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# Microstructure Formation in Porous Calcium Phosphate–Chitosan Bone Cements

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**Abstract**—We have studied the microstructure formation in composite cement materials consisting of amorphous calcium phosphate and chitosan and reinforced with sodium alginate granules, their compressive strength, and the kinetics of their dissolution in an isotonic solution. Hardening in air allowed us to obtain a cement stone with a strength of up to 18 MPa and limiting strain of 6–8%. During hardening in simulated body fluid, the maximum strength decreased to 0.8 MPa, and the strain increased to 25–30%, which was due to the dissolution of the granules and the increase in the elasticity of the chitosan framework. The calcium ion release to the isotonic solution varied nonmonotonically, with a maximum in the initial stage of hardening. The cements are intended for the fabrication of porous matrices in bone tissue regeneration.

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## INTRODUCTION

To restore damaged bone tissue, use is made of porous calcium phosphates, which can be produced via ceramic or cement route [1–4]. Pores provide space for biological flows, life activity of cells, and vascularization and may serve as reservoirs for targeted delivery of drugs: peptides, antibiotics, and antineoplastic and anti-inflammatory drugs. In the technology of porous calcium phosphate cements (CPCs), to a binder system, comprising a solid (powder) and a liquid phase, are added pore formers, which are removed after the curing of the binder system, for example, soluble salt crystals and sugar, ice, or water-soluble polymer particles [5–9]. Such additives are capable of ensuring a macroscopic porosity of up to 50 vol %. Porosity in cements can also be produced using foaming agents, for example, ammonium carbonate or calcium carbonate, which react with cement liquid (as a rule, orthophosphoric acid) to release carbon dioxide gas [10]. This approach may ensure higher porosity, but the pore size is in this case difficult to control.

Characteristically, the CPCs have low strength and high brittleness. This limits their potential clinical applications, especially for defect plastics in places with bleeding, tissue fluid circulation, or salivation and for defect filling in bones under mechanical load. To obviate the above drawbacks, considerable research effort has been concentrated on polymer–calcium phosphate composites in which the polymer component has the form of a framework that imparts a necessary deformation ability (elasticity) to the cement, whereas the presence of a calcium phosphate phase ensures biocompatibility and bioactivity. One of the most attractive biopolymers for this purpose is chitosan, a natural polysaccharide resulting from chitin deacetylation, which possesses many useful biological properties [11, 12]. The use of chitosan as a polymer component has made it possible to impart elasticity to an amorphous calcium phosphate (ACP) based cement and considerably improve the deformation ability of the material: after holding in a physiological salt solution, its compressive strain reached 25% [13, 14]. Chitosan is soluble in acid media but becomes insoluble at neutral or alkaline pH. This effect was used as the basis for the technology of proposed cements. The pH of cement liquid was controlled by adding tetracalcium phosphate (TeCP) as a major component.

The purpose of this work was to study the in situ porosity formation in calcium phosphate cements on the addition of granules soluble in human body fluids and to assess the strength, deformation ability, and dissolution kinetics of the materials.

## EXPERIMENTAL

The powder component of the CPCs consisted of ACP and TeCP in the ratio 1 : 1.3 [13]. To the ACP powder were added sodium alginate granules 100–200 or 300–500 μm in size (1, 3, or 5 wt %). The granules were prepared by grinding commercially available alginic acid (Aldrich, #180 947) and were then classified into size ranges by sieving. To the mixture thus prepared was added cement liquid: a 3% solution of high molecular weight chitosan (500 kDa) in orthophosphoric acid. Next, TeCP powder was added to the mixture, which changed pH to neutral. This led to chito-

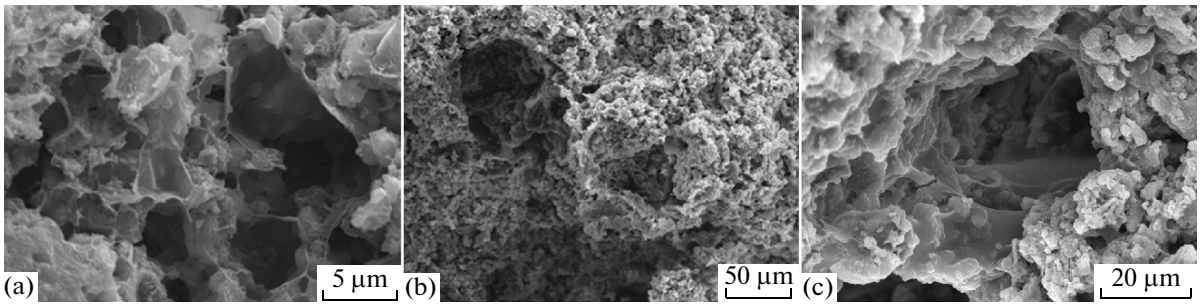


**Fig. 1.** SEM images of portions of the microstructure of the cement matrix hardened in air (a) and cement containing 3 wt % alginate granules after hardening in SBF for 28 h (b) and holding in an isotonic solution for 28 h (c).

