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SYNTHESIS OF RHENANITE (β-$NaCaPO_4$)-APATITIC CALCIUM PHOSPHATE BIPHASICS FOR SKELETAL REPAIR

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ABSTRACT

Biphasic rhenanite (β-$NaCaPO_4$)-apatitic calcium phosphate biomaterials for skeletal repair were prepared by using a one-pot, solution-based synthesis procedure at the physiological pH of 7.4, followed by low-temperature (300° to 600°C) calcination in air for 6 hours. Calcination was for the sole purpose of crystallization. An aqueous solution of $Ca(NO_3)_2{\cdot}4H_2O$ was rapidly added to a solution of Na_2HPO_4 and $NaHCO_3$ at room temperature, followed by immediate filtration of gel-like, poorly-crystallized precursor precipitates from the mother liquors of pH 7.4. Freeze-dried precursors were found to be nanosize with an average particle size of 45 nm and surface area of *ca.* 125 m^2/g. Upon calcination in air, precursor powders crystallized into biphasic (60% HA-40% rhenanite) biomaterials, while retaining their submicron particle sizes and high surface areas. Phase-pure rhenanite powders were also synthesized by solid-state reactive firing. Rhenanite is a high solubility sodium calcium phosphate phase. Samples were characterized by XRD, FTIR, SEM, ICP-AES, TG, DTA, DSC, and BET surface area measurements.

INTRODUCTION

In orthopedic, oral and maxillofacial surgery, a variety of synthetic bone grafts have been used to fill skeletal defects originating from tumor resection, trauma or infection [1-3]. Synthetic calcium phosphates, such as calcium hydroxyapatite [HA; $Ca_{10}(PO_4)_6(OH)_2$], β-tricalcium phosphate [β-TCP; β-$Ca_3(PO_4)_2$] and biphasic mixtures of these two have found use as bone substitutes [4-6]. HA or β-TCP implants exhibit relatively good tissue compatibility, and new bone is formed directly on the implants with no fibrous encapsulation (by the fibroblasts) [7]. However, sintered and well-crystallized HA ceramics usually demonstrated minimal in vivo resorption, with resorption times lagging the new bone formation rates [8-12]. Kilian et al. [13] showed that nonsintered HA could even be phagocytized and dissolved by macrophages and osteoclasts, while sintered ceramics were not degraded and remained at the site of implantation for years following the surgery. β-TCP, on the other hand, has a significantly high solubility [14, 15] and typically fades away from the defect site even before the completion of new bone formation. An ideal skeletal repair implant should readily take part in the bone remodeling processes, and also allow for the direct anchorage by the bony tissues surrounding it (osteoconduction) [16]. If the skeletal repair implant itself causes the in situ formation of the mineral part of the bone tissues (osteoinduction) rich in carbonated, apatitic calcium phosphates [17], while it is continuously resorbing (in vivo osseointegration), this could be its most affirmative contribution to the defect site [18-21]. Therefore, efforts in the direction of developing new calcium phosphate-based bone substitutes of higher in vivo resorbability and osteoinductive/osteoconductive capabilities are strongly needed.

In stark contrast to sintered HA ceramics [10, 11], calcium phosphate (CaP) self-setting cement formulations, which intentionally employed poorly-crystallized apatite as their major powder component, were shown [22-24] to have significant in vivo resorbability (i.e., with resorption rates in excess of 98% in 26 weeks following the implantation in the case of, for instance, α-BSM™, Etex Corp., Cambridge, MA). These cements rapidly took part in the bone remodeling processes by going through phagocytosis under the action of macrophages and osteoclasts [22]. Besides these special

orthopedic cements, such high resorption rates with calcium phosphates have only been encountered when the tested (in vivo) materials comprised nanoapatites [25].

The presence of the noncrystalline calcium phosphate phase in bones has been detected even by the very first electron microscope studies [26]. The earlier work of Posner *et al.* [27-34] set the foundation for the synthesis and characterization of amorphous or poorly-crystallized calcium phosphate powders. The cytoplasmic calcium phosphate mineral was found to have a structure built up of close-packed ion clusters of about 10 Å similar to those of $Ca_9(PO_4)_6$ present in synthetic amorphous calcium phosphates. Short-range order existed in these amorphous clusters (i.e., Posner clusters) but no long-range order was detected as crystalline hydroxyapatites have [35]. The work of Eanes *et al.* [35-42] and Rey *et al.* [24, 43-51] on the preparation of poorly-crystallized calcium phosphates should also be underlined in this context.

β-Rhenanite (β-$NaCaPO_4$), is an alkali calcium orthophosphate, which was recently shown to support cellular proliferation together with expression of osteogenic markers at a level higher than β-TCP [52], and $NaCaPO_4$ was, therefore, suggested to possess a higher potency to enhance osteogenesis than β-TCP. Ramselaar *et al.* [53-56] were the first to investigate the biodegradation rate of $NaCaPO_4$ implants in direct comparison to HA and β-TCP from six weeks to three months in vivo. Knabe *et al.* [57] noted the remarkably high solubility (1.0 g per liter of H_2O at pH 7 [53]) of $NaCaPO_4$ samples in a comparative set of in vitro rat bone marrow cell culture tests performed on a number of calcium phosphates. Suchanek *et al.* [58] discovered the formation of $NaCaPO_4$ interphase layers of high biocompatibility during the hot pressing of hydroxyapatite and bioactive glass powders together. Glass ceramics which contained $NaCaPO_4$ as the crystalline phase were also reported to be bioactive [59-61].

