A New Rhenanite (β-NaCaPO₄) and Hydroxyapatite Biphasic Biomaterial for Skeletal Repair

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Abstract: Biphasic β-rhenanite (β-NaCaPO₄)–hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) biomaterials were prepared by using a one-pot, solution-based synthesis procedure at the physiological pH of 7.4, followed by low-temperature (300–600°C) calcination in air for 6 h. 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, followed by immediate removal 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 a surface area of 128 m²/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. β-rhenanite is a high solubility sodium calcium phosphate phase. Samples were characterized by XRD, FTIR, SEM, TEM, ICP-AES, TG, DTA, DSC, and surface area measurements. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 80B: 304–316, 2007

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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. Synthetic calcium phosphates, such as calcium hydroxyapatite [HA; Ca₁₀(PO₄)₆(OH)₂], β-tricalcium phosphate [β-TCP; β-Ca₃(PO₄)₂] and biphasic mixtures of these two have found use as bone substitutes. HA or β-TCP implants exhibit relatively good tissue compatibility, and new bone is formed directly on the implants with no fibrous encapsulation. However, sintered and well-crystallized HA ceramics usually demonstrated minimal in vivo resorption, with resorption times lagging the new bone formation rates. Kilian et al. 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 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). 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, then it is continuously resorbing (in vivo osseointegration), this could be its most affirmative contribution to the defect site. Therefore, efforts in the direction of developing calcium phosphate-based bone substitutes of higher in vivo resorbability and osteoinductive/osteoconductive capabilities are still needed.

In stark contrast to sintered HA ceramics, calcium phosphate (CaP) self-setting cement formulations, which intentionally employed poorly-crystallized apatite as their major powder component, were shown 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, Cambridge, MA). These cements rapidly took part in the bone remodeling processes by going through phagocytosis under the action of macrophages and osteoclasts. Besides these special orthopedic cements, such high resorption rates with calcium phosphates have only been encountered when the tested (in vivo) materials comprised nanoapatites.

Bone is a connective tissue with extracellular substance consisting of a carbonated, apatitic calcium phosphate nano-size mineral dispersed in what is essentially a hydrated collagen matrix. The mineral portion comprises two intimately mixed calcium phosphate phases; (i) noncrystalline or amorphous calcium phosphate and (ii) a poorly-crystallized apatitic calcium phosphate. In the formation of bone mineral, the amorphous phase is laid down first by the bone-depositing cells, i.e., osteoblasts, and subsequently a significant portion

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of this is then converted in the physiological environment to the carbonated, Na-, K-, and Mg-doped, Ca-deficient bioapatite during the in vivo maturation of bones. The presence of the noncrystalline calcium phosphate phase in bones has been detected even by the very first electron microscope studies. The earlier work of Posner and coworkers 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$_4$(PO$_4$)$_3$OH 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. The work of Eanes and coworkers and Rey and coworkers were the first on the preparation of poorly-crystallized calcium phosphates should also be cited 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. and NaCaPO$_4$ was, therefore, suggested to possess a higher potency to enhance osteogenesis than β-TCP. Ramselaar and coworkers 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. noted the remarkably high solubility (1.0 g per liter of H$_2$O at pH 7.54) 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. 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.

On the other hand, “Rhenania process” is a well-known procedure mostly used in the fertilizer industry to obtain a soluble phosphate material. In this process, the natural mineral of hydroxyapatite is 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$_2$ is added to prevent the occurrence of free CaO in the sintered product. These powder mixtures are then ground together and calcined in a rotary kiln at about 1000–1200°C for about few hours. Rhenanite, NaCaPO$_4$, of high solubility, has been the major phase in the final product of the Rhenania process. As could be expected, NaCaPO$_4$ received such a high-temperature treatment has a rather low surface area, low surface reactivity, and larger powder particles.

Resorbable, granular bone graft substitutes based on NaCaPO$_4$ formulations have already been commercialized and marketed for the orthopedic surgeons. Setting cements based on NaCaPO$_4$ are also available for the repair of bone defects. Nevertheless, the powders of such products have been produced by high-temperature (>1100°C) processes.

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. Specifically, the apatitic calcium phosphate powders are known to lose their carbonate ions when and if a temperature higher than 700°C was used. The soluble component of these biphasic mixtures (i.e., NaCaPO$_4$), 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.