san polymerization, the formation of a chitosan framework, and the setting and hardening of the cement. The solid : liquid ratio in the CPCs was 1 : 1, because at a lower cement liquid content we failed to obtain homogeneous cement paste, whereas at a higher cement liquid content the cement paste had too thin a consistency and its setting time reached several hours. Setting took place in air or in simulated body fluid (SBF). The SBF composition was as follows (g/L): NaCl, 6.547; $NaHCO_3$, 2.268; KCl, 0.373; $Na_2HPO_4 \cdot 12H_2O$, 0.178; $MgCl_2 \cdot 6H_2O$, 0.305; $Na_2SO_4$, 0.071; $CaCl_2 \cdot 2H_2O$, 0.368; Tris buffer, 6.057.

The samples were characterized by scanning electron microscopy (SEM) (TeScan VEGA II microscope) and X-ray diffraction (Shimadzu XRD-6000 diffractometer). Open porosity was measured by hydrostatic weighing. Cylindrical specimens were tested in axial compression on an Instron 5581 tensile tester. Solubility was determined according to the R ISO 10993-14-2001 standard by measuring calcium ion concentration in solution. The samples were held in an isotonic 0.1 M NaCl solution for up to 28 days at a constant liquid phase volume (closed system), pH 7.4, and a temperature of 37°C. The calcium concentration in the liquid phase was measured using an Optima inductively coupled plasma atomic emission spectrometer. The uncertainty in our measurements was 0.01 mg/L.

## RESULTS AND DISCUSSION

The setting time of the cement paste ranged from 30 min to 2 h. The initial pH of the system was 7.1. Figure 1 shows SEM images of a portion of the microstructure of a cement matrix after hardening in air and the cement with granules after hardening in SBF and after holding the air-hardened cement in the isotonic solution. The cement matrix was porous, and the chitosan formed a framework (Fig. 1a). The pores were interconnected and ranged in size up to 10 μm. Both hardening in SBF and holding of the air-hardened cement in the isotonic solution led to a further increase in porosity (Figs. 1b and 1c, respectively). The forming large macropores were interconnected. This ensured dissolution of the granules during holding in both liquids. As a result of the granule dissolution, the average open porosity increased from 33 to 52 and from 28 to 42% in the samples reinforced with sodium alginate granules 100–200 and 300–500 μm in size, respectively. After 14 days of hardening in air, the compressive strength of the CPC without granules was 6.2 MPa. The limiting strain corresponding to the maximum load in the stress–strain diagram was about 6%. Figure 2a shows stress–strain diagrams of the CPCs containing 1 wt % granules 100–200 μm in size. As the hardening time increases to above three days, a considerable hardening effect is observed. The hardening is essentially independent of the alginate granule size (Fig. 2b) but decreases as the granule content increases to 5 wt % (Fig. 2c). The breakdown strain of all the materials is 6–10%.

When the granule-reinforced cement breaks down, a crack propagates through the brittle matrix. Since the stress intensity necessary for crack propagation in a plane other than the normal fracture plane is always higher, a change in crack trajectory may lead to an increase in the strength of the material. Holding in SBF did not increase the strength of the material. Figure 3 shows stress–strain diagrams of the CPCs containing granules hardened in SBF. The strength of the materials is under 0.8 MPa, which may be caused by both the increase in porosity due to granule dissolution and the reduction in the strength of the chitosan framework in solution. At the same time, the deformation ability of the materials increases considerably, reaching 25–30%, owing to the elasticity of the chitosan in the isotonic solution. For comparison, the strength of a CPC having the same composition but containing no alginate granules was 1.2 MPa, and its limiting strain was 20–25%.

During the holding of the alginate-granule-containing CPC samples in the isotonic solution, the calcium ion concentration varied in an unusual manner. After holding for 24 h, the calcium concentration reached a maximum. Next, it dropped sharply and then slowly increased for 25 days (Fig. 4). The sharp drop can be accounted for by the binding of the cal-