On the other hand, "Rhenania process" is a well-known procedure mostly used in the fertilizer industry to obtain a soluble phosphate material [62]. In this process, the natural mineral of hydroxyapatite was mixed with Na_2CO_3 and SiO_2 whereas the molar ratio of Na_2CO_3/P_2O_5 fixed at 1.0. SiO_2 was added to prevent the occurrence of free CaO in the sintered product. These powder mixtures were then ground together and calcined in a rotary kiln at about 1000°-1200°C for about few hours. The calcined material was then ground to the desired particle size range. Rhenanite, $NaCaPO_4$, of high solubility, has been the major phase in the final product of the Rhenania process [62].

Resorbable, granular bone graft substitutes based on $NaCaPO_4$ formulations have already been commercialized and marketed for the orthopedic surgeons [63, 64]. Self-setting cements based on $NaCaPO_4$ are also available for the repair of bone defects [65]. Nevertheless, the powders of such products have been produced by high-temperature (>1100°C) processes [63].

The motivation for the present study stems from our interest in developing a robust synthesis route for the manufacture of biphasic nanopowders of $NaCaPO_4$ and carbonated, apatitic calcium phosphate using temperatures less than 700°C [66]. Apatitic calcium phosphate powders rapidly lose their carbonate ions when heated at a temperature higher than 700°C [67].

Therefore, our experimental approach to that end was framed around the following straightforward supposition: "amorphous or poorly-crystallized calcium phosphate powders are known to consist of nanoparticles of apatitic calcium phosphates [24], and if they were synthesized in the presence of a significant amount of aqueous Na^+ ions, then upon calcination at relatively low temperatures, the resultant powders should be a biphasic mixture of $NaCaPO_4$ and apatitic calcium phosphate." This work reports the preparation of nanosize calcium phosphate precursor powders that are able to transform into biphasic mixtures of β-$NaCaPO_4$ and apatitic calcium phosphate upon low-temperature (300°-600°C) calcination.

EXPERIMENTAL PROCEDURE

Rhenanite-apatitic CaP biphasics: The Na-containing poorly-crystallized apatitic calcium phosphate powders were synthesized by a procedure inspired by the work of Lee *et al* [68]. Two

solutions were prepared. Solution-A was prepared as follows; 86.4 g Na_2HPO_4 (>99%, Fisher Scientific, Fairlawn, NJ) was dissolved in 1.2 L of deionized water, followed by the addition of 60.0 g $NaHCO_3$ (>99%, Fisher), which resulted in a clear solution of pH 9 at RT (23±1°C). Solution-B was prepared by dissolving 70.0 g of $Ca(NO_3)_2{\cdot}4H_2O$ (>99%, Fisher) in 500 mL deionized water. Solution-B was then rapidly added into solution-A under constant stirring (at 250 rpm, with a 5 cm-long Teflon-coated magnetic fish) at RT. The pH of the resultant milky suspension (with a nominal Ca/P molar ratio of 0.49) was then rapidly raised to around 7.4, i.e., the physiological pH value, by adding 3 to 5 mL of concentrated NaOH solution. The suspension was immediately filtered by using a filter paper (No. 42, Whatman International Ltd., Maidstone, UK) placed in a vacuum-suction porcelain Buechner funnel assembly, and washed with 5 L of deionized water. The obtained CaP gels were first frozen at -80°C for 2 hours, and then lyophilized in a vacuum chamber (Freezone® 4.5, Labconco Corp., Kansas City, MO) kept at 5 x10-2 mbar at RT overnight. Freeze-dried powders were then calcined in a static air atmosphere (5°C/min heating and cooling rates) over the temperature range of 300° to 600°C, with 6 h of soak time at the peak temperatures.

$NaCaPO_4$ synthesis: Rhenanite powders were also synthesized by solid-state reactive firing. 2.12 g of Na_2CO_3, 4.00 g of $CaCO_3$ and 5.28 g of $(NH_4)_2HPO_4$ were dry mixed and ground in a glass mortar by using a glass pestle for about 30 minutes. The powder was calcined in an alumina crucible at 900°C for 12 h (heating/cooling rate: 5°C/min), followed by regrinding and a second calcination at 650°C for 18 h (heating cooling rate: 5°C/min). This synthesis procedure was adapted here directly from the ICDD (Int. Centre for Diffraction Data), Powder Diffraction File (PDF) No. 29-1193. Phase-pure Rhenanite powders were synthesized to facilitate better XRD and FTIR characterization of the $NaCaPO_4$ phase found in the biphasic powders mentioned above.

