

Preparation of porous apatite granules from calcium phosphate cement

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Abstract A versatile method for preparing spherical, micro- and macroporous (micro: 2–10 and macro: 150–550 μm pores), carbonated apatitic calcium phosphate (Ap-CaP) granules (2–4 mm in size) was developed by using NaCl crystals as the porogen. The entire granule production was performed between 21 and 37 $^{\circ}\text{C}$. A CaP cement powder, comprising $\alpha\text{-Ca}_3(\text{PO}_4)_2$ (61 wt.%), CaHPO_4 (26%), CaCO_3 (10%) and precipitated hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (3%), was dry mixed with NaCl crystals varying in size from 420 μm to 1 mm. Cement powder (35 wt.%) and NaCl (65 wt.%) mixture was kneaded with an ethanol– Na_2HPO_4 initiator solution, and the formed dough was immediately agitated on an automatic sieve shaker for a few minutes to produce the spherical granules. Embedded NaCl crystals were then leached out of the granules by soaking them in deionized water. CaP granules were micro- and macroporous with a total porosity of 50% or more. Granules were composed of carbonated, poorly crystallized, apatitic CaP phase. These were the first spherical and porous CaP granules ever produced from a self-setting calcium phosphate cement. The granules reached their final handling strength at the ambient temperature through the cement setting reaction, without having a need for sintering.

1 Introduction

Synthetic calcium phosphate (CaP)-based skeletal repair or hard tissue regeneration materials in the form of granules of well-defined geometry, preferably in the shape of spheres with variable diametrical production sizes, can provide the orthopedic or periodontal surgeon with an ease in filling the bone defects/voids of irregular and patient-specific shapes. In comparison, sintered and pre-shaped CaP prismatic blocks can be difficult to fill voids of irregular dimensions. Sintered CaP bioceramic blocks typically comprise of pure calcium hydroxyapatite (HA: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), pure β -tricalcium phosphate (β -TCP: $\beta\text{-Ca}_3(\text{PO}_4)_2$) or the biphasic mixtures (HA-TCP) of the two [1–3]. It shall be remembered that sintered, well-crystallized HA, TCP, and biphasic HA-TCP are good platforms for osteoconduction.

An overwhelming majority of the early examples of CaP granule synthesis routines involved a high-temperature sintering step, which resulted in the production of well-crystallized CaP granules [4–12]. High-temperature sintering as a means of production was again quite popular in the later examples of CaP granule manufacturing. An abridged version of the literature survey is given below. Fabbri et al. [13] formed millimeter-sized HA granules by first dripping a CaP-containing aqueous suspension into liquid nitrogen, followed by sintering the formed beads, which were devoid of any significant macroporosity. Apatitic granules of coralline-origin were prepared and tested in vivo by Holtgrave and Donath [14] and Baran et al. [15]. Luo and Nieh [16] and Kawai et al. [17] used the spray granulation process to produce only micron-sized CaP granules, which were too small to be handled by the orthopedic or periodontal surgeons. However, this technique also necessitated a follow-up sintering step. Liu [18]

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modified the dripping procedure originally developed by Fabbri et al. [13], and added polyvinylbutyral (PVB) into the ceramic suspensions. Granules were then sintered at 1,200 °C to strengthen the wet granules, as well as to burn off of the polymer, PVB. Well-crystallized CaP granules synthesized by Liu [18] had porosity from 24 to 75 vol.%, pore sizes from 100 to 400 µm, and granular sizes from 0.7 to 4 mm. The use of PVB, as the porogen, was also tested by Zyman et al. [19] in forming porous granules. The complete burn off of PVB always necessitated high temperatures (<1,150 °C). Maruyama and Ito [20] formed a paste consisting of HA powders, CaO, ZnO, chitosan and malic acid, and wet granules were first formed out of this paste, followed by sintering at 1,150 °C. Gauthier et al. [21] hydrolyzed brushite (CaHPO₄·2H₂O) powder in a basic NaOH solution to obtain apatitic calcium phosphate (Ap-CaP) powders and sieved the water-soaked Ap-CaP powders to form granules in the size range of 200–500 µm. However, these soft granules were then heated over the temperature range of 550–950 °C. Oonishi et al. [1, 22] tested the in vivo response of granules of sintered hydroxyapatite. Granules obtained by Oonishi et al. [1, 22] were produced by simply sieving (to 100–300 µm size range) the finely ground chunks of previously sintered hydroxyapatite ceramics. Merckx et al. [23] examined the porous (trabecular) bovine apatite granules in an in vivo goat model. Paul and Sharma [24] first prepared the HA powders by reacting Ca(OH)₂ with H₃PO₄, then formed viscous aqueous suspensions in the presence of chitosan and paraffin, and obtained granules upon stirring those thick suspensions. Formed granules were fired at 1,100 °C to achieve the strength required for handling. Patel et al. [25] used a somewhat similar procedure, to that used by Paul and Sharma [24], to produce Si-substituted apatite granules and tested their in vivo behavior. The clinical study of Schwartz et al. [26] employed 55%HA–45%TCP biphasic, macroporous and well-crystallized, sintered (at 1,200 °C) granules with sizes varying from 2 to 3 mm in vivo resorbability of the sintered but macroporous granules of excellent pore connectivity was found to be quite poor even after a follow-up period of 36 months [26]. A similar high-temperature process for granule production was reported by Tanaka et al. [27], as well. In a quite different approach to form spherical CaP granules, Barinov and coworkers [28, 29] first prepared an aqueous suspension by using precipitated CaP powders, and then mixed it with a 10% gelatin solution. Upon stirring this CaP–gelatin suspension into a bath of vegetable oil, spherical granules of micron size were formed. Wet granular aggregates were then collected and sintered at 1,200 °C to provide those with the necessary handling strength. Rodriguez-Lorenzo et al. [30] prepared apatite suspensions containing soluble starch, and utilized the swelling of starch to form pores,

