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Sodium citrate as an effective dispersant for the synthesis of inorganic–organic composites with a nanodispersed mineral phase

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ABSTRACT

Although extensive efforts have been devoted to the development of polymer–ceramic composites for bone repair, those developed thus far were not able to mimic the nanostructure of bone, partly because of the aggregated, microscale organization of the mineral component. As a consequence, homogenization and intermixing of organic and inorganic components remain a major engineering challenge for the development of functional, biomimetic bone-substituting composites. In the current study, various dispersants were evaluated for their potential to be used as biocompatible dispersants in the synthesis of biomimetic composites with a nanodispersed mineral phase. Based on sedimentation experiments, tribasic sodium citrate was selected as the most effective dispersant for the stabilization of calcium phosphate (CaP) suspensions. Specific adsorption of citrate anions onto CaP nanocrystals was shown to result in a strong increase in the negative surface charge of the CaP particles and consequently increased repulsive interparticle forces that were able to overcome attractive van der Waals forces. Using sodium citrate as dispersant at a CaP/citrate ratio of 4.0, CaP–gelatin nanocomposites were fabricated which displayed a nanostructured mineral phase without occurrence of microscale CaP particles. Consequently, aggregation and sedimentation of CaP mineral phase was reduced considerably.

1. Introduction

Despite four decades of biomaterials research, synthetic bone-substituting materials are still largely inferior to auto- or allografts as the gold standard in orthopedic and dental surgery. The clinical success of the current generation of bone-substituting materials is disappointingly limited, since they lack the high functionality of bone tissue in terms of biological and mechanical properties. This lack of biofunctionality is related mainly to an engineering attitude towards design of novel biomaterials that lacks a biological mindset [1,2]. Only recently it has been recognized that bioinspired design of biomaterials holds strong promise for creating novel implant materials with a performance level close to that of natural bone [3,4].

Bone tissue is best described as a nanocomposite consisting of ~30 wt% collagen (a protein-based hydrogel template) reinforced by a finely dispersed mineral phase (70 wt%) consisting of nanosized, apatitic calcium phosphate (CaP) crystals. The organic components of bone give it flexibility and resilience, while the inorganic components give bone its hardness and rigidity. In order to mimic these unique properties, it is hypothesized that the optimal synthetic bone tissue equivalent will also consist of an organized and dense polymeric matrix containing dispersed apatitic nanoparticles of low crystallinity.

Although extensive R&D efforts have been devoted to bone-substituting polymer–ceramic composites, those developed thus far were not able to mimic the nanostructure of bone, partly because of the aggregated microscale organization of the mineral component [5]. Generally, the greatest stumbling block to commercialization of nanocomposites is the lack of cost-effective methods for controlling the dispersion of the nanoparticles in polymeric matrices [6], since hydrophilic ceramics are extremely difficult to intermix with polymeric solutions dissolved in hydrophobic, organic solvents. The nanoscale particles typically aggregate, which negates any benefits associated with the nanoscopic dimension [6].

In view of this critical need to establish effective nanocomposite synthesis strategies, several approaches have been proposed in the literature, including (co)precipitation of apatite crystals within organic solvents [7], use of ultrafine ceramic apatite crystals [8] or using polymer-functionalized nanoparticles [6]. Several drawbacks are related to these approaches, such as limited amount of mineral loading, poor development of apatitic phases, contamination with acid CaP phases, or the introduction of possibly cytotoxic components by nanoparticle functionalization which are often expensive and synthetically challenging. As a consequence, homogenization and intermixing of organic and inorganic components remains a

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major engineering challenge for the development of functional, biomimetic bone-substituting composites in the field of bioceramics.

The current study presents a novel approach to preserving the dispersion of nanoparticles in bone-substituting nanocomposites by exploiting dispersants as a simple, cheap and safe alternative to polymeric nanoparticle surface modifications. Dispersants are frequently used for ceramic processing of ceramic slips and slurries [9–12], but their usefulness for the preparation of biomimetic, bone-substituting nanocomposites with a preserved nanostructure has not been explored to the best of the authors' knowledge. To investigate the efficacy of these dispersants for this purpose, a hydrogel matrix were selected as model system. Hydrogels are ideal tissue engineering scaffolds and drug delivery vehicles, owing to their intrinsically high water content, which makes hydrogels biocompatible, biodegradable and injectable [13]. More importantly, however, it is hypothesized that the intrinsic high water content of hydrogels allows better control over the dispersion of hydrophilic, inorganic nanoparticles within an organic polymer matrix.