Samples were characterized by powder X-ray diffraction, XRD (Model XDS 2000, Scintag, Sunnyvale, CA), scanning electron microscopy, SEM (Model S-4700, Hitachi Corp., Tokyo, Japan), Fourier-transform infrared spectroscopy, FTIR (Model Nicolet 550, Thermo-Nicolet, Woburn, MA), inductively-coupled plasma atomic emission spectroscopy, ICP-AES (Model 61E, Thermo Jarrell Ash, Woburn, MA), thermogravimetry, TG/DTA (Model 851e, Mettler-Toledo Inc., Columbus, OH) and differential scanning calorimetry, DSC (Model SDT 2960, TA Instruments, New Castle, DE) analyses, and surface area (BET) measurements (Model ASAP 2020, Micromeritics Corp., Norcross, GA).

RESULTS AND DISCUSSION

The XRD data for the freeze-dried CaP gels were given in Figure 1a, and this trace was characteristic of poorly-crystallized CaP [24, 46-52] also similar to the biological apatites [69, 70]. BET surface area of freeze-dried powders was 128±5 m^2/g. The FTIR spectra of the freeze-dried CaP gels (i.e., they were gels prior to freeze drying) were given in Figure 1b. The symmetric and anti-symmetric stretching of the PO_4^{3-} group were observed at 1020, 964, 604 and 565 cm^{-1}. Bands of CO_3^{2-} ions were observed at 1470-1420 and 874 cm^{-1}. The weak IR band at 920 cm^{-1} and the weak shoulder at around 1300 cm^{-1} were attributed to the presence of HPO_4^{2-} ions [71]. The synthesis procedure of this study was able to produce hydrated and carbonated CaP precursors, which also contained (Fig. 1b) a trace amount of protonated orthophosphate (HPO_4^{2-}) ions, similar to human fetal bones.

Chemical analyses results are given in Table 1. Freeze-dried CaP gel precursors gave the following medians: Ca: 21.27±0.02%, P: 13.27±0.01% and Na: 9.10±0.01 wt%, which corresponded to a molar Ca/P ratio of 1.239 (Table 1), and a molar (Na+Ca)/P ratio of 2.163 attained in these powders. It is not so surprising that even if one started with a mother solution with a Ca/P molar ratio of around 0.5, the precipitates formed at or near the physiological pH would still be Ca-deficient apatitic CaP. C analyses proved that the precursor powders were carbonated, and the carbonate content decreased with an increase in the calcination temperature, while the Ca/P molar ratio and the Na content remained almost the same.

Table 1 Results of ICP-AES and C analyses (in weight%, average of 3 runs)

Sample	Ca	P	Ca/P molar	Na	C	CO_3 (*calc*)
Freeze-dried	21.27	13.27	1.239	9.10	0.82	4.10
300°C	28.93	17.99	1.243	8.98	0.58	2.90
400°C	28.49	18.06	1.222	9.02	0.39	1.95
500°C	28.36	17.88	1.226	9.35	0.32	1.60
600°C	29.17	18.30	1.231	9.16	0.21	1.05
1000°C	28.64	18.04	1.227	9.09	0.01	0.05

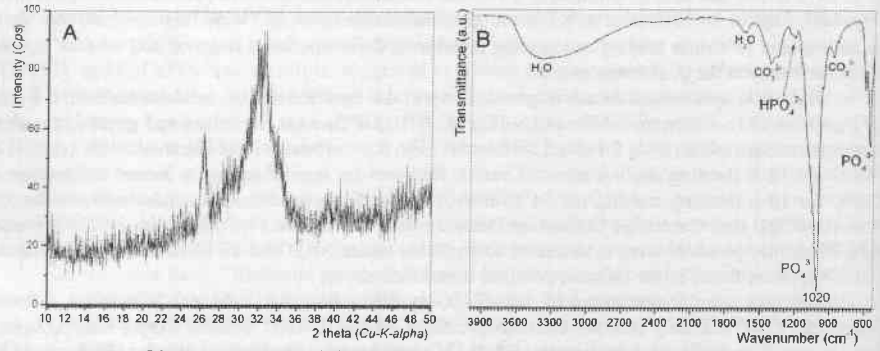

Fig. 1 (a) XRD and (b) FTIR traces of freeze-dried CaP precursors

The SEM morphology of the freeze-dried powders was shown in Figure 2a. TG/DTA/DSC analyses of the freeze-dried CaP precursors (Figure 2b) indicated that upon heating to 155°-160°C the samples first lost around 7.5% of their initial weight. This corresponded to the adsorbed water.