followed by sintering at 1,100 °C and granulation. In brief, research related to the production of calcium phosphate granules is gaining pace in recent years [31–39].

Pore sizes in human cortical bones are known to range from 1 to 100 µm, whereas in trabecular bones the pores were varying from 200 to 700 µm [2]. The use of NaCl as the porogen to achieve such significant pore sizes in bio-materials was rather seen in the production of porous scaffolds of polymeric origin, such as polycaprolactone [40], gelatin [41], polyurethane [42, 43], silk fibroin [44], poly(lactic-co-glycolic acid) (PLGA) [45, 46], poly-L-lactic acid (PLLA) [47, 48], polypropylene fumarate [49], copoly-L-lactide-epsilon-caprolactone [50], and polymethylmetacrylate [51].

Granulation, as it is understood and commonly used in the pharmaceutical industry, is the process in which powder particles are made to adhere (with the help of a binder that locks the powders together as granules and prevents disintegration) to form larger entities called granules. Pharmaceutical granules [52–55] typically assume sizes between 0.2 and 4.0 mm, and in orthopedic surgery granules between 1 and 2 mm [36, 38], whereas in periodontal surgery granule sizes of 0.25–1 mm are preferred [56]. Therefore, it would not have been unusual to inspire from the granulation processes and procedures of pharmaceutical industry to produce CaP granules for skeletal repair and biomedical applications.

Pharmaceutical industry typically uses planetary mixers, fluidized-bed granulators, spheronizers, rotors, extruders and pelletizers in forming granules from wet masses containing the desired ingredients [57]. However, sieving (or screening), in pharmaceutical granulation technology, is only regarded as a secondary tool of transforming the as-formed granules into monosized granules.

The spherical granules of the current study, which used a well-tested and documented CaP cement powder as the starting material [58–61], did not need any sintering or high-temperature calcination to develop their handling strength. The granules of carbonated and Ca-deficient Ap-CaP stoichiometry were formed and in situ hardened at the ambient temperature by the cement setting reaction. Cement reaction directly took place on an automatic sieve shaker and this sieve shaker was the sole granulation device.

The porous granules reported in this study are believed to be the first spherical granules produced at the ambient temperature from a self-setting CaP cement dough by simple sieving.

2 Materials and methods

Powders of self-setting cement Calcibon[®] (Biomet-Merck Biomaterials GmbH, Darmstadt, Germany) were used to

form the porous granules. The cement powder [57–60] consisted of a mixture of 61% α -TCP, 26% CaHPO₄, 10% CaCO₃ and 3% HA, by weight. A 3.5 wt.% solution of Na₂HPO₄ powder (99.9% pure, Merck KGaA, Darmstadt) in deionized water was its initiator liquid. NaCl (99.9%, Merck KGaA, Darmstadt) was used as the porogen. The as-received NaCl powder was first screened by using two sieves, mesh no. 18 (1.0 mm sieve opening) and no. 40 (420 μ m sieve opening). Therefore, the NaCl porogen crystals used in granule synthesis were varying in size between 420 μ m and 1 mm, and about 85% by weight of the NaCl crystals were smaller than 500 μ m. The particle size distribution of the NaCl porogen powders was determined by sieve analysis.

