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Calcium phosphate nanoparticles functionalized with a dimethacrylate monomer

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ABSTRACT

The synthesis of calcium phosphate nanoparticles may include modifying agents to tailor particle size, reduce agglomeration and add specific functionalities. This study describes the synthesis of dicalcium phosphate dihydrate (DCPD) nanoparticles functionalized with triethylene glycol dimethacrylate (TEGDMA), added to one of the reacting solutions, with the purpose of reducing agglomeration and improving the compatibility with vinyl-based resin matrices. The nanoparticles were characterized by X-ray diffraction (XRD), Fourier-transformed infrared spectroscopy (FTIR), elemental analysis, thermogravimetric analysis (TGA), transmission electronic microscopy (TEM), dynamic light scattering (DLS), and surface area (BET). As controls, proprietary DCPD nanoparticles and nanoparticles synthesized without the addition of TEGDMA ("bare") were subjected to the same analytical methods. XRD revealed a similar crystalline structure of the synthesized materials in comparison to the proprietary nanoparticles. The presence of a TEGDMA layer was confirmed by elemental analysis and TGA, corresponding to a mass fraction of 8.5%. FTIR analysis of the functionalized nanoparticles revealed the suppression of some absorbance bands found in the neat TEGDMA. A chemisorption mechanism between TEGDMA and the surface of primary particles by ion–dipole interaction involving TEGDMA oxyethylene, and also an interaction mechanism between the particle surface and terminal-CH_3 groups are proposed. Functionalized nanoparticles showed 3 to 11 times higher surface area than the controls, in agreement with DLS data, indicating lower agglomeration.

1. Introduction

Calcium orthophosphates have been extensively studied in the last decades due to their role in the mineralization of bones and teeth, as well as in pathological calcifications [4,8]. Recently, nano-structured calcium phosphates have been tested in several applications, including biomimetic remineralization [37], as fluorescent labels [14], non-viral vectors for gene [23] and drug delivery [10], bioactive coating for implants [18], in customized 3D-printed structures for bone augmentation [29,30], or as a means to increase the biocompatibility of acrylic bone cements [6].

Depending on the intended use, the synthesis of calcium phosphate (CaP) nanoparticles may include modifying agents to tailor particle size and crystallinity [5], reduce agglomeration [2] and add specific functionalities. Examples of functionalized nanoparticles include those conjugated with fluorescent dyes, antibodies [14], peptides [19], amino acids [5], and other unsaturated molecules, such as organosilanes [7] and methacrylates [3].

Functionalization of CaP nanoparticles is usually accomplished by stirring the particles in a solution containing the functionalizing agent, for periods varying from 8 to 48 h [3,14,17,20,26]. It has been demonstrated that this method results in the adsorption of molecules on the particle surface [9] and, to some extent, allows for the functional groups to bond to hydroxyl groups available on the surface of the particle [17]. However, because functionalization takes place after particle growth, it does not contribute to reduce agglomeration. The presence of large clusters has been shown to negatively affect ion release [33] and mechanical strength of resin-based biomaterials [27], due to their low cohesive strength and the poor filler/matrix interaction.

Particularly in load-bearing applications, such as restorative dentistry and orthopedics, the lack of a strong chemical interaction between the polymer matrix and the non-functionalized nanoparticles is critical, as it may significantly reduce the material's ability to withstand mechanical stresses. In order to overcome this problem, functionalization

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of CaP particles with different organic compounds has been investigated [3,15,21,32,33]. Organosilanes presenting a polymerizable vinyl group, such as γ-methacryloxypropyltrimethoxysilane, have been extensively studied as functionalizing agents over the past two decades [15,20,26,32,33]. Overall, the use of silanated micro- or nanoparticles in dimethacrylate-based polymer matrices showed a 20% to 70% increase in strength compared to non-functionalized controls [15,20,33]. On the other hand, the hydrophobic character of the silane molecule poses a problem, as the access of water to the CaP is delayed, significantly reducing ion release [33]. Less hydrophobic vinyl monomers, such as acrylic and methacrylic acids, were tested as coupling agents in hydroxyapatite particles, with an increase in strength of approximately 40% [3].

The present study describes the synthesis of dicalcium phosphate dihydrate nanoparticles (DCPD, $CaHPO_4.2H_2O$) functionalized with triethyleneglycol dimethacrylate (TEGDMA), which was added to one of the reacting solutions. By doing so, it is expected that the monomer would attach to the surface of the nanoparticle at early stages of its growth, contributing to reduce agglomeration. Additionally, functionalization with TEGDMA would increase the compatibility between the nanoparticles and vinyl-based resins, improving the mechanical properties of the resulting composite, in comparison to non-functionalized nanoparticles. Analytical techniques were used to characterize the nanoparticles, in addition to a synthesized bare nanoparticle and a commercial control. It is reasonable to assume that chemisorption rather than physical adsorption is favored when functionalization occurs concomitantly to the precipitation of the nanoparticle, when reactivity is at its maximum.