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, and if those powders were synthesized at the physiological pH in the presence of a rather significant amount of Na$^+$ ions, then upon calcining those powders over the temperature range of 300–600°C, the resultant material should be a biphasic powder mixture of NaCaPO$_4$ and apatitic calcium phosphate with a high surface area.” This work, to the best of the knowledge of authors, reports for the first time the aqueous preparation of nanosize calcium phosphate precursor powders, which are able to readily transform into biphasic powder mixtures of β-NaCaPO$_4$ and carbonated hydroxyapatite upon low-temperature calcination.

**MATERIALS AND METHODS**

**Synthesis of Calcium Phosphate Powder Samples**

The synthesis method used to form the Na-containing poorly-crystallized apatitic calcium phosphate powders simply consisted of preparing two solutions, and the procedure adapted here was inspired by the work of Lee et al. Solution-A was prepared as follows: 86.4 g Na$_2$HPO$_4$ (disodium hydrogen phosphate, >99%, Fisher Scientific, Fairlawn, NJ) was dissolved in 1.2 L of deionized water. Solution-B was then rapidly added into solution-A and stirred vigorously (at 250 rpm, with a 5 cm long, Teflon-coated magnetic fish) at room temperature. 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 3–5 mL of concentrated NaOH solution. The solids were immediately filtered by using a filter paper (No. 42, Whatman International, Maidstone, UK) placed in a vacuum-suction porcelain Buchner funnel assembly, and washed with 4 L 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 room temperature. 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 3–5 mL of concentrated NaOH solution. The solids were immediately filtered by using a filter paper (No. 42, Whatman International, Maidstone, UK) placed in a vacuum-suction porcelain Buchner funnel assembly, and washed with 4 L of deionized water. Upon filtering and washing the precipitates in the funnel one obtains a gel-like calcium phosphate precipitate body (i.e., CaP gel). Some portion of the recovered gels was placed in a glass Petri dish and dried at 37°C for 72 h in static air in a drying oven, while the remainder of the sample was first frozen at −80°C for 2 h, and then lyophilized in a vacuum chamber (Freezone® 4.5, Labconco, Kansas City, MO) kept heated at 100°C for 3 h.
at $5 \times 10^{-2}$ mbar at RT overnight. The above-mentioned synthesis recipe has been repeated six times, and the reproducibility in the composition of precursor powders was checked by consecutive triplicate chemical analyses.

To test the influence of solution pH on the phase composition of the resultant precipitates, in a number of experiments the pH of Solution-A was first decreased to 4.2 at room temperature, under vigorous stirring, by slowly adding aliquots of concentrated HNO$_3$ (69.2%, Fisher Scientific, Fair Lawn, NJ). The pH value of Solution-B was also reduced to 4.2 by adding HNO$_3$, followed by adding Solution-B into Solution-A rapidly, as described above. The precipitates were then immediately filtered out of the mother liquor in a fashion similar to that mentioned above.

CaP powders obtained at pH 7.4 placed in clean aluminum oxide crucibles or boats were calcined in a static air atmosphere in an electrically-heated muffle furnace (5°C/min heating and cooling rates) over the temperature range of 300–600°C, with 6 h of soak time at the peak temperatures. Calcined samples were lightly ground (only for few minutes) in an agate mortar by using an agate pestle. To test the sinterability of these rhenate–HA biphasic samples, we have uniaxially pressed (5000 kg/cm$^2$) eight pellets (each 1.5 mm thick) out of the 600°C-calcined biphasic powders. These pellets were then separately heated in an electrical resistance, chamber furnace to 1000°C at the rate of 5°C/min, held at that temperature for 6 h, and then cooled back to room temperature within the furnace either at the rate of 5°C/min or 1°C/min.

### Powder Characterization

Samples were characterized, at all stages, by powder X-ray diffraction (XRD Model XDS 2000, Scintag, Sunnyvale, CA), scanning electron microscopy (SEM, Model S-4700, Hitachi, Tokyo, Japan), transmission electron microscopy (TEM, Model HD200, Hitachi), 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, Columbus, OH) and differential scanning calorimetry (DSC, Model SDT 2960, TA Instruments, New Castle, DE) analyses, and surface area measurements (Model ASAP 2020, Micromeritics, Norcross, GA).