Granule preparation method comprised the successive steps of

- (1) dry mixing 40.0 g of the cement powder with 70.0 g of NaCl in a Turbula[®] T2F mixer for 90 min in a polyethylene bottle,
- (2) wetting the powder with an appropriate volume of a mixture of high-purity ethanol (99.9%, Merck KGaA, Darmstadt) and 3.5 wt.% Na₂HPO₄ aqueous solution (i.e., 6.7 mL ethanol mixed with a 12 mL aliquot of 3.5 wt.% Na₂HPO₄ solution),
- (3) kneading the wet powder body for 4 min to form a dough with a special mixer in a glass container,
- (4) immediately sieving that wet dough within the following 2 min in an automatic sieve shaker, with multiple plastic sieves, to in situ form granules of desired sizes,
- (5) drying the as-formed granules in static air for 1 h,
- (6) leaching out the NaCl porogen by soaking the granules in a sterile glass container with circulating distilled water at room temperature for 48 h,
- (7) checking the efficiency of leaching (of NaCl) by measuring the conductivity of the washing water at regular intervals, and
- (8) drying the washed granules at 37 °C in an air-circulated oven for 24 h in glass trays.

In a typical sieving/granulation run, the following stack of sieves was used: mesh no. 18 (1.0 mm), mesh no. 16 (1.19 mm), mesh no. 10 (2.0 mm), mesh no. 7 (2.83 mm) and mesh no. 5 (4.00 mm). The nominal sieve openings were given above in parenthesis next to the mesh numbers.

Conductivities of the NaCl leach-out solutions were measured by using a commercial electrolytic conductivity meter (Hanna Instruments, Model 98311, Kehl am Rhein, Germany). 1,000 ppm NaCl-containing solutions had a conductivity of around 2,000 μ S/cm at 21 °C. Washing of the granules was continued until a conductivity reading of 1 μ S/cm reached, which was a value close to that of

distilled water. Granule washing baths reached that level of low conductivity after 24–32 h of soaking.

Granules were characterized by scanning electron microscopy (SEM, Model JEOL630, Jeol Corp., Tokyo, Japan), energy-dispersive X-ray spectroscopy (EDXS, Thermo-Keveex, San Jose, CA), powder X-ray diffraction (XRD, Cu K α radiation, Model D5000, Siemens GmbH, Karlsruhe, Germany), Fourier-transformed infrared spectroscopy (FTIR, Model Nicolet 550, Thermo-Nicolet, Woburn, MA), ICP-AES analyses (Model 61E, Thermo Electron, Madison, WI), density (Pycnometer, Model AccuPyc 1330, Micromeritics, Norcross, GA), compressive strength measurements (Model 4500, Instron Deutschland GmbH, Germany), and BET surface area measurements (Model ASAP 2020, Micromeritics Corp., Norcross, GA). The water absorption percentage determination required the granule samples to be first boiled in water and then remain submerged while the water was naturally cooling and filling the pores. The samples were first weighed dry, then weighed while suspended in water, and weighed after superficial drying to obtain the desired information. This testing method was described in ASTM Standard Test Method C20-92 [62].

Samples were coated with a 50–70 nm-thick layer of carbon prior to SEM imaging. EDXS analyses were performed on the carbon-coated granule samples to detect any residual chlorine, after the washing step. XRD data were gathered over the 2θ range of 10–60°, by using a step size of 0.02 and a preset time of 1 s. 35 mA and 40 kV were the respective power and voltage settings of the X-ray diffractometer. FTIR analyses, on the other hand, were performed after diluting the pulverized (into a fine powder) granule samples in IR-grade KBr at the sample-to-KBr weight ratio of 1:100, followed by pelletizing in a 1 cm steel die at 25 MPa. Granule densities were measured by using a standard gas pycnometer. During the ICP-AES analyses of dried granules, 50 mg portions of samples were dissolved in 5 mL of concentrated HNO₃ solution. Compressive strength measurements were performed after filling a 2.5 cm-diameter stainless steel die with granules (just enough to form a monolayer of granules), followed by gentle tapping of the die to facilitate the even packing of granules. An automated Instron universal testing machine was used to push the upper punch into the die cavity at the crosshead speed of 1 mm/min. The BET surface area of the 2–2.8 mm granules was determined by applying the standard Brunnauer–Emmet–Teller method to the nitrogen adsorption isotherms obtained at –196 °C using the Micromeritics ASAP 2020 instrument.

To test the compressive strength of the as-synthesized granules, about 20 granules (2–2.8 mm in diameter) were evenly placed, in the form of a monolayer as much as possible, onto the surface of the lower punch inserted from

bottom into the circular cavity of a stainless steel die (2.5 cm in diameter), followed by gently placing the upper punch directly onto the granules. For measuring the compressive load-bearing capacities of granules, the above-mentioned die assembly was placed between the load plates of the Instron machine. The applied force versus displacement curves of granules originated from seven different production batches were recorded at 20 ± 1 °C, 50% relative humidity and a crosshead speed of 1 mm/min under compression.