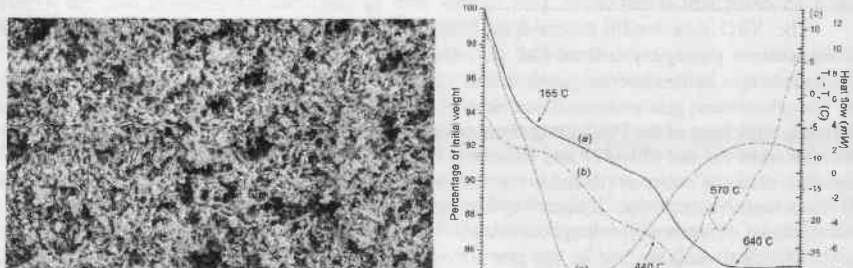

Fig. 2 (a) SEM micrograph and (b) TG-DTA-DSC traces of freeze-dried CaP precursors

Therefore, the water content of the precursor powders was deduced to be around 7 to 7.5%. With continued heating to 415°C, another gradual weight loss of about 2.5% was observed, and this was

probably due to the volatilizatio
ions were to be found at 1440-13
quite difficult to identify those
carbonate bands over the same ra
1b can be ascribed to the nitrate
removal of carbonate ions that w
the total weight loss to 15%. 640°

β-Rhenanite, i.e., β-NaCa
temperature calcination of the sa
DSC spectrum given in Fig. 2b
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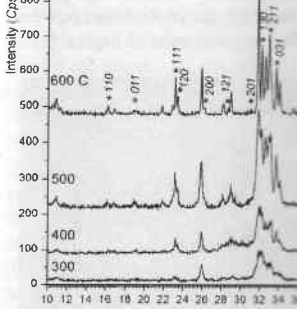

Fig. 3 (a) XR

also be written as $CaNaPO_4$) has
parameters of a=6.797, b=9.165,
$NaCaPO_4$ at 650°C) is also isostru
phase-pure $NaCaPO_4$ powders ca
molar ratio) of Na_2CO_3, $CaCO_3$ a
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then Na_2CO_3) will not be able to y
powders [75]. The peaks denoted
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1193. Upon heating at 600°C, Ca
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intense peak of hydroxyapatite (at
600°C for 6 hours can therefore be

FTIR traces of the same, c
even at the low temperature of 30
3572 cm^{-1}, and this band became

probably due to the volatilization of the remnants of nitrate ions. Characteristic IR bands for nitrate ions were to be found at 1440-1300 and 1070-1030 cm^{-1} [72], but in the IR spectra of Figure 1b it was quite difficult to identify those nitrate bands due to severe overlapping with the phosphate and carbonate bands over the same range. However, the weak bands at around 2200 to 2030 cm^{-1} in Figure 1b can be ascribed to the nitrates [73]. Further heating at above 415°C, up to 650°C, displayed the removal of carbonate ions that was accompanied with a weight decrease of around 5 wt%, bringing up the total weight loss to 15%. 640°C was the temperature when one reached constant weight (Fig. 2b).

β-Rhenanite, i.e., β-$NaCaPO_4$, phase in these gel precursors started to crystallize upon low-temperature calcination of the samples over the temperature range of 300° to 600°C. Especially, the DSC spectrum given in Fig. 2b showed that there were two exothermic events taking place over the temperature range of 440° to 570°C. The starting points of these exothermic events were indicated with arrows in Fig. 2b. It should be noted that DSC is a dynamic process taking place at a heating rate of 5°C/min, and under isothermal heatings the starting points of those exothermic events would be slightly lower than those indicated by the TG/DTA/DSC spectra. XRD spectra of Figure 3a showed the crystallization of $NaCaPO_4$ in a matrix of apatitic calcium phosphate. β-$NaCaPO_4$ (occasionally it may

