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# Porous, Biphasic $CaCO_3$-Calcium Phosphate Biomedical Cement Scaffolds from Calcite ($CaCO_3$) Powder

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Calcite ($CaCO_3$) is a geologically abundant material, which can be used as a starting material in producing biomedical scaffolds for clinical dental and orthopedic applications. Bone-filling applications require porous, biocompatible, and resorbable materials. Commercially available $CaCO_3$ powders were physically mixed, for 80–90 s, with an orthophosphoric acid ($H_3PO_4$) solution, which was partially neutralized to pH 3.2 by adding NaOH, to form biphasic, micro-, and macroporous calcite-apatitic calcium phosphate (Ap-CaP) cement scaffolds of low strength. The resultant carbonated and Na-doped Ap-CaP phase in these scaffolds crystallographically and spectroscopically resembled calcium hydroxyapatite. Upon mixing $CaCO_3$ powders and the setting solution, carbon dioxide gas was *in situ* generated and formed the pores. Thus formed scaffolds contained pores over the range of 20–750 μm. Scaffolds were also converted to single-phase Ap-CaP, without altering their porosity, by soaking them in 0.5 *M* phosphate buffer solutions at 80°C for 36 h in glass bottles. Soerensen's buffer solution was also shown to be able to convert the calcite powders into single-phase Ap-CaP powders upon soaking at 60–80°C. This robust procedure of synthesizing Ap-CaP bioceramics is simple and economical.

## Introduction

Synthetic, implantable bone-like materials are useful in many different biomedical applications. For example, bone-like scaffolding material can be implanted to fill large bone defects caused by trauma or removal of cancerous or otherwise diseased bone or cysts.[1,2] Ideally, such scaffolding should be formed to have a structure and composition compatible with that of natural bone.[3–7] The ideal biomaterial should also have a chemical and structural design so as to induce a response similar to that of natural fracture healing when placed in an osseous defect, including initial invasion by mesenchymal cells, fibroblasts, and osteoblasts before new trabeculae of bone infiltrate into the porous structure of the implant from the walls of the defect.[8–19] Bone mineral consists of small crystals (3–5 nm in thickness and 40–100 nm in length, aligned parallel to the collagen fibrils of natural bones[4–7,20–22]) of $CO_3$-containing (5.5 wt%), alkali and alkaline earth ion-doped, Ca-deficient, non-stoichiometric, apatitic calcium phosphate (Ap-CaP) with a large and reactive surface area of 100–200 $m^2/g$.[23] Bone mineral is similar to, but far from being identical to, the mineral Ca-hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$). In other words, HA shall only be regarded as the "prototype" of one of the major inorganic constituents of bones and teeth.[4] A synthetic bone substitute should have high porosity dominated by macropores similar to the structure of trabecular bones,

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with pore sizes ranging from 100 to 700 μm.[24] The macroporosity of bones is there to allow for vascularization and cellular habitat, especially during the *in vivo* remodeling processes, whereas microporosity acts as channels for nutrient supply and transmission.

The development of calcium phosphate cements (CPC) started almost 3 decades ago with the formulation of *in situ*-setting calcium phosphate pastes for dental repair and restoration applications.[25–28] Cements provide the surgeon with the unique ability of manufacturing, shaping, and implanting the bioactive bone substitute material on a patient-specific base, in real time in the surgery room. Moreover, during their preparation in the surgical theater, such cement pastes can be impregnated with the patient's own bone marrow cells or platelet-rich plasma to enhance their *in vivo* osteogenetic properties.[29] Early CPCs commonly used either α-tricalcium phosphate (α-TCP: α-$Ca_3(PO_4)_2$) or tetracalcium phosphate (TTCP: $Ca_4(PO_4)_2O$), or both, in their powder components. The very first calcium phosphate cement paste, comprising α-TCP, was disclosed in 1974 by Driskell *et al.*[25] α-TCP and TTCP do share a useful property through which both undergo *in situ* hydrolysis into nanocrystalline, non-stoichiometric Ap-CaP or carbonated, calcium-deficient hydroxyapatite (CDHA: $Ca_9(HPO_4)(PO_4)_5OH$), upon contact with aqueous solutions at 20–37°C.[30–40]