Additionally, granules pre-packed in the bone grafting system VacPac™ (Biomet, Inc., Warsaw, IN) were soaked with citrated blood. In brief, six times 9 mL of whole blood of healthy donors of either gender was drawn into 10 mL Monovettes™ (Sarstedt, Germany) containing 1 mL of 0.106 M sodium citrate solution commercially available VacPac™ provided 10 cm³ of dry cancellous allograft pre-packed under vacuum suction that could easily be hydrated completely with, for instance, PRP (platelet-rich plasma). For the experiments each 5 cm³ of granules were pre-packed in a VacPac™ pouch and sterilized by gamma irradiation. Citrated blood was activated by adding the donor's autologous thrombin to a ratio of 1:10 and quickly each 6 mL of the activated blood product were used to soak the granules. Autologous thrombin was produced by collecting 9 mL of whole blood into a Serum Gel Monovette™ (Sarstedt, Germany) and centrifugation of the clot for 5 min at 1,800 rcf. The serum supernatant containing the autologous thrombin stabilized by the fibrin

clot [63] was carefully recovered and calcified with a 10% (w/w) CaCl₂ solution (1:4) prior to use. For clot formation and retraction the VacPacs™ were incubated at 37 °C for 1 h and overnight at 4 °C.

3 Results and discussion

The photograph of Fig. 1a showed the as-formed granules in two different sizes (2.8–4 mm and 2–2.8 mm, respectively). The size distribution of NaCl-containing granules obtained in one typical setting-sieving batch (i.e., wet cement dough was set in situ during sieving) was given in Table 1. The weight percentages given in Table 1 were the average values obtained from ten separate runs. Size distribution could be slightly altered by varying the L/P (liquid-to-powder) ratio of the NaCl-containing cement dough placed onto the sieve shaker (data not shown). The discussion of the effect of L/P ratio was left outside the scope of this manuscript. NaCl leachout did not change the granule size distributions.

Figure 1b exhibited the low-magnification SEM photomicrograph of the obtained spherical granules, and the cubic imprints of the embedded NaCl porogen crystals were visible. NaCl porogen crystals were totally leached out upon washing. In other words, NaCl crystals left behind their footprints. Those square nests formed (after dissolution of these NaCl crystals) in the calcium phosphate cement matrix were like the “replicas” of those crystals,

Fig. 1 (a) Photograph of porous granules shown at two different sizes, (b) low magnification SEM image showing the nests of NaCl porogen crystals, (c) and (d) high magnification images detailing the microstructure of carbonated, apatitic CaP microporous cement matrix

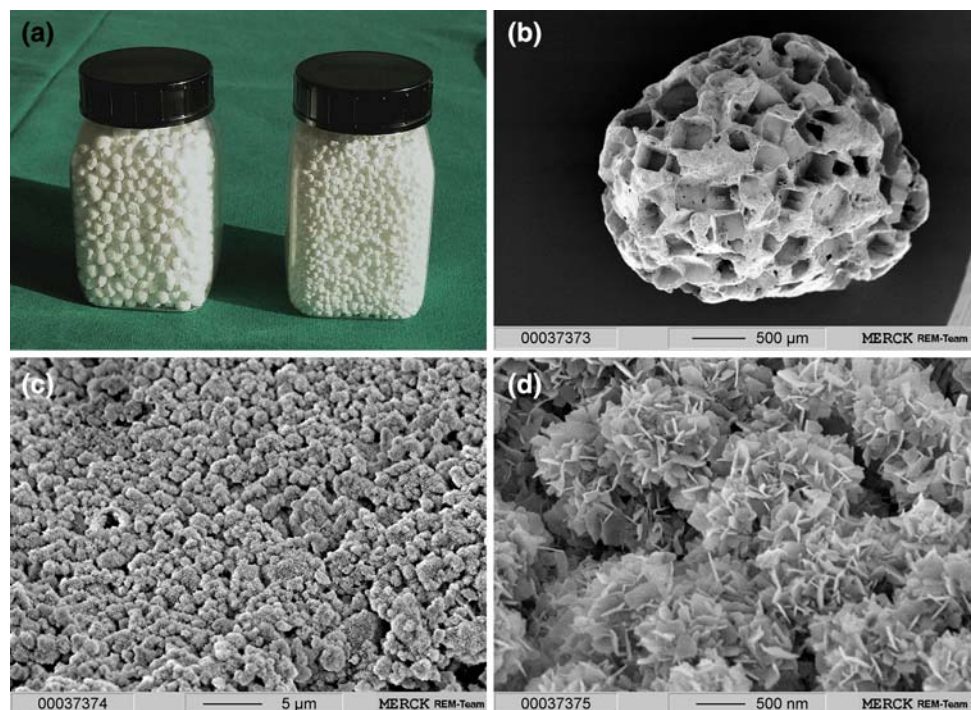


Table 1 Granule size distribution^a

Granule size range ^a (mm)	Weight % ($\pm 3\%$)
2.83–4	15
2–2.83	35
1.19–2	36
1–1.19	4
<1	0

