Hydroxyapatite Nanoparticle Synthesis and In- Vitro Study of Dentinal Tubule Occlusion

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ABSTRACT

A hydrothermal method of synthesizing hydroxyapatite by heating a precipitate, formed by mixing $Ca(NO_3)_2{\cdot}4H_2O$ and $(NH_4)_2HPO_4$ with distilled water, in a hydrothermal reactor at 200 ^oC for 24-72 hrs is described. A treatment time of 24 hrs produced single phase (as shown by XRD) hydroxyapatite powder, however for longer treatment times XRD patterns were indicative of the presence of a secondary phase, monetite ($CaHPO_4$). SEM examination of the treated powders displayed particles of rod-like morphology with dimensions 100-500 nm in length and 10-60 nm in diameter. Preliminary results on the use of the particles for the infiltration of dentine tubules are presented.

Keywords: hydroxyapatite, hydrothermal, dentine, hypersensitivity

1 INTRODUCTION

Hydroxyapatite (HAp) with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ has the attractive feature of biocompatibility and intergrowth with bone and teeth and has been used extensively in medicine and dentistry [1]. However due to its poor mechanical properties, HAp ceramics cannot be used for heavy load bearing applications [2], but common uses include bone graft substitution and coatings on metallic implants [1,2].

The properties of HAp can be influenced by control of particle size, chemical composition and morphology and can be synthesized by a variety of methods such as solid state reaction [3], co-precipitation [4], sol-gel [5] and mechanico-chemical [6]. Hydrothermal synthesis has been used to transform slurries, solutions, or gels into the desired crystalline phase under mild reaction conditions typically below 350 ^oC [7]. Typical powders synthesized by this method have been shown to consist of needle-like particles between 20 - 40 nm in diameter and 100-160 nm in length [7].

The motivation here for synthesising HAp by hydrothermal means is to obtain nanosized particles for infiltration of dentinal tubules for the alleviation of hypersensitivity, a common problem for millions of children and adults worldwide. Most commercial treatment procedures are based on desensitising agents for which the proposed mode of action is that of tubule occlusion and/or nerve desensitisation. Approaches include tubule occlusion by a precipitation reaction via the application of potassium oxalates to form insoluble calcium oxalate [8]; tubule occlusion and nerve desensitisation by appropriately formulated toothpastes; slow-release fluoride agents [9].

2 EXPERIMENTAL METHOD

Stock solutions, 1.0 M, were prepared by dissolving calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) (11.807 g) (Fisher Scientific ACS grade) and di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (6.603 g) (Fisher Scientific analytical reagent grade) in 50.0 ml distilled water. The stock solutions were diluted further with distilled water to give 0.10 M solutions, and subsequently mixed to yield suspensions with Ca/P molar ratios of 1.67 by the dropwise addition of 10.0 ml of 0.10 M di-ammonium hydrogen phosphate to 16.7 ml of 0.10 M calcium nitrate tetrahydrate. The mixed suspension had a pH = 5, this was adjusted in the range 5 –12 using ammonium hydroxide solution (measured using a glass electrode).

The solutions were either transferred to a 125 ml Teflon-lined hydrothermal reactor (Model 4748, Parr Instrument) and the reaction performed by placing the unit in a laboratory oven for between 24 and 72 hours at a temperature of up to 200 ^oC, and then cooling to

room temperature naturally. Other samples were prepared in a larger 1.6 L vessel with programmable computerised heating and mechanical stirring and computerised internal pressure monitoring. Following all experiments the reacted powders were washed by suspending the sample in distilled water using an ultrasonic bath for 5 mins before sedimenting the particles by means of centifugation. At the end of each spin cycle the liquid was decanted and replaced by an equal volume of fresh distilled water, this process was repeated 6 times, or more until the pH had returned to 7. In some cases a final wash was performed using methanol to limit agglomeration of HAp particles during drying. Samples for phase analysis were dried in a laboratory oven at ~ 50 ^{o}C for ~ 4 hours. X-ray diffraction (XRD) of all prepared powders was performed, in the range 5 – 70 $^{o}2\theta$ at a scan speed of 0.05^{o} s^{-1}. HAp particle sizes were determined using a field emission scanning electron microscope (LEO 1530 FEGSEM) at 3 kV with a working distance of 3-4 mm.