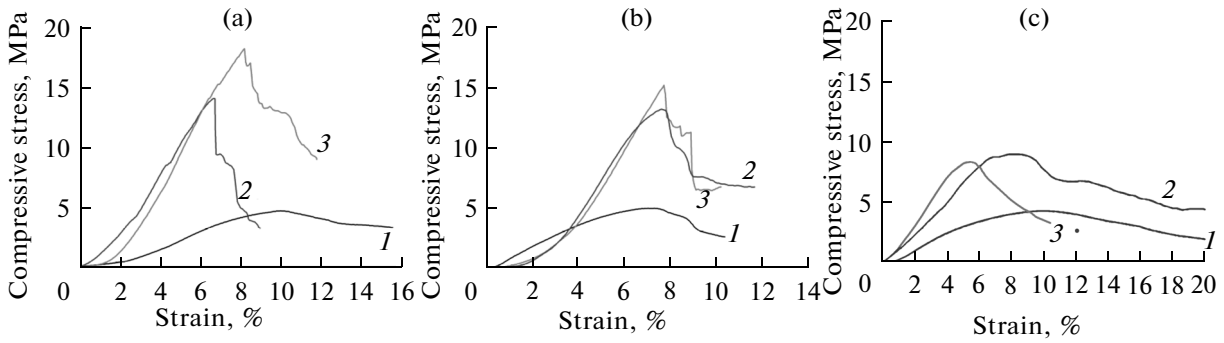


**Fig. 2.** Compressive stress–strain diagrams of air-hardened cements containing granules: (a) 100–200 μm, 1 wt %; (b) 300–500 μm, 1 wt %; (c) 300–500 μm, 5 wt %; hardening for (*1*) 3, (*2*) 7, and (*3*) 14 days.

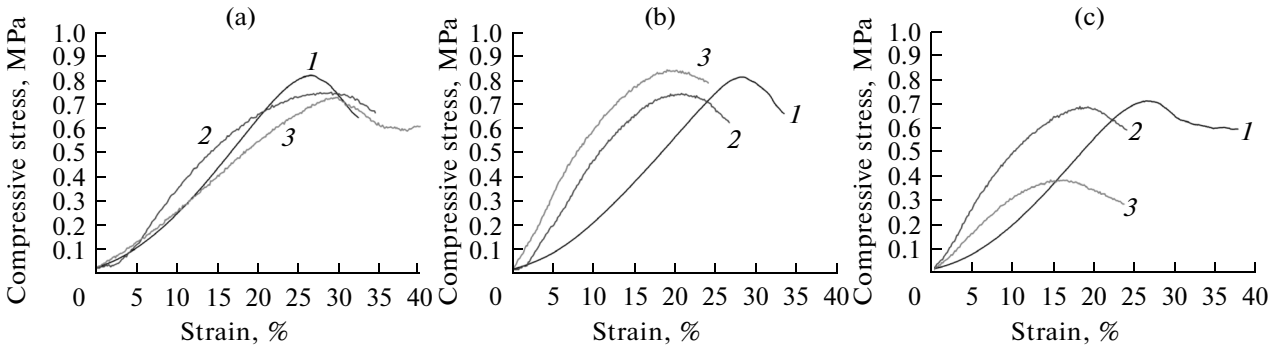


**Fig. 3.** Compressive stress–strain diagrams of SBF-hardened cements containing granules: (a) 100–200 μm, 1 wt %; (b) 100–200 μm, 3 wt %; (c) 300–500 μm, 5 wt %; hardening for (*1*) 3, (*2*) 7, and (*3*) 14 days.

cium ions by the alginate dissolving in the isotonic solution. The subsequent increase in calcium concentration in solution is probably due to the calcium release from the dissolving cement matrix, which increases over time.

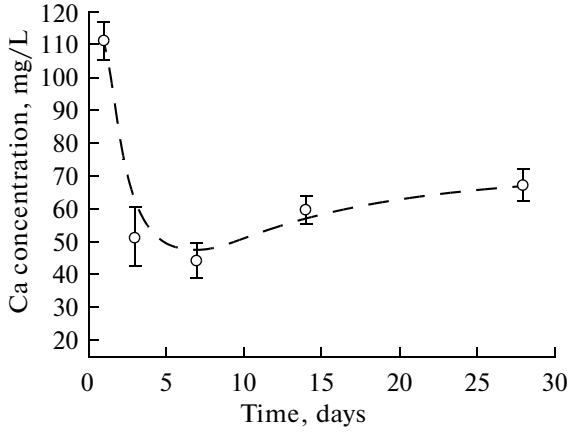


**Fig. 4.** Time dependence of the calcium ion concentration in an isotonic solution during holding of cement containing 3 wt % granules 300–500 μm in size.

## CONCLUSIONS

The addition of sodium alginate granules to cements consisting of ACP, TeCP, and a chitosan solution in orthophosphoric acid has a significant effect on the strength and deformation ability of the cement stone. The strength increases over time during hardening in air and remains essentially unchanged during hardening in SBF. We have produced materials with a fracture strain of 6–10% in air and 25–30% in SBF. Granule dissolution during the holding of the composite material in the isotonic solution models in situ porosity formation in the human body. The described materials can be used as matrices in cell technologies for bone tissue regeneration.

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