^a Sizes were given in accord with the mesh numbers of sieves used

and thus the product could be identified according to its manufacturing process. Macropores, being simply dependent on the crystal size of NaCl used in processing, could be readily varied over the range of 50–800 μm . However, for the sample shown in Fig. 1b, pore sizes were from 50 to 400 μm .

High-magnification SEM photomicrographs of the granules given in Fig. 1c, d consecutively zoomed into the microporous matrices (i.e., the dense looking areas of Fig. 1b) of the granules. The three-dimensional microporosity ranged in size from 1 to 4 μm , as seen in Fig. 1c. Since the cement of this study, i.e., Calcibon[®], was an α -TCP-based cement [64–66], with the major additives of CaHPO_4 and CaCO_3 , upon setting within 7–8 min, it formed a web of interlocking and intermingling nanosize platelets (as shown in Fig. 1d) of Ca-deficient apatitic CaP with a Ca/P molar ratio of 1.52 ± 0.1 . This Ca/P molar ratio was determined by ICP-AES analyses and reported as the average of seven different samples. Granulation process used in this study did not alter or destroy the cement stoichiometry (by ICP-AES and XRD data not shown) either prior to or after granulation.

“Biological” or “bone-like” apatite, which constitutes the bone mineral, is known to be a carbonated (4–10 wt.%), poorly crystallized, alkali (i.e., Na and K) and alkaline earth (Mg) element-doped, non-stoichiometric, calcium-deficient apatitic phosphate with a Ca/P molar ratio variable over the range of 1.50–1.70 [67–69]. The CaP cement used in the production of the porous granules of this study was previously shown to yield a fast deposition of new bone at the cement surface in its paste form [70].

XRD analysis of these granules yielded the characteristic poorly crystallized apatitic CaP spectra, as shown in Fig. 2a. The complete setting reaction for this high strength (>55 MPa under compression) cement has been described elsewhere in detail [64–66]. However, to mention briefly, the major component of this cement, i.e., α - $\text{Ca}_3(\text{PO}_4)_2$, went through a hydrolysis reaction upon its contact with the setting solution of pH 9. The major end-product of the hydrolysis reaction was Ca-deficient apatitic CaP. CaCO_3