For a comprehensive assessment of commercialized CPC formulations, classified with respect to their end products (i.e., after setting), the reader is referred to a recent study of Bohner *et al.*[41] It could thereby be seen (from Table I of Bohner *et al.*[41]) that the current CPCs can be divided into two categories: (i) apatitic and (ii) brushitic cements. Apatitic cements typically used α-TCP and/or TTCP in their starting powders, with only one exception of α-BSM®/Biobon®/Embarc®,[42,43] (Etex, Cambridge, MA) which used a cement powder consisting of a special mixture of poorly crystallized (or amorphous) calcium phosphate nanopowder (ACP[44–46]) and brushite crystals (dicalcium phosphate dihydrate, DCPD: $CaHPO_4 \cdot 2H_2O$). Conventional apatitic cements which contained α-TCP and/or TTCP phases in their powder components produced high compressive strength (from 30 to 70 MPa) cement products. On the other hand, brushitic cements, whose end product is the brushite phase, did not use α-TCP or TTCP powders,[41] and could only reach compressive strengths quite inferior to those of apatitic cements.[47–51] However, it is a well-known fact that brushite is more soluble than apatite under physiological conditions.[52] The higher *in vivo* resorbability of brushitic cements, in direct comparison with one of the most widely used apatitic cements (i.e., Norian SRS,® Synthes, West chester, PA), has recently been confirmed in a sheep model by Apelt *et al.*[50] It is not surprising to note that the only commercially available apatitic CPC, which does not contain any α-TCP or TTCP in its powder component (i.e., α-BSM® or Biobon® Etex, Cambridge, MA), has a higher *in vivo* resorbability than that of apatite,[17,53] simply owing to the higher solubility of ACP present in its starting powder. *In vitro* solubility of the α-BSM® material was also experimentally found[54] to be higher than that of both Norian CRS® or BoneSource® (refer to Table I of Bohner *et al.*[41] for further formulation details of these commercial cements). Commercialized brushitic cements (chronOS® [Synthes, Bettlach, Switzerland], Eurobone®, [Heimsbrunn, France] and VitalOS® [Products Dentaires, Vevey, Switzerland]) contain β-TCP as the major phase of their powder components,[41] and the reader is hereby referred to the results of *in vivo* studies independently performed by Wiltfang *et al.*[55] and Yuan *et al.*[32] for a direct comparison between the resorbability of α-TCP and β-TCP. Nevertheless, in the case of α-TCP (which is a quenched material and may need some particle size reduction operations before its use in CPC formulations), it will also be essential to take into account the crystallinity and reactivity (with aqueous solutions) of the surfaces of its particles.[56] $CaCO_3$ has not yet been seen as a dominant starting material in commercialized CPCs. The only exception to this was seen in the formulations of *Calcibon*® and *Norian*®,[41] which both used about 10% to 12% of $CaCO_3$ in their powders for the purpose of obtaining carbonated apatite as the end product. Constantz[57] and Edwards *et al.*[58,59] disclosed in their patent texts the use of much higher quantities of $CaCO_3$ (in comparison with the above-mentioned commercialized cement formulations), mixed with calcium phosphate phases such as TTCP or HA, to form new cements with increased porosity. Commercial CPCs,[41] which have such low $CaCO_3$ contents, do not yet possess any macroporosity.

Very recently, Combes and colleagues[60,61] reported the world's first experimental $CaCO_3$ cement, which had a biphasic starting material of amorphous calcium carbonate phase (doped with either strontium or magnesium) and crystalline vaterite.[62] The end products of these low-compressive strength cements were again calcium carbonate (vaterite+aragonite) because of the use of 0.9% (w/w) NaCl isotonic solution as their cement

liquid. This solution was not able to transform $CaCO_3$ into Ap-CaP.[62] The *in vitro* resorption of $CaCO_3$ (of calcite form) by the human or rat osteoclasts, in simultaneous comparison with HA and β-TCP bioceramics, has been previously studied by Monchau *et al.*[13]

The purpose of the present study was to develop a low-compressive strength but macroporous and inexpensive $CaCO_3$+Ap-CaP biphasic scaffold for biomedical use by using single-phase $CaCO_3$ powders. Commercially available calcite powders were used as received, and the powder component of the cement did not contain any additive whatsoever. The cement liquid or setting solution basically contained a solution of $H_3PO_4$ partially neutralized by the addition of concentrated NaOH solutions to pH 3.2. Powder X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR) and electron (and optical) microscopic investigations were used to monitor the purity and morphology of the biphasic $CaCO_3$+Ap-CaP scaffolds obtained. Cell culture studies on these macroporous cements will be published later.

## Experimental Procedure

### *Cement Powder*

The powder component of the cement of this study consisted of single-phase $CaCO_3$. The cements were initially developed by using the reagent-grade precipitated $CaCO_3$ powders manufactured by Merck KGaA (Catalog No: 1.02076, Darmstadt, Germany). The optimized cement recipe was also tested by using the precipitated $CaCO_3$ powders supplied by Fisher Scientific (Catalog No: C63-3, Fairlawn, NJ) as well. The cement recipe successfully worked for both reagent-grade $CaCO_3$ powders.

### *Preparation of the Cement Liquid*

The setting solution was prepared as follows: first a 100 mL aliquot of concentrated $H_3PO_4$ (Merck, Catalog No: 1.00573, 85%, 14.8323 *M*) was placed in an unused erlenmeyer flask of 300 mL capacity together with a Teflon®-coated magnetic stir bar (Dupont, Wilmington, PA). This erlenmeyer flask was then placed on a magnetic stirrer plate at room temperature ($21 \pm 1$°C). A 138 mL portion of concentrated NaOH (Merck, Catalog No: 1.05590, 32%, 10.8 *M*) was taken into a burette and placed directly on top of the open mouth of the erlenmeyer containing $H_3PO_4$. The first 50 mL portion of the NaOH solution in the burette was slowly (in 20 min) added in a dropwise manner, to prevent excessive heating and boiling, with a magnetic stirring rate of about 300 rpm. Upon slowly (ca. 10 min) adding another 25 mL aliquot of NaOH, the pH of the solution in the erlenmeyer increased to 0.4 (at 66–67°C). It should be noted that until now, only 75 mL of NaOH was added to the erlenmeyer. The slow (ca. 10 min) addition of another 25 mL portion of NaOH increased the pH to 1.60 (at 75°C). With the subsequent addition of another 25 mL aliquot of NaOH, pH became 2.60 (at 75°C). Finally, after adding (again slowly in about 10 min) the last 13 mL portion of NaOH in the burette to the erlenmeyer, the pH increased to 3.2 (at 45–50°C). The total volume of the viscous liquid thus formed in the erlenmeyer was completed to 250 mL by adding deionized water. The solution was cooled to room temperature under constant stirring, and transferred into a 250 mL capacity Pyrex® media bottle (with a cap) for long-term storage. This solution has been confirmed to preserve its constant pH value at 3.2 (at room temperature) for more than 2 years of storage on the laboratory bench. One milliliter of this solution (called afterwards as "SS" as an abbreviation of "setting solution") thus contained 0.0059 mol of phosphor.