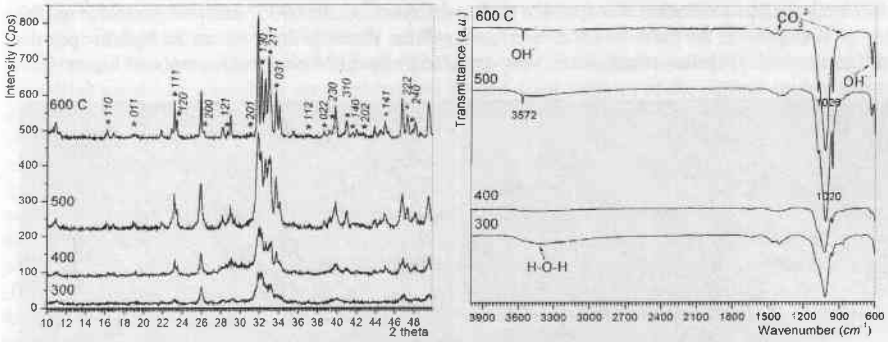

Fig. 3 (a) XRD and (b) FTIR traces of calcined CaP precursors

also be written as $CaNaPO_4$) has an orthorhombic (space group Pnam (62)) unit cell with the lattice parameters of a=6.797, b=9.165, and c=5.406 Å [74]. This phase (which will transform into α-$NaCaPO_4$ at 650°C) is also isostructural with β-K_2SO_4. The most straightforward way of synthesizing phase-pure $NaCaPO_4$ powders can be the solid-state reactive firing of the powder mixtures (in a 1:2:2 molar ratio) of Na_2CO_3, $CaCO_3$ and $(NH_4)_2HPO_4$ at 900°-950°C (see below) [74]. However, such a synthesis route (which involves the formation of liquid phases upon melting of first $(NH_4)_2HPO_4$ and then Na_2CO_3) will not be able to yield nanosize, therefore, high surface area and high surface reactivity powders [75]. The peaks denoted by * (and their respective hkl reflections) were those of β-$NaCaPO_4$, and the two-theta positions of such peaks were in close agreement with those given in ICDD PDF 29-1193. Upon heating at 600°C, CaP gel precursors of this study crystallized about 40±3% β-$NaCaPO_4$. This value was calculated from the data of Fig. 3a by using the relative intensity ratio of the most intense peak of hydroxyapatite (at 31.78° 2θ) to that of $NaCaPO_4$ (at 32.59° 2θ). The samples heated at 600°C for 6 hours can therefore be named as 40% $NaCaPO_4$-60% HA biphasic biomaterials.

FTIR traces of the same, calcined samples were depicted in Figure 3b. CaP precursors calcined even at the low temperature of 300°C were able to exhibit the characteristic OH^- stretching vibration at 3572 cm^{-1}, and this band became more pronounced with the increase in calcination temperature at or

above 500°C. The OH^- bending vibration was also recorded at 634 cm-1 [76]. These bands proved that the freeze-dried apatitic calcium phosphate phase (which originally lacked the OH vibrations) present in the gel precursors completely converted into hydroxyapatite upon calcination. Precipitated apatitic calcium phosphate precursors most probably used the humidity present in the calcination atmosphere to transform into Ca-hydroxyapatite during heating [77-81]. The relative humidity in our laboratories was at around 65-70% during those calcination runs. Characteristic FTIR spectrum of pure β-$NaCaPO_4$ was previously given by Driessens et al. [55]. The orthophosphate stretching bands for the 500°C-calcined samples were observed at 603 (ν_4), 962 (ν_1), 1020 and 1089 (ν_3) cm^{-1}, which were contributed both by crystalline β-rhenanite and apatitic calcium phosphate. Loong *et al.* [82] demonstrated the significant deficiency of OH^- ions in the Ca-deficient, nonstoichiometric apatitic crystals of rat and bovine bones by using inelastic neutron-scattering spectroscopy.

An IR band at 1020 cm^{-1} can be attributed to the ν_3 vibration of PO_4^{3-} in nonstoichiometric or Ca-deficient and/or carbonated apatitic calcium phosphates; however, a band at 1030 cm^{-1} is pinpointing to the ν_3 vibration of PO_4^{3-} in stoichiometric hydroxyapatite [83]. The relative ratios of 1020/1030 bands in the FTIR spectra could provide a measure of maturity in bone minerals or apatitic calcium phosphates [76, 84]. While the samples calcined at 300°C were displaying that ν_3 vibration at 1020 cm^{-1}, the same vibration was found to shift to 1026 cm^{-1} in the 600°C-calcined sample (Fig. 3b).

Variations in the grain size and morphology of the rhenanite-hydroxyapatite biphasic powders, with increasing calcination temperature, were depicted by the SEM photomicrographs of Figure 4.

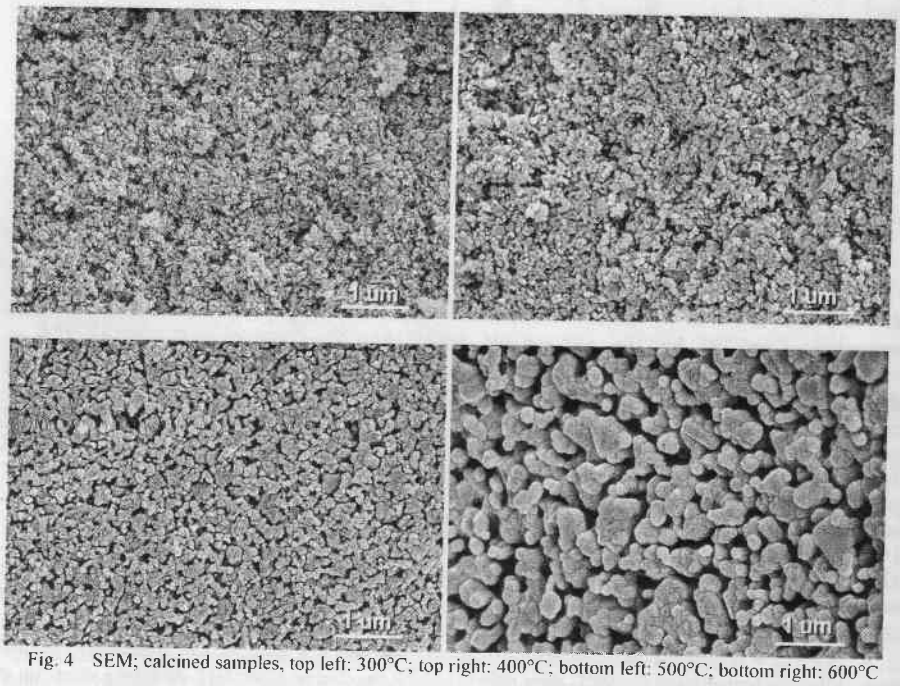

Fig. 4 SEM; calcined samples, top left: 300°C; top right: 400°C; bottom left: 500°C; bottom right: 600°C

Grain sizes directly measured from the
these powders, are given in Table 2.