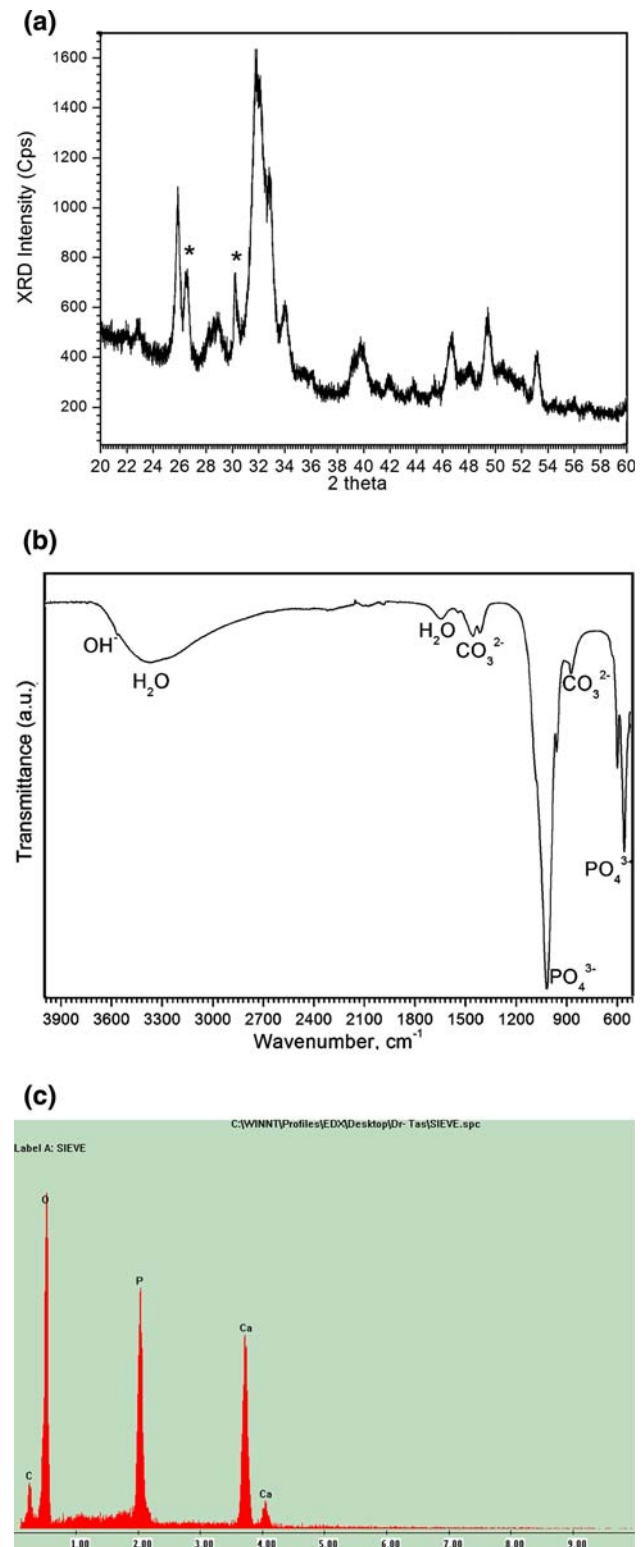


Fig. 2 (a) characteristic XRD trace of granules immediately after production (* CaHPO_4 peaks, rest: apatitic CaP peaks), (b) FTIR spectrum of granules and (c) EXDS spectrum of granules

present in the cement powder rapidly participated in this hydrolysis process facilitating the formation of carbonated, apatitic CaP [71]. Although a slow process, CaHPO_4 itself also underwent a similar hydrolysis procedure to the apatitic CaP, and the small amount of precipitated HA present in the cement formulation acted as an accelerator for those hydrolysis processes, which continued at the body temperature till the completion of the cement setting. The XRD data given in Fig. 2a belonged to the freshly produced porous granules after washing off of the NaCl porogen crystals in deionized water at 37 °C for 48 h, i.e., after step 7, but prior to step 8. CaHPO_4 was present as a secondary phase in the as-formed granules, which was also the case for the setting of NaCl-free cement samples [61]. Presence of NaCl crystals in granule production by using this cement did not affect the cement setting process. The absence of NaCl peaks in the XRD traces proved the efficiency of the washing process.

The carbonated and bone-like nature of the porous granules was strongly indicated by the characteristic CO_3^{2-} bands seen at 873 and 1,450 cm^{-1} in the FTIR data of Fig. 2b. The weak band seen at 3,571 cm^{-1} belonged to the OH stretching. Orthophosphate bands were also observed at their characteristic positions [72].

EDXS analyses performed on the washed granules showed that they did not contain any Na^+ or Cl^- ions originating from the use of NaCl as the porogen. EDXS results (accurate to ± 0.5 wt.%), shown in Fig. 2c, only exhibited the extraneous C peaks due to the carbon coating of granules prior to the analysis.

The formed granules (between 2 and 4 mm sizes) had a pycnometrically determined density of $1.60 \pm 0.15 \text{ g/cm}^3$, which corresponded to about 50% total porosity, considering the fact that the density of the fully set, fully dense Calcibon[®] cement was around 3.2 g/cm^3 [64–66]. Porous granules also possessed a water absorption percentage of 150, measured according to the aforementioned ASTM standard [62]. This property itself imparted the granules a significant wicking ability [73]. Percentage macro- and microporosity values would have best been determined by using, for instance, computerized tomography techniques; however, based on our available density and water absorption percentage data the total porosity in all the granule synthesis batches (either at the laboratory-scale or in the up-scaling batches) was never recorded to be less than 50%.

Compressive strength measurements performed on monolayers of around 20 granules ($2 < x < 2.8$ mm in diameter) are presented in Fig. 3. For each test a more or less jagged (zigzag) graph was initially recorded. Due to differences in sizes between the assembled granules, the method used to measure the compressive strength of granules would inevitably result in not all of the granules

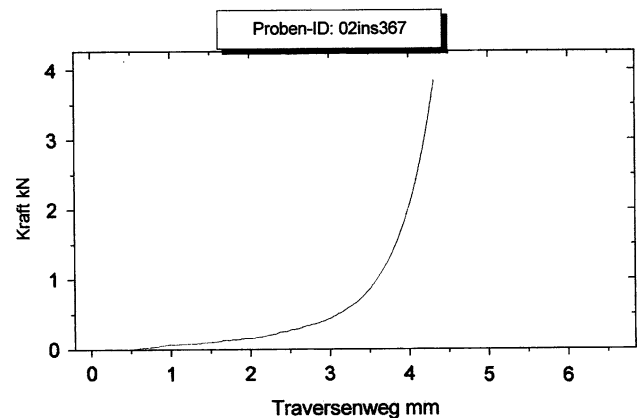


Fig. 3 Applied load (Kraft) versus displacement (Traversenweg) curve of as-synthesized granules