Although the above procedure was the preferred way of preparing the cement liquid, another preparation route was also considered. According to this alternative route, 108.33 g of $Na_2HPO_4$ (Merck, Catalog No: 1.06585) was first placed in a clean beaker of 400 mL capacity, followed by the addition of 80 mL of deionized water. Upon magnetically stirring and heating the beaker contents on a hot plate to about 85°C, a transparent solution was obtained. While cooling down to room temperature, a 25 mL aliquot of $H_3PO_4$ (Merck, Catalog No: 1.00573) was added in a dropwise manner (in 10 min). Following stirring for 15 min, another 25 mL portion of $H_3PO_4$ was added in the same fashion. The total volume was finally completed to 250 mL by adding deionized water. The pH of this solution was again stable at 3.2 during long-term storage at room temperature. This setting solution gave the same results as the above-mentioned SS.

### *Cement Preparation*

A plastic cup of cylindrical geometry (approximately 8 cm tall with a diameter of 6.5 cm) was used to mix the calcite powder with the SS to form the cement paste.

$CaCO_3$ powder of appropriate quantity was first weighed and then placed this cup. SS of proper volume was measured with a pipette and then placed in a clean glass bottle. At the moment of mixing the $CaCO_3$ powder and the SS in the glass bottle was immediately poured into the plastic cup containing $CaCO_3$. Mixing was manually performed by using a plastic spatula. Mixing was continued for 80–90 s in all trials, and at the end of mixing the formed paste was cast into a polyethylene, square-shaped weighing boat. Therefore, upon setting (which took place in 4 min), porous cement blocks readily took the shape of those weighing boats. Cement blocks were then soaked in deionized water at room temperature for 3 h, followed by drying at 24°C in an air-ventilated oven.

### *Preparation of the 0.5 M Phosphate Buffer Solution*

0.5 *M* phosphate buffer solution of neutral pH was prepared as follows: 800 mL of deionized water was placed in a 1 L-capacity beaker, followed by adding 39.749 g of $Na_2HPO_4$ (Merck, Catalog No: 1.06585) at room temperature (RT). Upon stirring, a transparent solution was obtained. To this solution 16.559 g of $NaH_2PO_4 \cdot H_2O$ (Merck, Catalog No: 1.06346) was added to increase the pH to about 7.2. Solution was then transferred into a 1 L-capacity media bottle and stored in a refrigerator when not in use. This solution was used to convert the biphasic $CaCO_3$+Ap-CaP porous scaffolds/blocks into single-phase Ap-CaP at a later stage of processing.

### *Conversion of Cement Scaffolds to Ap-CaP*

Cement blocks with the approximate dimensions of 2 cm × 2 cm × 2 cm (each weighing about 4.8 ± 0.1 g) were placed into 250 mL-capacity Pyrex® media bottles containing 200 mL of 0.5 *M* phosphate buffer solution (one block into one bottle), and then placed into a microprocessor-controlled oven maintained at 80°C. The entire phosphate buffer solution in the bottles was replenished with an equal volume of unused solution every 6 h. By the end of 36 h of soaking, the solution pH became stable at 7.2 ± 0.1 at 80°C. Blocks were finally washed with 2 L of water at RT (by soaking in water for 2 h), followed by drying in a static air atmosphere at 80°C for 17 h.

### *Preparation of Soerensen's Buffer*

Soerensen's buffer solution of pH 7.4 was prepared as follows: 1000 mL of deionized water was placed in a 1.5 L-capacity beaker, followed by adding 5.26 g $KH_2PO_4$ (Merck, Catalog No: 1.04873) at RT. A few minutes of stirring resulted in a transparent K-phosphate solution. To this solution 8.65 g of $Na_2HPO_4$ (Merck, Catalog No: 1.06585) was added to increase the pH to about 7.4. Soerensen's buffer was then transferred into a 1 L-capacity media bottle and stored in a refrigerator when not in use. This Na- and K-containing solution was used to convert pure $CaCO_3$ powders into Ap-CaP.

### *Sample Characterization*

Samples were characterized by powder XRD(Model D5000, Siemens GmbH, Karlsruhe, Germany), scanning electron microscopy (SEM; Model 630, Jeol Corp., Tokyo, Japan and Model S-3500, Hitachi, Tokyo, Japan), FTIR(Nicolet 550, Thermo-Nicolet, Woburn, MA), water absorption,[63] density measurements (Pycnometer; AccuPyc 1330, Micromeritics Corp., Norcross, GA), inductively coupled plasma atomic emission spectroscopy (ICP-AES; Model 61E, Thermo Jarrell Ash, Woburn, MA), and compressive strength measurements (Model 4500, Instron Deutschland GmbH). Percentage water absorption values of the porous cement scaffolds ((wet weight—dry weight)/dry weight)*100) were measured in accord with the ASTM standard C20-92.[63] Dry weights of the samples were recorded after drying at 24°C for 72 h, whereas the wet weights were recorded immediately after soaking the cement samples in deionized water (at 21°C) for 3 h. Pore sizes of the porous scaffolds were determined by using the linear intercept method on the photomicrographs.

