SYNTHESIS OF RHEANITE (β-NaCaPO₄)-APATITIC CALCIUM PHOSPHATE BIPHASIC FOR SKELETAL REPAIR

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ABSTRACT

Biphase rhenanite (β-NaCaPO₄)-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°C to 600°C) calcination in air for 6 hours. Calcination was for the sole purpose of crystallization. An aqueous solution of Ca(NO₃)₂·4H₂O was rapidly added to a solution of Na₂HPO₄ and NaHCO₃ at room temperature, followed by immediate filtration of gel-like, poorly-crystallized precursor precipitates from the mother liquor of pH 7.4. Freeze-dried precursors were found to be nanosized with an average particle size of 45 nm and surface area of ca. 125 m²/g. Upon calcination in air, precursor powders crystallized into biphase (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 resulting from tumor resection, trauma or infection [1-3]. Synthetic calcium phosphates, such as calcium hydroxyapatite [HA; Ca₁₀(PO₄)₆(OH)₂], β-tricalcium phosphate [β-TCP; Ca₃(PO₄)₂] and biphase mixtures of these two have found use as bone substitutes [4-9]. 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]. Kilan et al. [13] showed that nonsintered HA could even be phagocytosed 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 osteointegration), 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-HSM™, Exx 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 nanoclayes [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 extracellular 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\(_{10}\)(PO\(_4\))\(_6\) [35] 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 hydroxyapatite have [35]. The work of Eanes et al. [35-42] and Rey et al. [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\(_2\)O at pH 7 [53]) of NaCaPO\(_4\) samples in a composite setting 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\(_2\)CO\(_3\) and SiO\(_2\) whereas the molar ratio of Na\(_2\)CO\(_3\)/P\(_2\)O\(_5\) 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 15 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 (>1000°C) processes [66].

The motivation for the present study stems from our interest in developing a robust synthesis route for the manufacture of biphasic nanophosphates of NaCaPO\(_4\) and carbonated, apatic calcium phosphate using temperatures less than 700°C [66]. Apatic 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 apatic 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 apatic 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 apatic calcium phosphate upon low-temperature (300°-600°C) calcination.

### EXPERIMENTAL PROCEDURE

Rhenanite-apatic C\(_{4}\)P biphasics: The Na-containing poorly-crystallized apatic 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 NaHPO₄ (~99%, Fisher Scientific, Fair Lawn, NJ) was dissolved in 1.2 L of deionized water, followed by the addition of 69.0 g NaClO₃ (~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(NO₃)₂·4H₂O (~99%, Fisher) in 500 mL of 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 CaP 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 Buchner 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 x 10⁻² 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°C to 600°C, with 6 h of soak time at the peak temperatures.

NaCaPO₄ synthesis: Rhenane powders were also synthesized by solid-state reactive firing. 2.12 g of Na₂CO₃, 4.00 g of CaCO₃ and 5.28 g of (NH₄)₂HPO₄ were dried 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 Rhenane powders were synthesized to facilitate better XRD and FTIR characterization of the NaCaPO₄ 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 618, Thermo Jarrell Ash, Woburn, MA), thermogravimetry, TG/DTA (Model 851e, Mettler-Toledo Inc., Columbus, OH) and differential scanning calorimetry, DSC (Model DTA 2960, 1A 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 m²/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 antisymmetric stretching of the P-O₅ group were observed at 1039, 964, 604 and 565 cm⁻¹. Bands of CO₃²⁻ 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 HPO₄²⁻ ions [71]. The synthesis procedure of this study was thus designed to produce hydrated and carbonated CaP precursors, which also contained (Fig. 1b) a trace amount of protonated orthophosphate (HPO₄²⁻) ions, similar to human fetal bones.