Dentine sections were prepared from human teeth and etched using 35 wt% phosphoric acid for 60s to reveal the dentinal tubules.

3 RESULTS AND DISCUSSION

XRD patterns of the powders synthesized at 200 ^{o}C for 24 hrs from a starting reaction solution of pH 5, Figure 1 (a), only contained peaks arising from HAp, as listed in the relevant JCPDS reference file [10,11]. However the XRD patterns of the powders treated for >24 hrs showed peaks attributed not only to HAp, but also to monetite (CaHPO4) [12,13]. For all treatment times the XRD peaks were well defined, indicating the samples were well crystallized.

Scanning electron microscopy (SEM) was used to investigate the morphology and particle size of the synthesized powders. Typical SEM images of synthesized powders for treatment times of 24 hrs and 48 hrs hrs are shown in Figure 2. Both treatment times showed rod-like particles. For each synthesized powder the length and diameter of 100 particles was measured and the aspect ratio (i.e. length/diameter) determined. For all three treatment times the aspect ratio was measured to be between 10 – 30, with the particle length and diameter measured to be between 100-600 nm and 10-60 nm respectively. The median length was in the size increment 350 - 400 nm and the

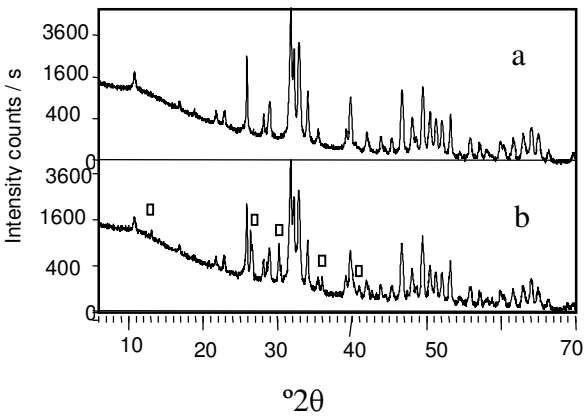

Figure 1. XRD patterns of powders prepared hydrothermally at 200 ^{o}C for (a) 24 hrs, (b) 48 hrs. Squares indicate $CaHPO_4$, un-marked peaks are HAp.

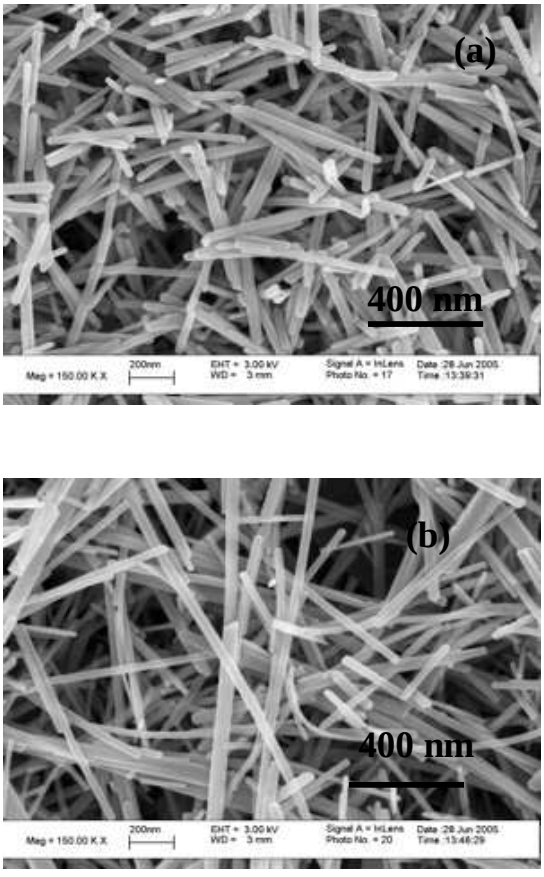

Figure 2 SEM micrographs of powders synthesized for different treatment times at 200 ^{o}C: (a) 24 hrs, (b) 48 hrs.