To give more details on the characterization runs, 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 to form a thin layer, followed by tapping to remove excess powder. The XRD was operated at 40 kV and 30 mA with monochromated Cu$K\alpha$ radiation. XRD data (over the typical range of 20–45° 2$\theta$) were collected with a step size of 0.03° and a preset time of 1 s at each step. FTIR samples were first ground in a mortar, in a manner similar to that used in the preparation of XRD samples, and then mixed with KBr powder in a ratio of

1:100, followed by forming a pellet by using a uniaxial cold press. One hundred and twenty eight scans were performed at a resolution of $3\,cm^{-1}$. Powder samples examined with the SEM were sputter coated with a thin Au layer, to impart surface conductivity to the samples. The Brunnauer–Emmet–Teller (BET) surface area of powder samples was determined by applying the standard BET method to the nitrogen adsorption isotherms obtained at $-196^{\circ}C$ using an ASAP 2020 instrument (Micromeritics Corp., Norcross, GA). Powder samples used in the ICP-AES analyses were first dissolved in nitric acid before the measurements. Cylindrical cement samples (1 cm diameter, 2 cm height) were loaded under compression until failure, at a crosshead speed of 1 mm/min, with a 1.5 kN load cell, to determine compressive strength. The ASTM standard C1424-04 was observed in strength measurements.

## Results and Discussion

### *Porous Cement Preparation*

This cement was designed to have a unique property; calcium was supplied by its powder component, whereas phosphor was received from the setting solution. As this inexpensive, low-strength cement[64] had a powder component comprised of single-phase $CaCO_3$, the development of a self-setting cement out of pure calcite solely relied on the manufacture of a proper cement liquid or the SS. If one used concentrated phosphoric acid alone, the reaction of the acid with calcite would be too severe and instantaneous (resulting in the formation of a mixture of acidic phosphates, such as $Ca(H_2PO_4)_2$ and $Ca(H_2PO_4)_2 \cdot H_2O$), and the handling or shaping of the material into a specific shape would be almost impossible. Partial neutralization of phosphoric acid with concentrated sodium hydroxide solution, in forming the SS, thus seemed to be a viable alternative. Pore formation in this biphasic cement proceeded with the conversion of only a certain fraction of $CaCO_3$ into Ap-CaP upon coming into contact with the mildly acidic SS, and the by-product of this limited conversion reaction was $CO_2$ gas. The pore cavities were the direct result of physically entrapped bubbles of the evolved $CO_2$ gas in the setting body.

SEM photomicrographs of Figs. 1a and 1b show the starting morphology of the powder component (i.e., $CaCO_3$) of this cement. Calcite powders used in this study were commercial powders (supplied by Merck KGaA and Fisher Scientific Corp., Pittsburgh, PA) and both of these precipitated chalk powders were morphologically similar to one another. These powders were used as received, without any further treatment. Both calcite powders were found to have a surface area of about $6.15 \pm 0.25\,m^2/g$ (BET runs in triplicate on each), and similarly consisted of 0.5-μm-thick and 1.5-μm-long spindle-shaped agglomerated particles.

Table I lists three optimal recipes developed for the preparation of these cements. The nominal, starting Ca/P molar ratio in these cements could be adjusted either by simply varying the amount of calcite powder or the volume of the SS to be used. However, as the SS developed for calcite powders was so mild, the conversion of $CaCO_3$ to Ap-CaP would never reach to completion. It should be apparent from Table I that when one keeps the mixing time limited to between 80 and 90 s, there would not be enough time for a complete reaction between calcite and the SS. If one increased the mixing (or agitation) time to about 4 or 7 min, more calcite would convert to Ap-CaP, and total porosity and pore sizes would significantly decrease (data not shown). Therefore, we intentionally stopped mixing after 80–90 s and proceeded with casting into a mold.

Figures 2a and 2b show the optical micrographs of the porous cement scaffolds produced at the nominal Ca/P molar ratio of 1.51. Figures 3a–3d, on the other

**Table I. Optimal Recipes for the Preparation of Biphasic ($CaCO_3$+Ap-CaP) Porous Cements by Using Pure Calcite Powders**

| Powder (g) | SS (mL) | SS/P (mL/g) | Mixing time (s) | Nominal Ca/P (*M*) | %$CaCO_3$ left* (rest Ap-CaP) |
|---|---|---|---|---|---|
| 29.75 | 30 | 1.0084 | 80 | 1.67 | $82 \pm 3$ |
| 26.90 | 30 | 1.1152 | 85 | 1.51 | $79 \pm 3$ |
| 22.30 | 30 | 1.3453 | 90 | 1.25 | $77 \pm 4$ |

*Determined by using the ratio of XRD peak intensities of calcite and Ap-CaP phases.
XRD, X-ray diffraction; SS, setting solution; Ap-CaP, apatitic calcium phosphate.

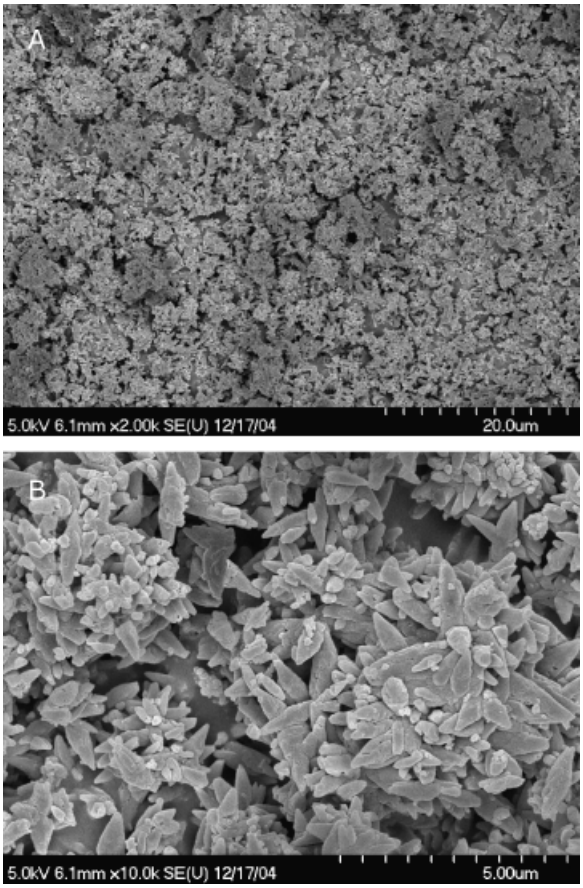



*Fig. 1. Scanning electron microscopy photomicrographs of starting $CaCO_3$ (calcite) powders, (a) low magnification, (b) high-magnification image.*