Powder samples for XRD analyses were first ground in an agate mortar using an agate pestle and then sprinkled onto ethanol-damped single crystal quartz sample holders as a thin and flat layer, followed by tapping to remove the excess of powder. The X-ray diffractometer was operated at 40 kV and 30 mA with monochromated Cu Kα radiation. XRD data were collected in the step mode over the 2θ range of 3°–50°, with a scanning speed corresponding to 0.01° per minute. FTIR samples were first ground in a mortar, in a manner similar to that used in the preparation of XRD samples, and then placed onto the diamond ATR holder of the FTIR spectrometer. This spectrometer was equipped with an Endurance Foundation Series single-bounce diamond ATR (50° incidence angle), and 32 scans were performed at a resolution of 4 cm$^{-1}$. Powder samples examined with the scanning electron microscope (SEM) were sputter-coated with a thin Pt layer, just prior to imaging, to improve the conductivity of samples. For the transmission electron microscope (TEM) investigations, small aliquots of respective powder samples were first dispersed in pure ethanol, and then few drops of those suspensions were dried on the sample holder grids. The BET surface area of powder samples was determined by applying the standard Brunauer–Emmet–Teller method to the nitrogen adsorption isotherm obtained at $-196^\circ$C using the Micromeritics ASAP 2020 instrument. Powder samples used in the ICP-AES analyses were first dissolved in nitric acid prior to measurements. For carbon analyses, 200 mg of powder samples was combusted at around 1200°C in oxygen atmosphere by using a C analyzer (Model Vario Macro, Elementar, Mt. Laurel, NJ). In this process, carbonaceous moieties were converted into CO$_2$, and after catalytic postoxidation, drying, and cleaning of carrier gas, all carbon oxides are reduced to molecular carbon over a reducing agent and transported by the carrier gas to the thermoconductivity cell for the final quantitative determination of carbon content. Carbon content was then reported by the computer of the instrument as wt % C present in the samples. We converted the measured C contents into CO$_2$ percentages. Thermogravimetric analyses (TGA) of the CaP gel (or powder) samples were performed in Pt crucibles in a static air atmosphere with a heating rate of 5°C/min from RT to 800°C. Water content of the precursor powders were directly deduced from the TGA traces. DTA and DSC runs were also performed in Pt crucibles in flowing air atmosphere with a heating rate of 5°C/min, and Al$_2$O$_3$ powder was used as the reference sample in these experiments.

### RESULTS AND DISCUSSION

The synthesis procedure described in *Synthesis of Calcium Phosphate Powder Samples* was able to produce apatitic calcium phosphate powders when the synthesis solution pH was maintained at 7.4. The results and data given below are all for pH 7.4 samples, unless otherwise noted. XRD data for 37°C- and freeze-dried CaP gels were given in the comparative chart of Figure 1(a). Both traces were characteristic of poorly-crystallized CaP powders, although drying at 37°C seemed to cause a slight increase in the overall crystallinity with respect to freeze drying. In freeze drying, the water initially present was gradually sublimed, whereas in 37°C drying that water present at the end of the washing step helped the crystallization to proceed at a low rate. Freeze-dried powders were found to be fluffier with respect to those dried at 37°C, therefore, after the preliminary experiments all of the powders obtained by aqueous synthesis procedure were freeze-dried. Surface area measurements also confirmed this observation, and the BET surface area of freeze-dried powders was 128 ± 5, whereas that of the powders dried at 37°C for 72 h was $62 \pm 3$ m$^2$g. 

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Adult human bones yield XRD data very similar to those given in Figure 1(a). If the syntheses were to be carried out under refrigeration, e.g., at around 4°C, the XRD charts obtained would consist of, more or less, X-ray amorphous traces. The hydrated nature of CaP gel samples dried at 37°C were also confirmed by the wide water bands displayed in their FTIR spectra given in Figure 1(b). On the other hand, in the FTIR spectra of freeze-dried samples, the intensities of those water bands have been significantly reduced [Figure 1(b)]. Therefore, drying at 37°C did not completely eliminate the water present in those precipitated precursor gels. The symmetric and antisymmetric stretching of the PO₄³⁻ group were observed at 1097, 1043, 964, 604, and 565 cm⁻¹. Bands of CO₃²⁻ ions were observed at 1470–1420 and 875 cm⁻¹. The very weak IR band at around 919 cm⁻¹ and again the weak shoulder at around 1297–1310 cm⁻¹ were attributed to the smaller presence of HPO₄²⁻ ions. HPO₄²⁻ ions do also have a band at 874 cm⁻¹, which partially overlaps with that of CO₃²⁻ ions rendering the distinction between HPO₄²⁻ and CO₃²⁻ ions more difficult. In contrast to our findings, Wu et al. previously noted that unique HPO₄²⁻ group present in the human bone mineral was not seen in synthetic CaP samples produced under conditions similar to those of this study. The synthesis procedure adopted and used in this study was, therefore, able to produce a hydrated and carbonated CaP gel precursors, which contained a trace amount of protonated orthophosphate ions similar to the human fetal bones [Figure 1(b)].