median width 20 - 40 nm with no difference observed between the treatment reaction times. These particle dimensions fall within the range reported in the literature for HAp produced under similar hydrothermal conditions [7]. Although prolonging the treatment times may be expected to increase the crystallite size, multiple measurements of the crystallites did not show this to be the case.

Despite XRD showing there to be a second phase of monetite in the 48 hrs and 72 hrs samples, examination by SEM did not reveal corresponding second-phase particles. This implies that either monetite crystallises exist as discrete needle-like particles of indistinguishable morphology from those of hydroxyapatite, or that individual needles are polycrystalline and biphasic. The reasons for monetite forming after 24 h are uncertain; experiments are underway to monitor changes in pH as a function of reaction time, and to obtain more detailed information on the time-dependent evolution of particle morphology in the first few hours of the reaction.

Initial in-vitro occlusion trials were performed using 40 nm silica nanospheres suspended in methanol which showed promising tubule occlusion, Fig 3. A similar approach was subsequently used for the nano HAp particles synthesised by hydrothermal reaction. Particles synthesised at a pH > 10 proved to have a smaller aspect ratio than the particles shown in Fig 2 and were selected for the initial trials. This decision was based on the 'proof of principle' results for the spherical silica particles, which suggested that small equiaxed particles showed suitable tubule infiltration properties.

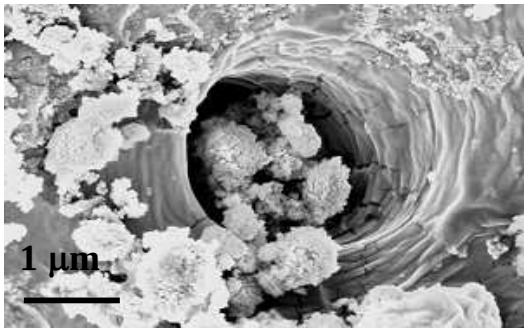

Figure 3 SEM micrograph of dentine section after tubule infiltration experiment using ~ 40nm silica nanospheres.

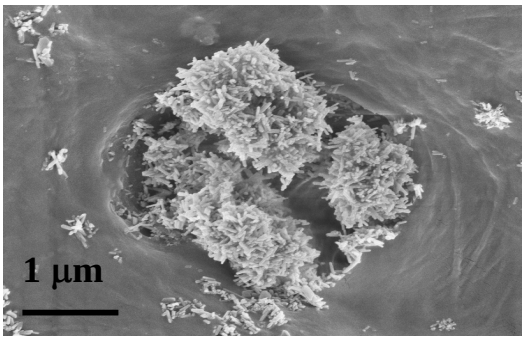

Figure 4 SEM micrographs of dentine section after tubule infiltration experiment synthesized HAp particles in the size range 100 – 200 nm.

4 CONCLUSIONS

Single phase hydroxyapatite (by XRD) crystallites with a rod-like morphology were synthesized by a hydrothermal method up to 200 ^{o}C under saturated water vapour pressure for 24 hrs from a precipitate formed by mixing Ca(NO3)2·4H2O, (NH4)2HPO4 and distilled water. Longer treatment times led to the production of a secondary phase, monetite, (CaHPO4). However the treatment time had no effect on the particle morphology or size within the reaction time range of 24-72 hrs. The crystallites were measured to be within the size range 100-600 nm in length and 10-60 nm in diameter. An increase in the pH of the starting reaction suspension to pH 10-12 had the effect of reducing the particle size and subsequent infiltration results indicate smaller, more equiaxed or spherical particles may be desirable for dentine tubule infiltration.

5 ACKNOWLEDGEMENTS

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