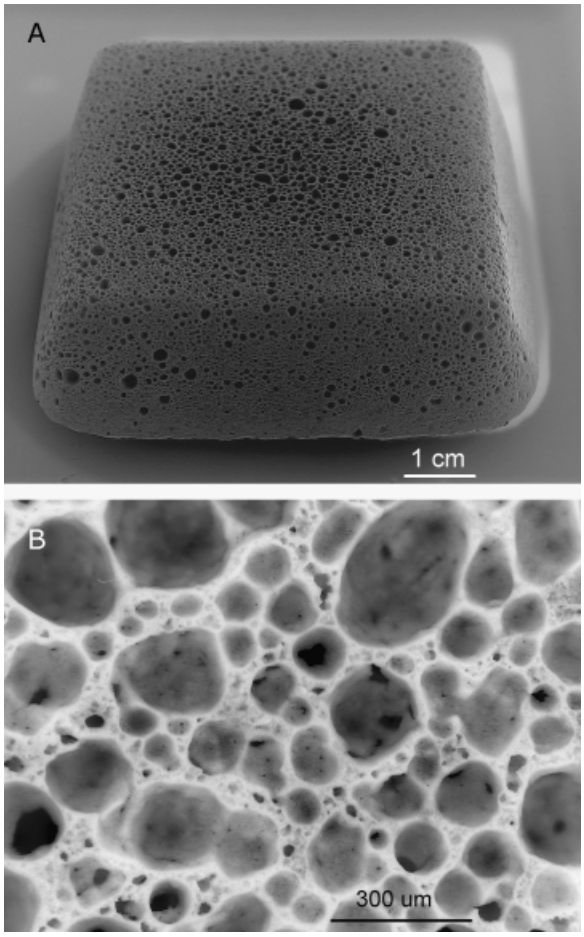



*Fig. 2. Optical micrographs of the set cement body (Table I; Ca/P = 1.51), (a) macro view of the body cast into a large weighing cup, (b) optical microscope image of the same.*

hand, depict the SEM photomicrographs of the same, recorded at consecutively increasing magnifications. Pores of these scaffolds are large enough to allow the attachment and proliferation of bone cells, that is, osteoclasts and osteoblasts. Average pore sizes of 150–500 μm are desirable for optimal bone cell ingrowth.[65] Pores in commercial CPCs cannot yet reach sizes larger than 10–20 μm.[41]

Upon casting the bubbling paste (after 80–90 s of agitation/kneading with a spatula) into a plastic weighing boat (Fig. 2a), the paste completed its setting within the next 4–5 min. After 4 min, the cement body was stable and workable. To determine the hardening time quantitatively, a stainless-steel needle (with a circular tip diameter of 2 mm) weighing 115 g was gently placed on the surface of the setting cement, and at the moment when it no longer left an indent mark (deeper than 1 mm) behind, the cement was deemed to set and harden. This was how we deduced the setting time in this study. The needle used was conformed to the specifications of a light Gillmore needle.[66] Gillmore needles measure one initial (I) and one final (F) setting time for cements. The clinical meaning of Gillmore needles is that the cement should be implanted before (I) and the wound can be closed after (F).[67] We reported the initial setting time (I) throughout this study. Aforementioned setting time did not change for all the three compositions listed in Table I.

The SEM photomicrograph of Fig. 3a shows the debris, resulting from the sample cutting operation, nestled in the macropores. Cracks were also formed as