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The ICP-AES and C analyses results are given in Table I. Analyses were performed in triplicate on all the samples. 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 I), and a molar (Na+Ca)/P ratio of 2.163 in these
powders. It is not so surprising that even if one started with a nominal precipitation solution 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.

When the pH values of the same precipitation solutions were fixed at around 4.2, then the formed powders only consisted of the brushite phase (DCPD, CaHPO$_4$·2H$_2$O), whose XRD and FTIR data are not shown here to save space. The size and shape of those brushite crystals were also perfectly the same as mentioned elsewhere.

The SEM morphology of the freeze-dried powders was shown, at two different magnifications, in Figures 1(c) and 1(d). The bright-field TEM photomicrographs given in Figures 2(a) and 2(b) depicted the nanostructure of the same powders. It should be noted that the selected-area electron diffraction inset of Figure 2(b) proved the nanocrystalline nature of those powders. TG/DTA/DSC analyses of the freeze-dried CaP precursors [Figure 2(c)] indicated that upon

<table>
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<th>Sample</th>
<th>Ca</th>
<th>P</th>
<th>Ca/P (molar)</th>
<th>Na</th>
<th>C</th>
<th>CO$_3$</th>
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<td>9.10</td>
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Figures 2. (a) & (b). TEM micrographs of freeze-dried CaP gel precursors.

Figure 2. (c) TG/DTA/DSC spectra of freeze-dried CaP gel precursors; (a) TG, (b) DSC, and (c) DTA spectra. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
heating to 155–160°C the samples first lost around 7.5% of their initial weight. This corresponded to the adsorbed water. 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 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, but in the IR spectra of Figure 1(b) it was quite difficult to identify those nitrate bands because of severe overlapping with the phosphate and carbonate bands over the same range. However, the weak bands at around 2200–2030 cm⁻¹ in Figure 1(b) can be ascribed to the nitrates. 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%. The temperature when one reached constant weight was 640°C.\(^{75}\) This phase (which will transform into \(\alpha\)-NaCaPO₄ at 650°C) is also isostructural with \(\beta\)-K₂SO₄. The most straightforward way of synthesizing phase-pure NaCaPO₄ powders can be the solid state reactive firing of the powder mixtures (in a 1:2:2 molar ratio) of Na₂CO₃, CaCO₃, and (NH₄)₂HPO₄ at 900–950°C.\(^{75}\) However, such a synthesis route (which involves the formation of liquid phases upon melting of first (NH₄)₂HPO₄ and then Na₂CO₃) will not be able to yield nanosize, therefore, high surface area and high surface reactivity powders.\(^{76}\) The peaks denoted by * (and their respective \(hkl\) reflections) were those of \(\beta\)-NaCaPO₄, and the \(2\theta\) 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% \(\beta\)-NaCaPO₄. This value was calculated from the data of Figure 3(a) by using the relative intensity ratio of the most intense peak of hydroxyapatite (at 31.78° 2\(\theta\)) to that of NaCaPO₄ (at 32.59° 2\(\theta\)). The samples heated at 600°C for 6 h can therefore be named as 40% NaCaPO₄–60% HA biphasic biomaterials.