Chemical analyses results are given in Table 1. Freeze-dried CaP gel precursors gave the following median: Ca: 21.2±4.02%, P: 13.2±3.011%, and Na: 9.1±3.01 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 CaP molar ratio of around 0.5, the precipitates formed at or near the physiological pH would still be Ca-deficient apatite CaP. Chemical analyses proved that the CaP powders were carbonated, and the carbonate content decreased with an increase in the calcination temperature, while the CaP 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ca</th>
<th>P</th>
<th>Ca/P molar</th>
<th>Na</th>
<th>C</th>
<th>CO₃ (calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried</td>
<td>21.27</td>
<td>13.27</td>
<td>1.239</td>
<td>9.10</td>
<td>0.82</td>
<td>4.10</td>
</tr>
<tr>
<td>300°C</td>
<td>28.93</td>
<td>17.99</td>
<td>1.243</td>
<td>8.98</td>
<td>0.58</td>
<td>2.90</td>
</tr>
<tr>
<td>400°C</td>
<td>28.49</td>
<td>18.06</td>
<td>1.222</td>
<td>9.02</td>
<td>0.39</td>
<td>1.95</td>
</tr>
<tr>
<td>500°C</td>
<td>28.36</td>
<td>17.88</td>
<td>1.226</td>
<td>9.35</td>
<td>0.32</td>
<td>1.60</td>
</tr>
<tr>
<td>600°C</td>
<td>29.17</td>
<td>18.30</td>
<td>1.231</td>
<td>9.16</td>
<td>0.21</td>
<td>1.05</td>
</tr>
<tr>
<td>1000°C</td>
<td>28.64</td>
<td>18.04</td>
<td>1.227</td>
<td>9.09</td>
<td>0.01</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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°C-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
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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°C 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°C 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

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 isomorphic 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\(_2\)CO\(_3\), CaCO\(_3\), and (NH\(_4\))\(_2\)HPO\(_4\) at 900°-950°C (see below) [74]. However, such a synthesis route (which involves the formation of liquid phases upon melting of first (NH\(_4\))\(_2\)HPO\(_4\) and then Na2CO\(_3\)) 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° 20) to that of NaCaPO\(_4\) (at 32.59° 20). 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
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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 need 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-NaCaPO₄] was previously given by Driesens et al. [55]. The orthophosphate stretching bands for the 500°C-calcined samples were observed at 603 (v₄), 962 (v₅), 1020 and 1089 (v₇) cm⁻¹, which were contributed both by crystalline β-rhenanite and apatitic calcium phosphate. Leong 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 spectrometry.

An IR band at 1020 cm⁻¹ can be attributed to the v₁ vibration of PO₄³⁻ in nonstoichiometric or Ca-deficient and/or carbonated apatitic calcium phosphates; however, a band at 1030 cm⁻¹ is pinpointing to the v₁ vibration of PO₄³⁻ in stoichiometric hydroxyapatite [83]. The relative ratios of 1020/1010 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 v₁ 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.

Table 2 Grain sizes and surface area are

<table>
<thead>
<tr>
<th>Sample</th>
<th>Grain size (nm)</th>
<th>Surf.</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried</td>
<td>45 ± 10</td>
<td>60 ± 10</td>
<td></td>
</tr>
<tr>
<td>300°C</td>
<td>60 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400°C</td>
<td>100 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500°C</td>
<td>150 ± 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600°C</td>
<td>300 ± 70</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Even after light calcination at temperatures, small grain sizes still in the nano- or sub- micrometer range were reported by Somrani et al. [51]. Apatitic calcium phosphate powders produced by reacting sodium hydrogen phosphate as the starting water precipitation solutions. Apatitic calcium phosphate can crystallize from tricalcium phosphate upon calcination (as shown in the micrographs of Fig. 2). The morphology with average dimensions of 100 nm within the size range of bone apatite crystals was observed for more than 5 decades ago [27, 85]. Johnstone et al. [86] reported that these powders (Fig. 2a and not shown TEM data of Fig. 4) such a tendency of nanosize globular particles. In this study, those initially plate-like or needleshaped crystals were platelike in shape with dimensions 40-500 nm and those moieties (Fig. 2a) actually being comprised of smaller plate-like crystals, as suggested by Molnar [87, 88] suggested that bone crystals have a true end-to-end relationship. An X-ray diffraction analysis of the bone apatite crystals within this range was suggested as a mosaic of microcrystals with a dimension of 10-60 nm, sodium-doped calcium phosphate gel precipitated by Nakahira et al. [90], in a study of hydroxyapatite, reported the formation of hydroxyapatite bioceramic samples upon treatment of hydroxyapatite and NaHCO₃ (at 10% level) by compaction, cold isostatic pressing and sintering those 1000°C-sintered samples by soaking them in solutions from 4 to 7 days. It is quite interesting to note how these conditions, according to Nakahira et al. [90] (in vitro and in vivo field application) were not showing any bone bonding. Even sodium-doped calcium phosphate gel precipitated hydroxyapatite powders were covered with a high abundance of bioactivity of NaCapO₄ phase than that of pure hydroxyapatite powders in an SBF-soaking study in this manuscript.
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Grain sizes directly measured from the SEM micrographs, as well as the respective surface areas of these powders, are given in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Grain size (nm)</th>
<th>Surface area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried</td>
<td>45 ± 10</td>
<td>128 ± 5</td>
</tr>
<tr>
<td>300°C</td>
<td>60 ± 10</td>
<td>79 ± 4</td>
</tr>
<tr>
<td>400°C</td>
<td>100 ± 10</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>500°C</td>
<td>150 ± 20</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>600°C</td>
<td>300 ± 70</td>
<td>34 ± 3</td>
</tr>
</tbody>
</table>