2. Materials and methods

2.1. Synthesis of the nanoparticles

DCPD nanoparticles were synthesized through the stoichiometric reaction between ammonium phosphate, $(NH_4)_2HPO_4$, and calcium nitrate, $Ca(NO_3)_2 \cdot 4H_2O$, by sol–gel process. Two solutions of equal concentrations (0.078 mol/L) were prepared using distilled and deionized water. Using a peristaltic pump (9 mL/min), 400 mL of the calcium nitrate solution was added drop-wise to the same volume of ammonium phosphate solution, which previously received 7 g of TEGDMA (2-methyl 2-propenic acid, Mw = 286 g/mol, ESSTECH, Technology Inc., Essington, PA), which structural formula is shown in Fig. 1. Precipitation occurred at room temperature under constant stirring, which was sustained for 30 min after dripping was finished. The pH of the final solution was 5.2. After particle decantation, they were rinsed in water to remove reminiscent ions and TEGDMA in excess. The resulting paste was freeze dried and a white powder was obtained. A second synthesis procedure was conducted as described above, but without the addition of TEGDMA to the ammonium phosphate solution. Also as a control, commercially available DCPD nanoparticles were used (Labsynth, Diadema, SP, Brazil).

2.2. Characterization of nanoparticles

X-ray diffraction (XRD) measurements were taken using nickel filtered Cu Kα radiation at 40 kV and 30 mA (MultiFlex, Rigaku Corp., Tokyo, Japan). The equipment geometry was θ/2θ. Readings were continuous at angles from 10° to 60° at 0.05° intervals, 10 s per interval. Fourier-transformed infrared (FTIR) spectroscopy was used to identify mid-infrared bands characteristic of CaPs, as well as those corresponding to the TEGDMA monomer (Vertex 70, Bruker Optik GmbH, Germany). Spectra were obtained in transmission mode between 3600 cm^{-1} and 500 cm^{-1} using KBr pellets, with a resolution of 4 cm^{-1}.

Elemental analysis (2400 CHNS/O Elemental Analyzer, Perkin-Elmer, Waltham, MA, USA) was used to determine the carbon content of the nanoparticles. Approximately 1 mg of the powder was heated to 925 °C under pure oxygen. Carbon content (from TEGDMA) was estimated based on the percentage of CO_2, with a resolution of 0.3%. Additionally, the TEGDMA mass fraction on the nanoparticles was determined by thermogravimetric analysis (TGA 2950, TA Instruments, New Castle, DE, USA). Ten milligrams of powder was heated to 600 °C (10 °C/min) under nitrogen atmosphere. In order to identify which part of the curve corresponded to the elimination of TEGDMA, the neat monomer was also analyzed.

Nitrogen adsorption isotherms were carried out at −196 °C using a volumetric adsorption analyzer (Quantachrome, model Nova 1200e, Boynton Beach, FL, USA). The surface area was determined according to the Brunauer, Emmet and Teller (BET) method. Prior to the analysis, the functionalized nanoparticles were sonicated and centrifuged in acetone to remove the organic layer, which could negatively affect the test accuracy. The powders were kept for 16 h at 150 °C under vacuum to remove adsorbed contaminants.

Nanoparticle morphology was observed under transmission electron microscopy (JEOL, model 1010, Tokyo, Japan), using an accelerating voltage of 80 kV. Powders were dispersed in isopropyl alcohol and after 24 h a few drops of the supernatant were placed on nickel grids (200 mesh) covered with a poly(vinyl formal) pellicle (Formvar). Nanoparticle dimensions (n = 30) were measured using the ImageJ software (National Institute of Health, Bethesda, MD, USA). Agglomerate size distribution was estimated by dynamic light scattering (DLS, Nanotrac 252, Microtrac, Montgomeryville, PA, USA). The powders were dispersed in isopropanol and sonicated for 10 min. Then, after 20 min, the supernatant was collected for analysis.

3. Results

X-ray diffraction (Fig. 2) revealed the same crystallographic structure for the synthesized and the commercial nanoparticles. The intensity peaks located at 11.6°, 20.9°, 23.6°, 29.3°, 35.5° and 48° were characteristic of DCPD (Joint Committee on Powder Diffraction Standards, PDF#09-0077). Fig. 3 shows the FTIR spectra of the

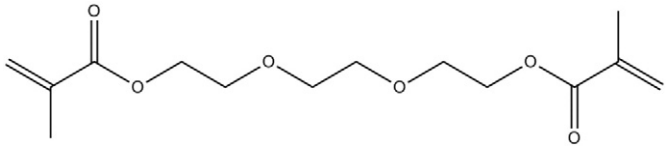

Fig. 1. Structural formula of triethyleneglycol dimethacrylate (TEGDMA), used as functionalizing agent.

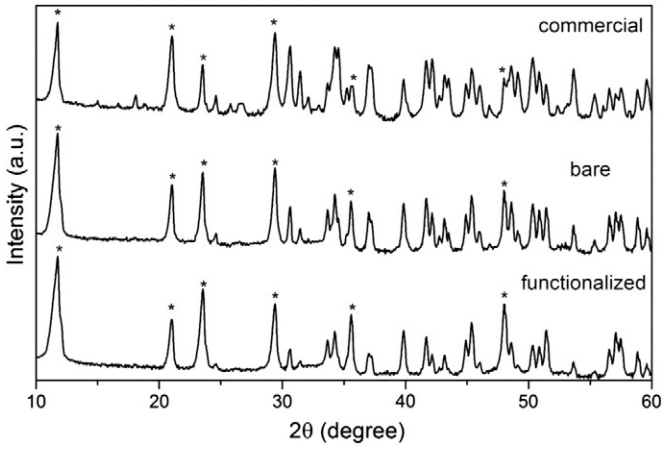

Fig. 2. Difractograms of the synthesized nanoparticles. Asterisks indicate diffraction peaks characteristic of the DCPD. "Bare" refers to the nanoparticles synthesized without additive, "commercial" refers to the commercial material, and "functionalized" refers to the TEGDMA-coated nanoparticles.