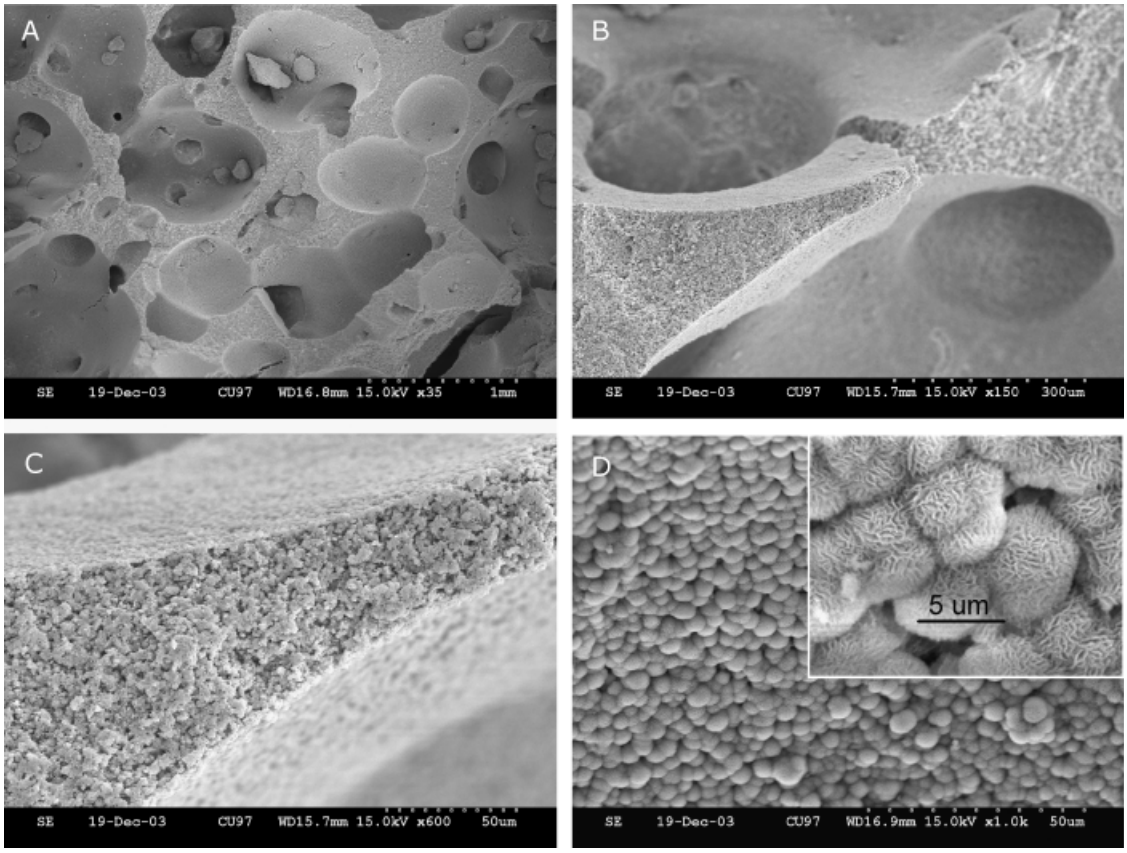



*Fig. 3. Scanning electron microscopy photomicrographs of set cement (Table I; Ca/P = 1.51); (a) to (c) pore morphology of the cement body, (d) close-up image of the Ap-CaP skin formed on the calcite-based aggregates of the bulk.*

a result of the same cutting operation (Fig. 3a). Figures 3b and 3c reveal the skin of Ap-CaP formed along the entire external surfaces. A close-up image of this skin is given in Fig. 3d. The micromorphology of the Ap-CaP skin shown in the inset of Fig. 3d resembled that of Ap-CaP biomimetically deposited via synthetic body fluid (SBF) solutions onto metals,[68,69] ceramics,[70] or polymers.[71,72]

The variation in the SS/P ratios was found to have a small but detectable effect on the degree of conversion of calcite to Ap-CaP. On the other hand, the change in the SS/P ratios used did not produce a significant change in the percentage water absorption, density and compressive strength of the resultant cement bodies (as shown in Table II), as well as total apparent porosity. Unfortunately, in this study, the percentage

**Table II. Variation of the Physical Properties of Biphasic ($CaCO_3$+Ap-CaP) Porous Cements**

| Powder (g) | SS (mL) | Setting time (min) | Water absorption (%) | Density* ($g/cm^3$) | Compressive strength* (MPa) |
|---|---|---|---|---|---|
| 29.75 | 30 | 4 | $172 \pm 5$ | 0.60 | 0.71 |
| 26.90 | 30 | 4 | $169 \pm 3$ | 0.63 | 0.67 |
| 22.30 | 30 | 4 | $179 \pm 6$ | 0.59 | 0.69 |

*Reported as the averages of measurements in triplicate.
SS, setting solution; Ap-CaP, apatitic calcium phosphate.

porosity was mainly estimated by visual inspection of the photomicrographs, in combination with the water absorption and density measurements. According to the results of measurements by using the linear intercept method, pore sizes in the samples were varying between 20 and 750 μm. Micropores (20–80 μm) were typically located around the macropores (90–750 μm), and the struts defining the macropores were porous, as well (Fig. 2b).

Owing to their low density, set cement blocks (such as those shown in Fig. 2a) were able to remain afloat when placed in deionized water (800 mL water contained in a 1 L beaker) immediately after 4 min of setting in the weighing boat. It took about 20 min for the cement blocks to sink to the bottom of the beaker at room temperature (with the invasion of pores by water). However, when the temperature of water was increased to 80°C, the blocks started to rise and remain afloat again. This was probably due to the decreased surface tension of water with an increase in temperature. Hot water was able to penetrate the micropores. It was also noticed that the cement blocks immersed in water formed visible lines of striations (which typically form in aqueous systems when two fluids of different viscosities come together) around them, and this was due to the passage of sodium phosphate species (present in the SS) to the water. Within the limited scope of this study, we were not able to quantify the amount of sodium/sodium phosphate species being released from the set cement blocks into the soaking water. It should be noted that when the whole cement block (as shown in Fig. 2a) was placed into 150 mL of deionized water (in a 400 mL capacity beaker) at RT, its pH was recorded as $6.4 \pm 0.2$. However, upon changing this water, after 10 min of soaking, with a fresh 150 mL aliquot, the pH of the water increased to $6.8 \pm 0.2$. After 1 h of soaking, the pH of the water became stabilized at $7.0 \pm 0.1$. This soaking step was deemed to be necessary to substantially remove the sodium-$H_2PO_4$ species originating from the mildly acidic SS.

The presence of citric acid (as well as, malic, fumaric, lactic, or succinic acids) in the SS may also be considered in forming these macroporous biphasic $CaCO_3$+Ap-CaP scaffolds.[58,59] As an example, an alternative SS can be prepared by dissolving 20.1 g of $NaH_2PO_4$ powder in a 40 mL portion of a 200 g/L citric acid (Merck, Catalog No: 1.00241) solution. This solution contained 0.1675 moles of phosphor. Upon mixing the above solution with 28.0 g of $CaCO_3$ ( = 0.2798 moles of calcium) for 90 s, followed by casting, one obtained the same porous cement scaffold as shown in Fig. 2. When citric acid was present in the SS, citrate groups were strongly coordinated to the calcium ions, yieding a relatively large complex molecule or species. Therefore, the reaction between Ca and P species was partially delayed. The product then entered into a gelation process.[73–75] However, when the formed porous scaffold was hydrothermally treated at 80°C in 0.5 *M* phosphate buffer solution, it was, again, fully converted into Ap-CaP. The presence of citric acid in the SS improved the consistency and the workability of the formed paste before its casting into the mold. Moreover, the setting time was increased to about 8 min in this case.