FTIR traces of the same calcined samples were depicted in Figure 3(b). 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.\(^{77}\) These bands proved that the freeze-dried apatitic calcium phosphate phase (which lacked the OH vibrations) present in the gel precursors completely converted into hydroxyapatite upon calcination. Precipitated apatitic calcium phosphate precursors need the humidity in the calcination atmosphere to transform into Ca–hydroxyapatite upon heating.\(^{78–82}\) The relative humidity in our laboratories was at around 65–70% during those calcination runs. Characteristic FTIR spectrum of pure \(\beta\)-NaCaPO₄ was previously given by Driessens et al.\(^{55}\) The orthophosphate stretching

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**Figure 3.** (a) XRD spectra of CaP gel precursors heated in air from 300°C to 600°C (* and the respective \(hkl\) indices denote the reflections of \(\beta\)-NaCaPO₄, all the other peaks belong to HA)

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**Figure 3.** (b) FTIR traces of CaP gel precursors heated in air for 6 h from 300°C to 600°C
bands for the 500°C-calcined samples were observed at 603 ($\nu_3$), 962 ($\nu_1$), 1020, and 1089 ($\nu_3$) cm$^{-1}$, which were contributed both by crystalline $\beta$-rnenanite and apatitic calcium phosphate.\textsuperscript{77} The absence of the $P$—$O$—$P$ vibrational mode of pyrophosphates at 740 cm$^{-1}$ [Figure 3(b)] proved that the calcined samples did not contain any traces of Ca$_3$P$_2$O$_7$.\textsuperscript{77} If the amount of HPO$_4^{2-}$ ions in the precursor powders were significant, then their conversion into pyrophosphate would have been inevitable through the reaction 2HPO$_4^{2-}$ $\rightarrow$ P$_2$O$_7^{4-}$ + H$_2$O, which takes place at 600°C.\textsuperscript{31} Moreover, Loong et al.\textsuperscript{83} definitively demonstrated the lack or significant deficiency of OH$^-$ ions occupying crystallographic sites in the Ca-deficient, nonstoichiometric apatitic crystals of rat and bovine bones by using inelastic neutron-scattering spectroscopy.

It is known that an IR band at 1020 cm$^{-1}$ can be attributed to the $\nu_3$ vibration of PO$_4^{3-}$ in nonstoichiometric or Ca-deficient and/or carbonated apatitic calcium phosphates; however, a band recorded at 1030 cm$^{-1}$ is pinpointing to the $\nu_3$ vibration of PO$_4^{3-}$ in stoichiometric hydroxyapatite.\textsuperscript{84} Therefore, the relative ratios of 1020/1030 bands in the FTIR spectra could provide a measure of mineral crystallinity and maturity in bone minerals or apatitic-looking calcium phosphates.\textsuperscript{77,85} While the samples calcined at 300°C or 500°C were displaying that orthophosphate $\nu_3$ vibration at 1020 cm$^{-1}$, the same vibration was found to shift to 1026 cm$^{-1}$ in the 600°C-calcined sample [see Figure 3(b)]. This can be ascribed to the transition from nonstoichiometric to stoichiometric apatite together with the crystallization of $\beta$-Na-Ca$_3$PO$_4$ phase. On the other hand, it is interesting to note here that for the gel precursor samples dried at 37°C the same $\nu_3$ vibration was recorded at 1027 cm$^{-1}$, whereas the freeze-dried samples had it at 1020 cm$^{-1}$, as shown in the IR traces of Figure 1(b). This means that drying those gel precursors at 37°C for 72 h may have a positive effect on the progress of crystallization or so-called “maturation.”

Variations in the grain size and morphology of the rhenanite-hydroxyapatite biphasic powders, as a function of increasing calcination temperature, were monitored by the SEM photomicrographs given in Figure 4. The cracked-like grain/particle surfaces seen especially in the high magnification micrographs of Figure 4 are the artifacts created by the Pt-coating layer. Grain sizes directly measured from the SEM micrographs, as well as the respective surface areas of these powders, is given in Table II.

