CHEMICAL TECHNOLOGY

Bone Cements in the Calcium Phosphate–Chitosan Systems Containing Magnesium and Zinc

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Abstract—Based on studying the possibility of the introduction of physiologically important cations into a cementing system, technology of fabrication of highly deformable calcium phosphate cements has been developed for bone tissue reconstruction in medicine. It has been elucidated that the method of introduction of metal cations (magnesium, zinc) into the cementing system has an effect on the formation of the microstructure and properties. The in vitro degradation of composite cements in simulated body fluids has been studied. Results have been obtained for the development of cements with tailored properties, which can be varied in different ranges.

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To fill bone tissue defects resulting from surgery, pathological diseases, or injuries, calcium phosphate cements (CPCs) are used [1, 2]. Of interest is the introduction of physiologically important cations, such as magnesium and zinc, into CPCs, which should provide a depot of these elements for maintaining physiological functions. Magnesium is actively involved in almost all physiological processes in the human body, acting as a regulatory factor. Magnesium competes with intracellular calcium, and its content has an effect on metabolism, protein synthesis, cell division, and generation of hormones playing an important role in the biomineralization [3]. Of special significance is zinc, which is involved in the synthesis of bone matrix [4]. It has been found that the defects of human bone system are due to zinc deficiency [5]. Earlier, there have been developed composite CPCs containing chitosan, which imparts enhanced elasticity and rupture deformation to hardened cements [6]. This paper presents the results of a study concerning the design of such deformable CPCs, but containing physiologically essential magnesium and zinc cations.

EXPERIMENTAL

The powder components of CPCs consisted of amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2 \cdot nH_2O$) and tetracalcium phosphate (TeCP, $Ca_4(PO_4)_2O$) in the 1 : 1.3 ratio. The initial ACP powder was modified by introduction Mg^{2+} and Zn^{2+} cations into it during synthesis (1, 2, and 5% as calculated for calcium). The synthesis was carried out as follows: ACP was synthesized by precipitation from aqueous solutions of calcium nitrate, ammonium hydrogen phosphate, and magnesium/zinc nitrate ($Mg(NO_3)_2$, $Zn(NO_3)_2$) according to the reaction

$$(3-x)Ca^{2+} + 2PO_4^{3-} + xMe^{2+} \rightarrow Ca_{(3-x)}Me_x(PO_4)_2, \quad (1)$$
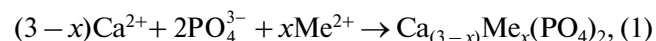
where $Me^{2+} = Mg^{2+}$ or Zn^{2+} (1, 2, and 5 wt % with respect to the calcium content). According to energy dispersive X-ray microanalysis, the cations were incorporated into ACP. To the resulting ACP powder where magnesium/zinc cations were substituted for part of calcium ions, a cement liquid (CL) was added in a 1 : 1 ratio. As the CL, a 3% solution of high-molecular-weight chitosan (500 kDa) in orthophosphoric acid was used. Then, a TeCP powder prepared by a ceramic method according to the reaction

$$2CaHPO_4 + 2CaCO_3 \rightarrow Ca_4O(PO_4)_2 + H_2O + 2CO_2 \quad (2)$$
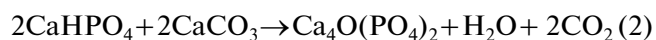
through mixing the initial components in a planetary mill in the presence of ethanol with subsequent calcination of the product at 1400°C.

The introduction of TeCP changes the pH of the cement mixture toward neutral reaction, which leads to chitosan polymerization and scaffold formation [6]. The hardening of the ACP samples took place in air or upon immersion into a solution mimicking the extracellular body fluid (SBF). The SBF composition (g/L) was 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.