### *Conversion of Cement Scaffolds to Ap-CaP*

The bottom trace of Fig. 4 shows one of the characteristic XRD spectra for the porous cement blocks soaked in water for 3 h, following the setting. It shows the presence of about 80% calcite (ICDD PDF 05-586 or 83-1762,[76] rhombohedral lattice[77]). The top XRD trace of Fig. 4 confirms the formation of single-phase Ap-CaP of low crystallinity upon soaking these biphasic cement scaffolds in a 0.5 *M* phosphate buffer solution at 80°C for 36 h. The XRD trace shown in Fig. 4b was

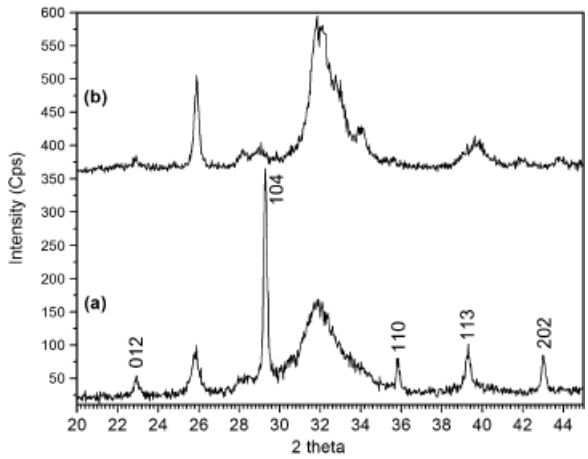



*Fig. 4. X-ray diffraction traces of cement scaffolds (Table I; Ca/P = 1.51 composition); (a) cement scaffold after 3 h of soaking in water at room temperature, peaks of calcite were indicated by their Miller indices and the rest belonged to Ap-CaP of low crystallinity, (b) cement scaffold after 36 h of soaking in 0.5 M phosphate buffer at 80° C.*

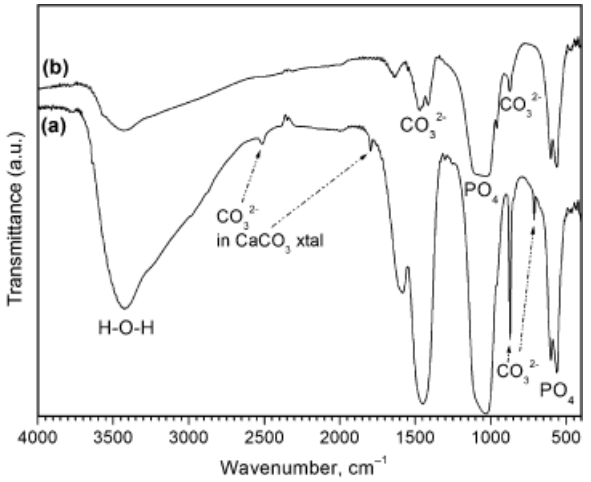



*Fig. 5. Fourier-transform infrared spectroscopy traces of cement scaffolds (Table I; Ca/P = 1.51 composition); (a) cement scaffold after 3 h of soaking in water at room temperature, (b) cement scaffold after 36 h of soaking in 0.5 M phosphate buffer at 80°C.*

obtained from the agate mortar-ground powders of these 0.5 *M* phosphate buffer-soaked and washed scaffolds.

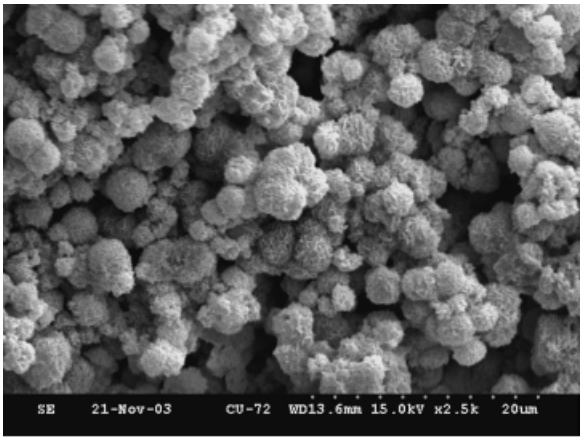
FTIR spectra of the same samples are plotted in Fig. 5, respectively. In Fig. 5a, the bands observed at 3643, 2513, 1797, 1587, 1450, 871, 848, and 713 $cm^{-1}$ were characteristic of calcite. On the other hand, in Fig. 5b, the weak stretch for $OH^-$, which was observed at 3571 $cm^{-1}$, indicated the presence of hydroxyl ions in Ap-CaP. Soaking of the porous cement scaffolds in a 0.5 *M* phosphate buffer solution at 80°C caused the conversion of calcite into Ap-CaP.