Table 2 Grain sizes and surface are

Sample	Grain size (nm)	Surf
Freeze-dried	45 ± 10	
300°C	60 ± 10	
400°C	100 ± 10	
500°C	150 ± 20	
600°C	300 ± 70	

Even after light calcination at temperature
small grain sizes still in the nano- or subm
to those reported by Somrani *et al.* [51]
apatitic calcium phosphate powders proc
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were platelike in shape with dimensions 40
this study, those initially plate- or need
powders (Fig. 2a and not shown TEM dat
(Fig. 4). Such a tendency of nanosize glob
those moieties (Fig. 2a) actually being com
by Molnar [87, 88] suggested that bone cr
end-to-end relationship. An X-ray diffract
dimension of the bone apatite crystals wa
regarded as a mosaic of microcrystals rath
sodium-doped calcium phosphate gel precu
68] consisted of poorly-crystallized apatitic
those of bone mineral.

Nakahira *et al.* [90], in a study of
hydroxyapatite, reported the formation of
hydroxyapatite bioceramic samples upon
hydroxyapatite and $NaHCO_3$ (at 10% level
compaction, cold isostatic pressing and sin
those 1000°C-sintered samples by soaking
solutions from 4 to 7 days. It is quite inter
conditions, according to Nakahira *et al.* [90]
field application) were not showing any bon
samples were covered with a high abundanc
bioactivity of $NaCaPO_4$ phase than that of p
an SBF-soaking study in this manuscript, the

Grain sizes directly measured from the SEM micrographs, as well as the respective surface areas of these powders, are given in Table 2.

Table 2 Grain sizes and surface areas of powders

Sample	Grain size (nm)	Surface area (m^2/g)
Freeze-dried	45 ± 10	128 ± 5
300°C	60 ± 10	79 ± 4
400°C	100 ± 10	70 ± 5
500°C	150 ± 20	53 ± 3
600°C	300 ± 70	34 ± 3

Even after light calcination at temperatures from 300° to 600°C, these materials retained their initially small grain sizes still in the nano- or submicron-range. These surface area data were quite comparable to those reported by Somrani *et al.* [51] in a study on the thermal evolution of poorly-crystalline apatitic calcium phosphate powders produced by using Ca-nitrate tetrahydrate and di-ammonium hydrogen phosphate as the starting water soluble reagents, in the absence of any Na ions in their precipitation solutions. Apatitic calcium phosphate samples of Somrani *et al.* [51] decomposed into crystalline tricalcium phosphate upon calcination. Freeze-dried samples of the current study consisted of (as shown in the micrographs of Figs. 1 and 2) particles (or moieties) having a needlelike morphology with average dimensions of 10 (thickness) and 70 (length) nanometer. These are very well within the size range of bone apatite crystals, which were documented by using electron microscopy for more than 5 decades ago [27, 85]. Johansen and Parks [86] reported that bone apatite crystallites were platelike in shape with dimensions 400 x 200-350 x 25-50 Å. Upon calcination of the samples of this study, those initially plate- or needle-like, longitudinal moeities present in the freeze-dried powders (Fig. 2a and not shown TEM data) tended to form more or less equiaxed or globular grains (Fig. 4). Such a tendency of nanosize globule formation upon heating can also be taken as a sign of those moieties (Fig. 2a) actually being comprised of very much smaller particles. Indeed, early studies by Molnar [87, 88] suggested that bone crystals are composed of chains of microcrystals fused in an end-to-end relationship. An X-ray diffraction study by Posner *et al.* [89] reported that the largest dimension of the bone apatite crystals was about 100 Å, and those apatitic crystallites should be regarded as a mosaic of microcrystals rather than as a continuously uniform, single crystal [31]. The sodium-doped calcium phosphate gel precursors of this study [enthused by the work of Refs. 24, 48, 68] consisted of poorly-crystallized apatitic microcrystals very similar in dimensions and appearance to those of bone mineral.

Nakahira *et al.* [90], in a study of testing the applied magnetic field on the bioactivity of hydroxyapatite, reported the formation of $NaCaPO_4$ as a second phase in 10% $NaHCO_3$-mixed hydroxyapatite bioceramic samples upon sintering those at 1000°C. These authors blended the hydroxyapatite and $NaHCO_3$ (at 10% level) powders by using a conventional ball-mill, followed by compaction, cold isostatic pressing and sintering. Nakahira *et al.* [90] also tested the bioactivity of those 1000°C-sintered samples by soaking them, at 37°C, in SBF (synthetic body fluid [91, 92]) solutions from 4 to 7 days. It is quite interesting to note here that, under the identical SBF soaking conditions, according to Nakahira *et al.* [90], while the pure hydroxyapatite samples (with no magnetic field application) were not showing any bonelike CaP deposits on their surfaces, $NaCaPO_4$-containing samples were covered with a high abundance of such deposits. This was again attributed to the higher bioactivity of $NaCaPO_4$ phase than that of pure hydroxyapatite [90, 93]. Although we did not include an SBF-soaking study in this manuscript, the strong evidence brought upon by the work of Nakahira *et*