Gelatin was chosen as the model hydrogel template, since it is biodegradable and has been studied extensively for pharmaceutical and medical purposes. The biosafety of gelatin has been proved through its long clinical applicability, and recent studies have revealed that gelatin-derived scaffolds are highly suitable as a drug delivery vehicle for the controlled release of osteogenic and angiogenic growth factors [14,15]. A specific benefit of gelatin with respect to the current mineral dispersion study relates to the fact that gelatin can be easily processed into porous hydrogel matrices by freezing liquid gelatin solutions followed by water removal using lyophilization [16–19]. Preliminary laboratory studies revealed that lyophilized CaP–gelatin nanocomposites were able to induce calcification *in vitro*, as indicated by strongly increased apatitic reflection intensities (using X-ray diffraction (XRD)) and phosphate absorptions (using Fourier-transform infrared spectroscopy (FTIR)) upon prolonged soaking in simulated body fluid. For lyophilization of large bulk samples, however, the freezing step is generally time-consuming, and may take up to 45 min at a freezing temperature of −20 °C [20]. As a result, sedimentation will occur when high contents of inorganic fillers are to be incorporated into gelatin matrices (such as is the case for bone-substituting composites), thereby producing inorganic–organic composites with a heterogeneous microstructure that can be neither upscaled nor applied in a clinical setting. A possible solution to avoid sedimentation of these inorganic fillers involves acceleration of the freezing rate, but it is known that the pore size of lyophilized hydrogels strongly decreases with increasing freezing rate [20]. Small pore sizes <10 μm are not suitable for bone tissue engineering purposes, where active ingrowth of bone tissue and blood vessels is required. Therefore, other approaches need to be explored which enable processing of lyophilized gelatin–apatite nanocomposites with preserved nanofunctionality.

In the current study, gelatin–apatite composite matrices were prepared using lyophilization of various gelatin–CaP formulations. These formulations were prepared by mixing gelatin solutions with pre-made, microscale CaP powders as well as suspensions of precipitated CaP nanocrystals. The aim of the study was to investigate the influence of various dispersants on (i) the stability of CaP suspensions and (ii) the final dispersion of CaP in porous gelatin hydrogels.

2. Materials and methods

2.1. Synthesis and characterization of CaP powders and suspensions

A well-known, wet-chemical precipitation method was used to synthesize apatitic nanoparticles at room temperature, involving the dropwise addition of ortho-phosphoric acid to an aqueous suspension of calcium hydroxide according to the following reaction [21,22]:

$$5Ca(OH)_2 + 3H_3PO_4 \rightarrow Ca_5(PO_4)_3(OH) + 9H_2O$$

This preparation method was chosen because water is its only by-product. The apatitic crystals were produced by slow dripping of 250 ml of a phosphoric acid solution (75 mM) to a basic suspension of 250 ml of $Ca(OH)_2$ (125 mM) at a rate of ~3–4 ml min^{-1} and continuous stirring, yielding an apatite content of 0.625% (w/v).

In order to obtain nanoscale CaP suspensions, the pH of the reaction mixture was set at a pH of 7.4 using concentrated HCl, followed by aging under continuous stirring for 15–18 h, and left until use.

Microscale CaP powders were prepared by aging the suspension under continuous stirring for 15–18 h at 25 °C followed by centrifuging at 4000 rpm, after which the precipitate was dried at 37 °C overnight, crushed using a mortar and pestle, and finally ball-milled for 5 min at 500 rpm. The resulting powders were analyzed using XRD (Philips, PW3710), attenuated total reflectance-transform infrared spectroscopy (ATR-FTIR; Perkin Elmer) and field emission scanning electron microscopy (FESEM; JEOL 6301) equipped with energy dispersive spectroscopy (EDS, EDAX).

2.2. Addition of dispersants to CaP suspensions

Several dispersants were selected from the literature [9–12] to evaluate their potential to stabilize CaP suspensions, including ammonium carbonate ($(NH_4)_2CO_3$, Sigma–Aldrich), citric acid ($C_6H_8O_7$, Sigma–Aldrich), and sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$). Citrate-based dispersants were selected because of the high levels of citrate in bone, where citrate might act as a native dispersant by mobilizing calcium transport [23]. Dolapix PC 75 (Zschimmer & Schwartz), which is a commercially available dispersant based on ammonium polyacrylate solution, was used as a reference, because it is frequently used as a deflocculant for thinning ceramic slips.

Based on the literature [10], all dispersants were added at a concentration of 0.1% (w/v), whereas a separate experiment was performed for sodium citrate concentrations between 1 and 10 mM to determine the optimum concentration for this specific dispersant.

The morphology of the CaP precipitates with/without dispersants was examined using transmission electron microscopy (TEM; JEOL 1010), whereas the conductivity and pH of the suspensions were measured using a conductivity meter (WTW Cond 315i) and a standard pH meter (Radiometer PHM210), respectively. Since the variation in conductivity and pH was negligible, only one sample was measured ($n = 1$). The ζ-potential of the suspensions, in contrast, displayed variation and was therefore determined in triplicate using a Zetasizer (Malvern Instruments Nanoseries ZS). To this end, samples were diluted 100-fold in both ddH_2O (unbuffered condition) or HEPES buffer (buffered condition, 5 mM, pH 7.4) prior to each measurement. The Smoluchowski approximation with Henry's function $f(ka)$ equal to 1.5 was used to calculate the ζ-potential from the electrophoretic mobility, since the samples were diluted aqueous suspensions with moderate electrolyte concentrations.

Sedimentation characteristics of the prepared suspensions were monitored by adding 50 ml of suspension in 50 ml tubes and recording the settling height in time up to 7 days.

2.3. Synthesis and characterization of CaP–gelatin hydrogel composites

In order to test the efficacy of sodium citrate to disperse CaP nanocrystals within organic matrices, CaP–gelatin (50:50 by