Water absorption and density measurements performed on these converted scaffolds showed that the values given in Table II did not change. However, the compressive strength of the converted scaffolds increased to $2.4 \pm 0.4$ MPa from the starting value of *ca.* 0.7 MPa. It should be noted that the compressive strength of trabecular bones is between 2 and 10 MPa, whereas that of cortical bones is in the vicinity of 100 MPa. The weak cement scaffolds of this study can only match the compressive strength of trabecular bones at its lower limit. Many commercial cements could reach compressive strengths in excess of 50 Mpa.[2,27,41,57] However, those high-strength cements, which used α-TCP or TTCP powders in their formulations (with lesser amounts of $CaCO_3$), were not able to show any significant overall *in vivo* resorbability even after 1 year of implantation.[55]

ICP-AES analyses of the agate mortar-ground powders of the converted cement scaffolds (from the samples of Figs. 4b and 5b) gave a Ca/P molar ratio of $1.56 \pm 0.3$, together with a Na concentration of $7700 \pm 250$ ppm. The reader is referred to the works of Yubao *et al.*,[78] Ivanova *et al.*,[79] and Kasten *et al.*[80] for more detailed treatises (including *in vitro* and *in vivo* experiments) on non-stoichiometric Ap-CaP.

Pure $CaCO_3$ powders (Fisher Scientific Corp., Catalog No: C63-3) could also be converted to Ap-CaP by simple soaking in a dilute phosphate solution at 60–80°C. We have previously shown that even the surfaces of $CaCO_3$ marble blocks would transform to Ap-CaP by using such a soaking procedure at 60°C.[81] To transform the calcite powders, this time we used Soerensen's buffer solution as described in Chapter II. Soaking experiments were performed in 250 mL-capacity Pyrex® media bottles, which contained 3.1 g calcite powder (whose morphology is given in Fig. 1) and 225 mL of Soerensen's buffer in each. Sealed bottles were first kept in a microprocessor-controlled oven at 60°C for 24 h, and then the solutions were replenished with an unused solution at every 24 h for the next 48-h period. For the last 48 h of the total soaking time of 72 h, the temperature of the oven was increased to 80°C. Powders in the media bottles were filtered out, washed with deionized water, and dried overnight at 80°C. The resultant powders had the morphology illustrated in Fig. 6a. Characteristic XRD spectra of these Ap-CaP powders was reproduced in Fig. 6b. ICP-AES analyses of these powders yielded a Ca/P molar ratio of $1.58 \pm 0.2$. BET surface area of these powders were measured as $40 \pm 2$ $m^2/g$. This procedure was presented here as a robust way of synthesizing Ap-CaP powders in bulk form. It also served to confirm the phase nature of the "Ap-CaP skin" observed on our porous cement scaffolds as depicted in Fig. 3d.

The interest in the conversion of $CaCO_3$ into Ap-CaP, to obtain porous biomaterials, started with the pioneering work of Prof. Della M. Roy in the early 1970s.[82–84] In these extremely competent studies, Roy *et al.*[83,84] investigated the conversion of natural coral skeletal aragonite (Porites) by using high temperatures (140–260°C) and high autoclave pressures (550–1055 $kg/cm^2$) in the presence of acidic (such as, $CaHPO_4$ and $Ca(H_2PO_4)_2 \cdot H_2O$) and basic (such as $(NH_4)_2HPO_4$) phosphates, as well as of sodium or potassium orthophosphates and acetic acid. Hydrothermal reactions were carried out from 12 to 48 h under the



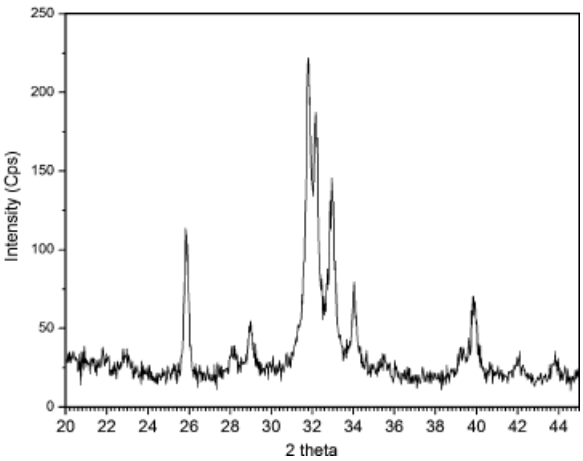




*Fig. 6. (a) Scanning electron microscopy photomicrograph of Ap-CaP powders synthesized by soaking calcite powders in Soerensen's buffer at 60–80°C for 72 h, (b) X-ray diffraction trace of the same powder.*

above-mentioned experimental conditions. Over the last 3 decades, the early studies of Roy and colleagues[82–84] were duplicated, improved, or inspired by, and the scientific literature related to these is immense, but just to name a few proficient examples, the works of Zaremba *et al.*,[85] Su *et al.*,[86] Ni *et al.*,[87] and Jinawath *et al.*[88] must be cited. Nowadays, converted coral and other marine skeletal species are also available as commercial porous implant biomaterials.[89,90] However, it should be noted that in most coral or marine species, the major phase was biological aragonite, and its conversion to Ap-CaP was more difficult than that of $CaCO_3$ of this study. This was apparent from the need for higher temperatures and higher autoclave pressures in converting biological aragonite to Ap-CaP. Experimental studies that started only with calcite powders and that produced macroporous Ap-CaP biomedical scaffolds from those were quite rare, and this study was conceived to fill this gap.

## Conclusions

(1) Single-phase calcite ($CaCO_3$) powders were used to form a macroporous, biphasic (calcite+Ap-Cap) and weak cement scaffold.

(2) Concentrated orthophosphoric acid, partially neutralized by NaOH to pH 3.2, was used as the setting solution of the above-mentioned cement.

(3) 0.5 *M* phosphate buffer solutions were able to fully convert the calcite+Ap-CaP biphasic scaffolds into pure Ap-CaP, upon soaking these weak scaffolds in those solutions at 80°C.

(4) Soerensen's buffer was used to synthesize single-phase Ap-CaP powders, with a Ca/P molar ratio of 1.58 and BET surface area $\geq 40\ m^2/g$, by soaking pure calcite powders in these solutions at 60° to 80°C.

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