al. [90] was considered to be sufficient to ascertain the apatite-inducing ability (in SBF solutions) of such $NaCaPO_4$-containing hydroxyapatite bioceramics. Moreover, the presence of Na ions that weaken the bond between Ca^{2+} and PO_4^{3-} in the crystal surface accounts for the high dissolution rate of β-$NaCaPO_4$. If the surface of a bioceramic sample inserted in an SBF solution exhibits such a significant ionic level dissolution phenomenon, then the Ca^{2+} and HPO_4^{2-} ions to be abundant on these surfaces will further trigger the aggregation, and the consequent surface segregation, of Posner's clusters found in those solutions [94]. Many times, especially for calcium phosphates implanted into non-bony sites (such as, muscles), ectopic formation of biological apatite crystals (as explained above) in the vicinity of those implanted calcium phosphates were incorrectly interpreted as osteoinductivity-caused by the implant itself.

β-$NaCaPO_4$ phase was recently reported by El-Ghannam [61] to form upon the calcination (180° to 800°C) of a new class of SiO_2-$CaHPO_4{\cdot}2H_2O$ physically-mixed powder blends initially wetted by rather concentrated NaOH solutions. In vivo studies performed by El-Ghannam [61] found that these materials were superior to Bioglass® in terms of protein absorption, enhancement of bone generation, and overall resorption. Gong *et al.* [60] reported that crystalline β-rhenanite in contact with SBF solutions may act as a nucleation precursor for the formation of apatitic calcium phosphates with respect to the following reaction:

$$3NaCaPO_4\,(aq) + 2Ca^{2+}\,(aq) + H_2O\,(aq) \rightarrow Ca_5(PO_4)_3OH\,(aq) + 3Na^{+}\,(aq) + H^{+}\,(aq) \qquad (1).$$

The solid-state reactive firing (SSRF) process we used [74] in this study to produce $NaCaPO_4$ powders was quite robust and reliable for synthesizing large quantities of this substance. Figures 5a and 5b respectively showed the XRD and FTIR traces of $NaCaPO_4$ produced.

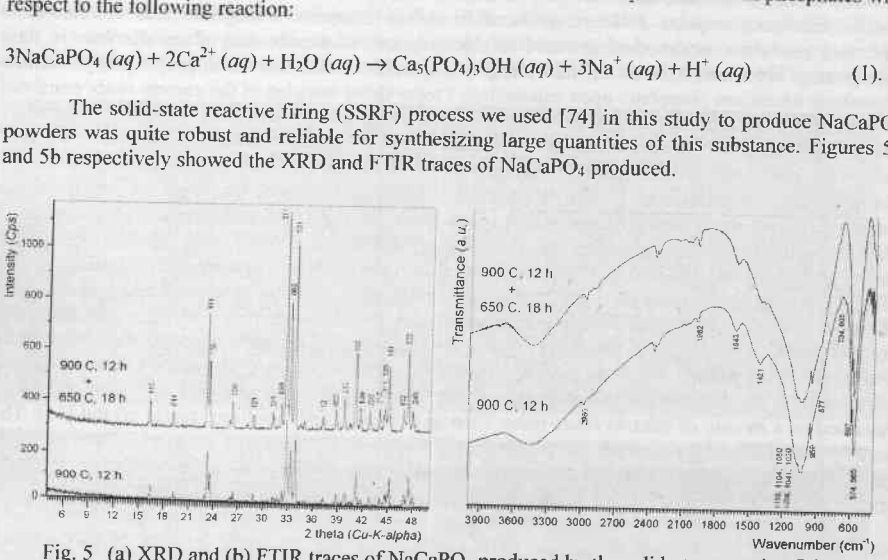

Fig. 5 (a) XRD and (b) FTIR traces of $NaCaPO_4$ produced by the solid-state reactive firing

Kangasniemi et al. [95] prepared β-rhenanite powders by sintering stoichiometric mixtures of $CaHPO_4$ and Na_2CO_3 at 1300°C, followed by sieving the ground sintered chunks to a size below 45 μm, and used those later as crystalline additives (from 20 to 30 wt%) in their experimental bioactive glass compositions. The same authors were then reported in a separate study [96] the dissolution behavior of crystalline β-rhenanite- or crystalline HA-containing bioactive glasses soaked in SBF from 5 hours to 6 days. Kangasniemi *et al.* [96] concluded that the β-rhenanite-containing composites had a very positive effect on the rate of apatitic CaP formation on the surfaces of samples soaked in SBF.

The earlier but quite comprehensive work of Ramselaar *et al.* [54-56] should be taken as a good reference for the strong potential of β-rhenanite in developing resorbable or so-called osteoinductive calcium phosphate bioceramics. The in vivo canine studies performed by Ramselaar *et al.* [56] demonstrated that statistically more bone deposition occurred on β-rhenanite particles than on hydroxyapatite particles.