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.\textsuperscript{51} in a study on the thermal evolution of poorly-crystalline apatitic calcium phosphate powders produced by using Ca-nitrate tetrahydrate and diammonium 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.\textsuperscript{51} decomposed into crystalline tricalcium phosphate upon calcination. Freeze-dried samples of the current study consisted of (as shown in the micrographs of Figures 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.\textsuperscript{27,86} Johansen and Parks\textsuperscript{87} reported that bone apatite crystallites were platelike in shape with dimensions 400 $\times$ 200$-$350 $\times$ 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 (Figures 1 and 2) tended to form more or less equiaxed or globular grains (Figure 4). Such a tendency of nanosize globule formation upon heating can also be taken as a sign of those moieties (Figure 2) actually being comprised of very much smaller particles. Indeed, early studies by Molnar\textsuperscript{88,89} 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.\textsuperscript{90} 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 uni-
The sodium-doped calcium phosphate gel precursors of this study, enthusiased 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., 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. also tested the bioactivity of those 1000°C-sintered samples by soaking them, at 37°C, in SBF (simulated/synthetic body fluid) 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., while the pure hydroxyapatite samples (with no magnetic field application) were not showing any bonelike 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. Although we did not include an SBF-soaking study in this manuscript, the strong evidence brought upon by the work of Nakahira et al. was considered to be sufficient to ascertain the bioactivity (in SBF solutions) of such NaCaPO₄-containing hydroxyapatite biocermics. Moreover, the presence of Na ions that weaken the bond between Ca²⁺ and PO₄³⁻ in the crystal surface accounts for the high dissolution rate of β-NaCaPO₄. If the surface of a bioceramic sample inserted in an SBF solution exhibits such a significant ionic level dissolution phenomenon, then the Ca²⁺ and HPO₄²⁻ 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.

The NaCaPO₄–HA biphasic powders of this study sintered well even after heating them at the low temperature of 1000°C for 6 h in air as pressed pellets. The SEM photomicrographs given in Figures 5(a) and 5(b) (insets showed a
higher magnification view) depicted the surface of the well-densified pellets heated at 1000°C for 6 hours after cooling at the rate of 5°C/min and 1°C/min, respectively. It is interesting to note that even the chemically synthesized, submicron HA-TCP biphasic powders do not show a densification rate (even after heating those at 1200°C) as high as the samples of this study.96 Cooling rate was found to have a pronounced effect on the NaCaPO₄–HA biphasic samples though. Samples cooled at the rate of 5°C/min [Figure 5(a)] showed a larger number of both inter- and intra-granular cracks, when the cooling rate was decreased to 1°C/min [Figure 5(b)] those cracks were reduced. The mismatch between the coefficients of thermal expansion of NaCaPO₄ and HA phases might be responsible for the formation of those cracks. Pellets sintered at 1000°C still had the same biphasic nature (i.e., 60% HA–40% NaCaPO₄) and their XRD spectra were in strong resemblance to those given in Figure 3(a) for the 600°C-calcined powder sample. Sintering the biphasic samples at 1000°C for 6 hours did not destroy the original phase constitution. However, as expected, the FTIR spectra of the 1000°C-sintered pellets did not exhibit the carbonate bands. Carbonate ions in apatitic calcium phosphate structures cannot easily persist at temperatures greater than 700–750°C. Nevertheless, a more detailed study of the sintering behavior of these biphasic powders, with the aim of forming dense, carbonate ion-free ceramics of low surface area, over the temperature range of >1000–1300°C was out of the scope of this study.

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**TABLE II. Grain Sizes and Surface Areas of Powders**

<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>

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**Figures 5.** (a) 600°C-calcined biphasic powders sintered at 1000°C for 6 h and cooled at the rate of 5°C/min

(b) 600°C-calcined biphasic powders sintered at 1000°C for 6 h and cooled at the rate of 1°C/min
The osteoinductive character reported\textsuperscript{99–101} for the biphasic \(\beta\)-TCP (40%) and HA (60%) biomaterials may also be expected for the \(\beta\)-rhenanite-HA materials of this study. Finally, to validate the above speculation and the clinical usefulness of the \(\beta\)-rhenanite + HA biphasic biomaterials of this work \textit{in vivo} studies must be performed, which we plan to report in a follow-up study.

**CONCLUSIONS**

Sodium-doped calcium phosphate precursors were produced at room temperature by using a robust aqueous synthesis procedure involving the use of Na\(_2\)HPO\(_4\), NaHCO\(_3\), and Ca(NO\(_3\))\(_2\) *4*H\(_2\)O. 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 120 m\(^2\)/g. Calcination of these samples in a static air atmosphere over the temperature range of 400–600°C for 6 h led to the production of \(\beta\)-rhenanite and hydroxyapatite biphasic biomaterials. Calcined powder samples had surface areas over the range 30 to 80 m\(^2\)/g, and consisted of nanosize grains.

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**REFERENCES**