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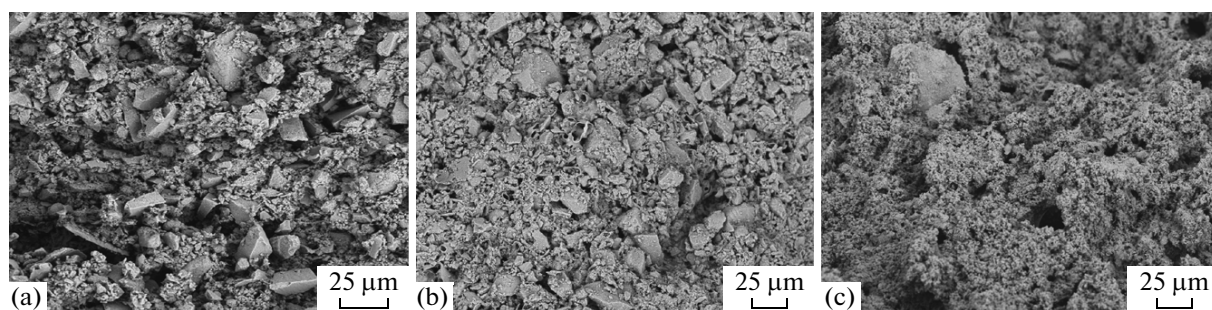*119991 Russia*
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Fig. 1. SEM images of the fracture surfaces of the CPCs kept in SBF: (a) without magnesium/zinc cations, (b) 5% magnesium substitution, and (c) 5% zinc (a) TCP and (b) sodium alginate beads after keeping in isotonic solution.
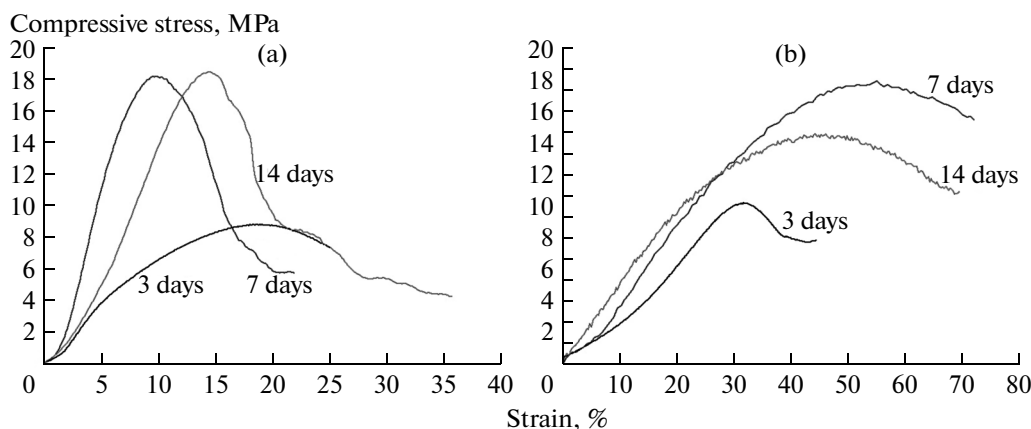

Fig. 2. Strain diagrams of the zinc-substituted CPCs: hardening in (a) air and (b) SBF.

RESULTS AND DISCUSSION

The samples were studied by scanning electron microscopy (SEM, a TeScan Vega II microscope) and X-ray diffraction (a Shimadzu XRD-6000 diffractometer). The mechanical tests were performed by axial compression of cylindrical specimens on an Instron 5581 testing machine. The solubility tests were carried out according to GOST R ISO 10993-14-2001 by measuring the content of calcium ions in solution. The samples were kept in an isotonic 0.1 M NaCl solution for up to 28 days at a constant volume of the liquid phase (closed system), pH 7.4, and 37°C. The calcium concentration in the liquid phase was measured by inductively coupled plasma atomic emission spectroscopy using an Optima spectrometer. The measurement error was 0.01 mg/L.

RESULTS AND DISCUSSION

The introduction of magnesium and zinc had no effect on the cement paste setting time, which was from 30 min to 2 h. For the cements of all developed compositions, the initial pH of the system was 7.1−7.3 and by the seventh day it increased to 7.5−7.6. According to powder X-ray diffraction, the major crystalline phase in the hardened cements is ACP.