This study showed that by simple calcination of a poorly-crystallized, Na-containing calcium phosphate gel precursor synthesized at the physiological pH it will be possible to form biphasic biomaterials consisting of a high solubility β-$NaCaPO_4$ and less soluble nanosize hydroxyapatite. Since the starting material is a gel precursor, it can be easily shaped (for instance, by extrusion, injection molding or solid freeform fabrication techniques) into any desired three-dimensional form before the full crystallization of the phases to take place during the final calcination step. The initial viscosity of such gels can be readily adjusted prior to the form fabrication. We have also observed that these gels can even be stored in ordinary zip-locked, air-tight polyetyhlene bags for more than a year (under refrigeration at 4°C), without resulting in any detectable changes in their XRD and FTIR patterns. Moreover, leachable porogen phases or particulates (such as, NaCl, ammonium carbonate, ammonium acetate, ice crystals, etc.) may also be incorporated into these gels to form porous bodies at the end of the fabrication processes. The only delicate step in the use of such preformed gels for forming 3D shapes would be the careful drying in a relative humidity-controlled environment that should avoid the formation of drying cracks due to the rapid removal of entrapped water.

The osteoinductive character reported [97-100] for the biphasic β-TCP (40%) and HA (60%) biomaterials may also be expected for the β-rhenanite-HA materials of this study. Finally, to validate the above speculation and the clinical usefulness of the β-rhenanite + HA biphasic biomaterials of this work in vivo studies must be performed, which we plan to report in a follow-up study.

The highly soluble component (i.e., $NaCaPO_4$) of these new biphasic mixtures, under the in vivo action of osteoclasts, is assumed to supply Ca^{2+} ions, as well as hydrogenated phosphate ions, to the surrounding tissues upon implantation. Such materials can, therefore, be expected to act like an osteoinductive stimulant in the body.

For the very interested reader, we should mention that the compound $NaCaPO_4$ was found in nature only once in a meteorite, specifically named as the Cape York iron meteorite, by Dr. Vagn Buchwald of Denmark. The tiny $NaCaPO_4$ crystals in that meteorite were later investigated by E. Olsen *et al.* [101], too. Correspondingly, the crystals of that mineral were named as "Buchwaldite" in honor of Dr. Buchwald, just to separate those natural crystals from the quite similar synthetic crystals obtained by using the fertilizer industry's well-known Rhenania process. The name rhenanite, on the other hand, was coined to $NaCaPO_4$, for the first time, by Spencer [102].

CONCLUSIONS

Sodium-doped calcium phosphate precursors were produced at room temperature by using a robust aqueous synthesis procedure involving the use of Na_2HPO_4, $NaHCO_3$, and $Ca(NO_3)_2{\cdot}4H_2O$. The precursors formed at the physiological pH of 7.4 were in the form of a gel. Upon freeze-drying, these precursor gels were found to consist of poorly-crystallized, nanosize apatitic calcium phosphates with a surface area in excess of 125 m^2/g. Calcination of these samples in a static air atmosphere over the temperature range of 400° to 600°C for 6 hours led to the production of β-rhenanite ($NaCaPO_4$) and hydroxyapatite biphasic biomaterials for the first time. Calcined powder samples had surface areas over the range 30 to 80 m^2/g, and consisted of nanosize grains.

ACKNOWLEDGEMENTS

Author was a Research Associate Professor at Clemson University (South Carolina, USA) from May 2003 to April 2006, and gratefully acknowledges the hands-on participation of the high school student Rosie M. Knotts, as a laboratory assistant for two weeks, at the start of this research project in June 2004 at Clemson University.

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NANOMATERIALS AS IMPROVE

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ABSTRACT

The response of host organisr different than that observed to co possess constituents (such as grains direction. This review will cover the on orthopedic) highlighting their pr vivo studies have highlighted th inflammation, inhibit infection, and Such reviewed studies will empha subsequently cells. In this manner, relationships unique for nano-sized m unique surface energetics to optimal forming cell) adhesion and subseque through the control of nanomaterials growth necessary for the next generati

INTRODUCTION

Nano-scale Materials

Nano-scale materials, also call very small components and/or structu one dimension in the range of 1-1(polymers, or composite materials w materials due to their nano-scale feat material assembly as their bulk and s not govern traditional bulk material be

Over the past two decades, na government, private enterprises and ac has resulted in the identification of magnetic, catalytic, optical, electrical formulations of the same material [1] increased interest in exploring numero

It has been shown that the rem with the unique properties of nanomat such as biosensors, sensitive diagn significantly improved performances. categories according to their geomet dimensional (or lamellar) forms. Select their use in biomedical applications are

Advances in Bioceramics and Porous Ceramics

Ceramic Engineering and Science Proceedings
Volume 29, Issue 7, 2008

Edited by
Roger Narayan
Paolo Colombo

Volume Editors
Tatsuki Ohji
Andrew Wereszczak

WILEY

ISBN 978-0-470-34494-1