being compressed at the exact same time. In other words, the smaller granules would not be compressed until the larger granules have been slightly compressed. The characteristic data presented in Fig. 3 corresponded to a maximum failure load of $3.87 \pm 0.11 \text{ kN}$ and to $3.83 \pm 0.09 \text{ mm}$ displacement at the maximum load. These values translated to a compressive strength of $10.19 \pm 0.16 \text{ MPa}$. The traces obtained from seven different batches of granules were almost perfectly reproducing one another; in other words, compressive strength tests were not alone able to tell the differences between production batches, if there were any.

As presented in Fig. 4, the granules were tightly fixed within a clotted citrated blood, and the visible macropores were readily filled with blood. Nevertheless, the granules were not completely soaked with citrated blood as the white color of the calcium phosphate could still be seen clearly. To test whether the granules were completely soaked with blood, the granules were recovered after maximal clot retraction, embedded in a resin and a polished

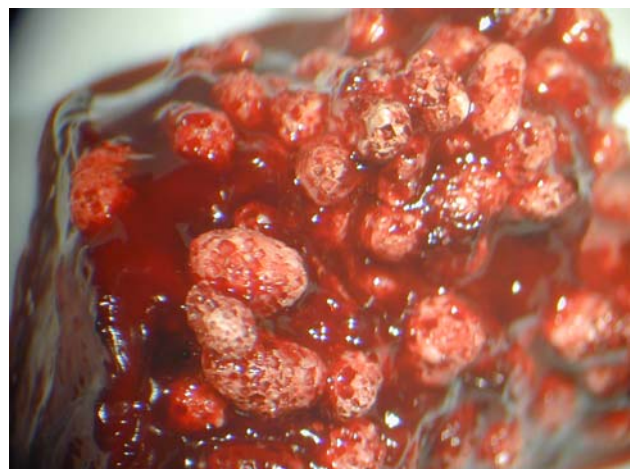


Fig. 4 Photograph of tightly fixed granules within clotted citrated blood

section was obtained by grinding (Fig. 5). As can be seen, the granules manually mixed (Fig. 5a) with citrated blood were soaked with less blood than the granules that had been pre-packed in the VacPac™ system (Fig. 5b).

The cement liquid (3.5 wt.% Na_2HPO_4 solution), when mixed with the cement powder of this study, resulted in a smooth and malleable calcium phosphate paste. This paste showed the ability of seamlessly embracing and surrounding the cubic NaCl crystals. The use of ethanol in the granule manufacturing was only for the purpose of retarding the setting reaction for a few more minutes to allow successful sieving. Ethanol used was either evaporated or washed away with water during the later, washing-drying stages of the granule manufacturing process. During leaching out of the embedded NaCl crystals, the effluent solutions were monitored with respect to their electrolytic conductivity on a real time basis. It is known that even ppm levels of dissolved NaCl in water would cause an increase in the conductivity values of such solutions. Washing operation was terminated when the conductivity of the effluent solutions reached the level of almost that of distilled water (i.e., $1 \mu\text{S}/\text{cm}$).

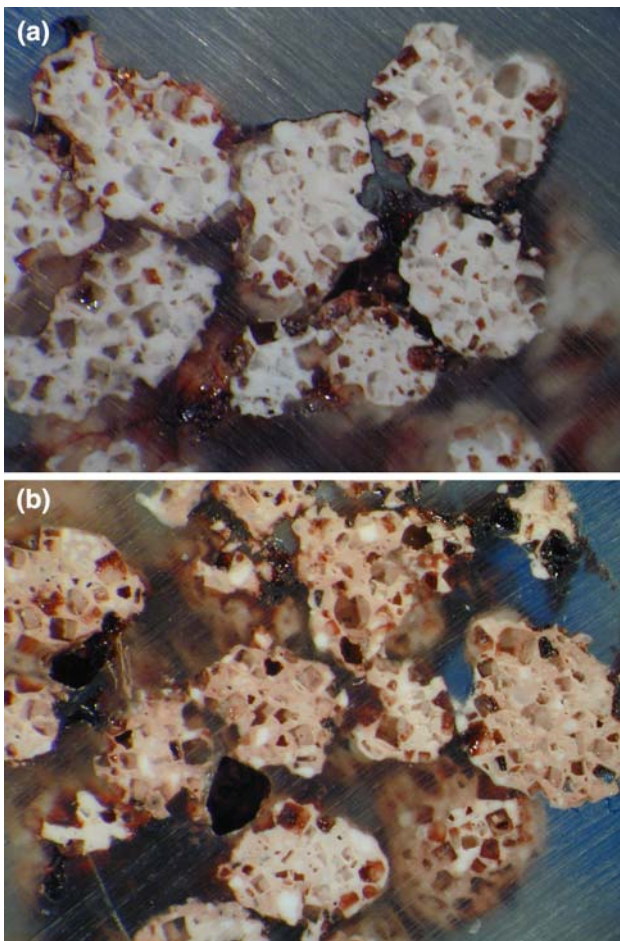


Fig. 5 Polished sections of granules (a) manually mixed with citrated blood and (b) pre-packed in the VacPac™ system