Figure 1 shows the SEM images of the fracture surfaces of the pristine CPC free of magnesium or zinc cations (Fig.1a), CPC with 5% zinc substitution (Fig. 1b), and CPC with 5% magnesium substitution (Fig. 1c) on the 14th day of hardening in SBF. The structure of the pristine cement without magnesium or zinc cations is homogeneous, being a chitosan-based cement stone with particles of less than 1 μm in size uniformly distributed all over the volume. However, in the case of CPC containing 5% Zn, long-term storage in SBF can lead to the precipitation of tabular crystals with morphology corresponding to dicalcium phosphate dehydrate crystals, which is presumably a result of ACP hydrolysis.

The developed cements have high compression deformability (up to 50%), which is due to the formation of the elastic chitosan scaffold. Figure 2 shows the strain diagrams of CPC specimens with 5% zinc substitution kept in air and in SBF. The compressive strength of such CPCs kept in air and in SBF is as large as 16 and 1.2−1.4 MPa, respectively. The content of metal cations has no noticeable effect on the strength characteristics. The porosity of such cements hardened in SBF changed from 17% (first day of hardening) to 26% (28th day of hardening) for magnesium-
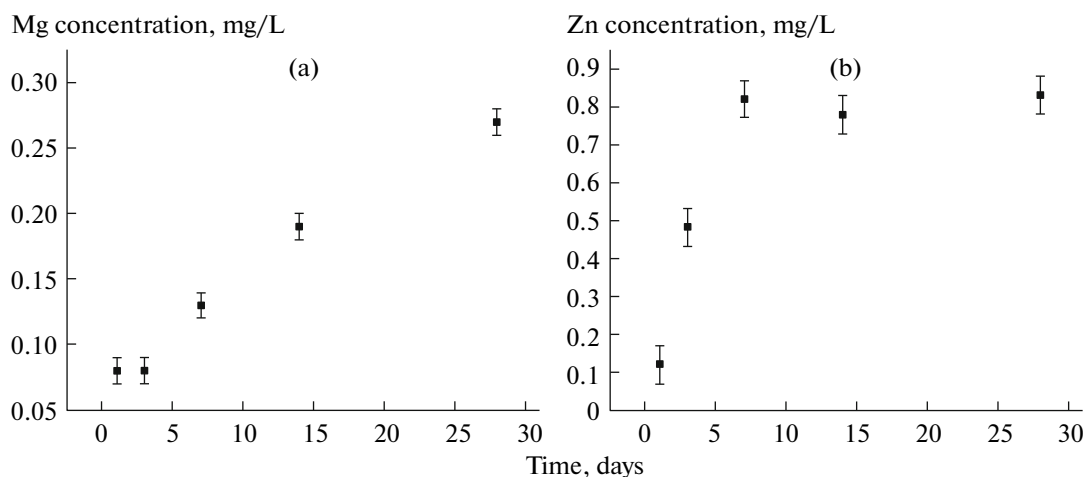
Fig. 3. Change in the concentration of (a) magnesium and (b) zinc cations when CPCs are kept in isotonic solution.

containing samples and from 16 to 19% for the zinc-containing samples.

Figure 3 shows the release of ions in solution when magnesium- or zinc-containing CPC is kept in an isotonic solution. In both cases, the kinetic law can be described by an exponential function, which corresponds to first-order kinetics (the rate of the change in the concentration is proportional to the current concentration).

CONCLUSIONS

Thus, physicochemical foundations of the design of highly deformable cements in the ACP–chitosan system containing physiologically essential Mg^{2+} and Zn^{2+} cations have been developed. The developed cements have ultimate compressive strain of up to 50%, which is due to the formation in it of an elastic chitosan scaffold. These highly deformable materials offer good prospects for the application in the bone tissue engineering, being simultaneously the source of zinc and calcium for the delivery of these physiologically important cations in the human body.

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