Even after light calcination at temperatures from 300°C 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 moieties 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 NaCaPO₄ as a second phase in 10% NaHCO₃-mixed hydroxyapatite bioceramic samples upon sintering those at 1000°C. These authors blended the hydroxyapatite and NaHCO₃ (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 bone-like CaP deposits on their surfaces, NaCaPO₄-containing samples were covered with a high abundance of such deposits. This was again attributed to the higher bioactivity of NaCaPO₄ 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
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The earlier but quite complex reference for the strong potential of calcium phosphate bioceramics was demonstrated that statistically hydroxyapatite particles might readily form when their corresponding bioactive systems were prepared. This study showed that calcium phosphate gel precursor systems in biomaterials consisting of a hydroxyapatite. Since the starting material is injection molding or solid free forming before the full crystallization of the gels can even be stored in ice-cold patterns. Moreover, leachable ammonium acetate, ice crystals, and ammonium acetate, ice crystals, serve as the end of the fabrication process. The resulting gel forming 3D shapes would be in a softer gel stage. The osteoinductive character of the above speculation and the clinical work in vivo studies must be performed to confirm the efficacy of the osteoinductive actions of in vivo osteoclasts, is associated with the surrounding tissues upon injection of an osteoinductive stimulant in the bone or cartilage matrix nature only once in a meteorite found in honor of Dr. Buchwald of Denmark. The time traveled by Olsen et al. [101], too. Correspondingly, the highly soluble component of the fertilizer is, obtained by using the fertilizer to nitrogenate only once in a meteorite found in honor of Dr. Buchwald, just to a solution that should avoid the formation of dense hard tissue. For the very interested reader, a nature only once in a meteorite found in honor of Dr. Buchwald, just to a solution that should avoid the formation of dense hard tissue. For the very interested reader, a nature only once in a meteorite found in honor of Dr. Buchwald, just to a solution that should avoid the formation of dense hard tissue.

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CONCLUSIONS

Sodium-doped calcium phosphate powders were prepared by sintering stoichiometric mixtures of CaHPO₄ and Na₂CO₃ 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-Rhenonite- or crystalline HA-containing bioactive glasses soaked in SBF for 5 hours to 6 days. Kangasniemi et al. [96] concluded that the β-Rhenonite-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 Ramseier 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 Ramseier 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-NaCaPO₄ 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 polyethylene 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., NaCaPO₄) of these new biphasic mixtures, under the in vivo action of osteoclasts, is assumed to supply Ca²⁺ 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 NaCaPO₄ 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 NaCaPO₄ 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 NaCaPO₄, 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 Na₂HPO₄, NaHCO₃, and Ca(NO₃)₂·4H₂O. 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 m²/g. Calcination of these samples in a static air atmosphere over the temperature range of 400°C to 600°C for 6 hours led to the production of β-rhenanite (NaCaPO₄) and hydroxyapatite biphasic biomaterials for the first time. Calcined powder samples had surface areas over the range 30 to 80 m²/g, and consisted of nanosize grains.
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References

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Synthesis of Rhenanite Apatitic Calcium Phosphate Biphasics for Skeletal Repair


[65] Calcigen™-NaPO4 Bone Void Filler. Biomet, Inc. Warsaw, IN, USA


NANOMATERIALS AS IMPROVED THERAPY DEVICES
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ABSTRACT

The response of host organisms to nanomaterials is drastically different than that observed to conventional materials (such as glasses). This review will cover the potential of these materials in orthopedic treatments highlighting their promise in bone repair and regeneration. In vivo studies have highlighted their potential to repair bone and reduce inflammation, inhibit infection, and promote bone growth. Such reviewed studies will emphasize the importance of understanding the surface properties of these materials through the control of nanomaterials' surface chemistry and their use in biomedical applications.

INTRODUCTION

Nano-scale Materials

Nano-scale materials, also called nanomaterials, are very small components and/or structures (less than 100 nm in one dimension in the range of 1-100 nm), or composite materials with such components as grains or single crystals. These materials due to their nano-scale features have attracted much attention recently, with their unique surface energetics to optimal cell adhesion and subsequent cell growth necessary for the next generation of biomaterials.

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