The wet, freshly formed granules hardened at the ambient temperature by an endothermic reaction. The chemical composition and crystalline structure of the cured material did mimic the mineral part of natural bone, as depicted especially in the IR spectrum of Fig. 2b.

Tadic et al. [74] used a NaCl porogen technique similar to ours to produce macropores with pore diameters in the range of 250–400 μm in CaP scaffolds. Nevertheless, their starting material was just a precipitated hydroxyapatite in powder form, which was far from being a self-setting cement. In order not to cause any undesired grain growth and a decrease in the surface reactivity of their powders, they apparently did not calcine their precipitated powders, and used such powders as received. Tadic et al. [74] unfortunately needed to compact their NaCl-embedded hydroxyapatite powder blocks by using cold isostatic pressing. Since they did not sinter their 3D objects, after porogen removal and consequent pore formation, it was not possible to mention any handling strength to be present in such devices for the orthopedic surgeon. The above-mentioned report [74] constituted a vivid example on the significance of using strong self-setting cements in forming porous CaP-based medical devices by the porogen technique. Formation of macropores in a TTCP ($\text{Ca}_4(\text{PO}_4)_2\text{O}$)-based cement paste was previously shown by Takagi and Chow [75] and they used either sucrose, NaHCO_3 or Na_2HPO_4 crystals (125–250 μm particles) as the porogen. The loading of the porogen crystals was much lower (33%) than that employed in the current study (65%). Moreover, Takagi and Chow [75] pressed (at 2 MPa) their porogen + cement pastes into well-defined cylindrical shapes by using a steel die. Xu et al. [76] used mannitol as the porogen to produce macropores in a TTCP ($\text{Ca}_4(\text{PO}_4)_2\text{O}$)-based cement. Lee et al. [77], on the other hand, used carbonated mixtures of $\text{Ca}(\text{OH})_2$ and NaCl to form macroporous CaCO_3 scaffolds. Guan and Davies [78] had tried PLGA as the porogen in a TTCP-monetite cement.

The granules of the present study regarded to be one-time skeletal repair implants which shall stay in place until their potential complete biodegradation (by the osteoclastic resorption) by the body. According to Bohner and Baumgart [79], the *in vivo* resorption rate of porous granules can be theoretically predicted to be much faster than the dense blocks or prismatic objects made out of the same material. Depending on the indication, accurate repositioning, as well as fixation or stabilization, of the granules should be performed as if using an autologous bone graft [80]. Such granules should be intended for filling and reconstruction of large, aseptic, metaphyseal, cancellous bone defects. These bone defects might be caused by trauma or other genesis, e.g., a benign tumor, from surgery or congenital.

Calcium phosphate bone substitutes impregnated with, for instance, bone marrow aspirate (BMA) [81], autologous platelet-rich plasma (PRP) [82], osteocalcin [83] or mesenchymal stem cells [84] were already shown to significantly improve the skeletal repair abilities of the otherwise pristine materials. Therefore, these granules are expected to perform better in vivo when they are first impregnated with PRP or BMA and then used.

4 Conclusions

Micro- and macroporous carbonated, apatitic calcium phosphate granules for skeletal repair were produced by the NaCl porogen technique by using a self-setting CaP cement powder as the starting material. The high strength CaP cement used here facilitated the economical production of porous granules.

- (1) The simple process used in granule manufacturing allowed the precise selection and specification of the final granule sizes over the range of 2–4 mm,
- (2) Pore sizes over the range of 50–550 μm were readily achieved,
- (3) Granules had about 50% porosity,
- (4) Granules were produced from totally synthetic materials, such as $\alpha\text{-Ca}_3(\text{PO}_4)_2$, CaHPO_4 , CaCO_3 and $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$,
- (5) Granules comprised of carbonated, calcium-deficient, poorly crystallized apatitic CaP phase with a Ca/P molar ratio of 1.52 ± 0.1 ,
- (6) Granules had a high water absorption percentage (ca. 150%),
- (7) Granules were produced at the ambient temperature.

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