the chemicals used were purchased form Fisher Scientific (Fair Lawn, NJ, USA). As-mixed pastes of DCPA and DCPA-mMWCNTs was shaped into spheres by hand and soaked into SLS at 37 °C immediately. This provides a very harsh condition for washout resistance evaluation and also simulates the actual application in treating an orthopedic emergency. The sample was considered to pass the washout resistance test if it did not visibly disintegrate in the SLS [23].

2.5. Setting time evaluation

Initial (t_i) and final (t_f) setting times of cement samples were determined by using Gillmore needle (Humboldt Mfg. Co., Schiller Park, IL) according to the international standard ISO 9917 for dental cement and ASTM C266-89 [24]. A record of t_i, indicating the time point that the cement is no longer injectable, was made and registered as the time in minutes when the light needle did not leave an indentation deeper than 1 mm on the cement surface, and t_f, indicating the time point that the cement is able to provide strength, was the time in minutes when the heavy needle failed to leave an indentation deeper than 1 mm on the cement surface. For statistic purpose, 6 specimens were performed for each sample type.

2.6. Mechanical testing

Before testing, each specimen was incubated in an incubator with 100% humidity at 37 °C for 24 h and soaked in DI water at 37 °C for 24 h before testing to remove all possible soluble salts in cement structure. Samples with a height of 2.54 cm and a diameter of 1.27 cm were tested to failure in uniaxial compression test using a universal testing machine with 50 kN load cell (model 5569; Instron, Norwood, MA, USA). Compressive load versus compression deflection plots and data were obtained from Instron compression tester too. The crosshead rate of loading, as American Dental Association suggested 0.75 ± 0.25 mm/min, was set to 0.5 mm/min. A minimum of six samples was prepared for each combination of reinforcement. The compressive modulus was determined from the slope of the linear region in the stress-strain diagram. Before the test, all of the sharp edges of samples were polished to decrease the effects of stress concentration in sharp edges.

2.7. Physical characterization

The changes in the crystallographic structures of the DCPA-mMWCNTs produced were examined using XRD (Ultima III, Rigaku, The Woodlands, TX, USA) with monochromated Cu Kα radiation at a speed of one degree per minute in continuous scan mode with 40 kV and 44 mA setting. The XRD data were collected for a 2θ range between 10° and 60°. To identify the phases present in the sample, the XRD pattern of the sample was compared with every calculated pattern in a powder diffraction file (PDF) database from the International Center for Diffraction Data (ICDD). Using the search-match capabilities of XRD software JADE (MDI, Livermore,