ISSN 1070-3632, Russian Journal of General Chemistry, 2010, Vol. 80, No. 3, pp. 666–674. © Pleiades Publishing, Ltd., 2010.
Original Russian Text © S.M. Barinov, 2010, published in Rossiiskii Khimicheskii Zhurnal, 2010, Vol. 53, No. 2, pp. 123–130.

Trends in Development of Calcium Phosphate-Based Ceramic and Composite Materials for Medical Applications: Transition to Nanoscale

S. M. Barinov

Baikov Institute of Metallurgy and Materials Science, Russian Academy of Sciences,
Leninskii pr. 49, Moscow, 119991 Russia
e-mail: barinov@imet@ac.ru

Received January 10, 2009

Abstract—Current status and lines of research were considered for calcium phosphate-based ceramic materials intended for application in new technologies for damaged bone tissue regeneration. The main trend is toward mimicking the composition, structure, and properties of biological bone tissues by ceramic materials. This necessitated developmental efforts on synthesizing polysubstituted, in particular nanocrystalline, biphasic calcium phosphates and on preparing ceramics and composite materials thereof. The progress achieved in this area was reviewed, and certain benefits associated with medical application of these materials were discussed.

DOI: 10.1134/S1070363210030461

INTRODUCTION

In recent years there has been major intensification of research into new bone surgery techniques, including those for removal of malignant tumors (osteosarcomas). These methods involve intervention into human body, which typically leads to extensive postoperative defects. There is a need in replacing the defects to restore the disturbed functions of organs and make the life comfortable for patients. Application for this purpose of auto- and allografts (bone tissue implants from the patient or a donor, respectively) entails certain difficulties (eliciting immune responses, generating the need for reoperations). Implants made of synthetic materials (plastic, metals, ceramics) typically do not yield satisfactory results because of inadequate biological and mechanical compatibility of the implant material with body tissues.

Nearly a decade ago, the concept of restoration, rather than replacement, of the removed segment of bone tissue was advanced. Restoration proceeds on porous resorbable matrices with bone-forming cells cultivated therein [1, 2]. Of key importance is the choice of the matrix whose material and architecture should satisfy a number of requirements. These include, in particular, biological compatibility, osteoconductivity, certain resorbability rate, and adequate mechanical to sustain physiological loads [1–3].

The most promising matrix materials to be used for damaged bone regeneration are those based of calcium phosphates [3–5]. Research and developmental efforts dedicated to medicinal applications of calcium phosphate-based materials have a fairly long history. Nevertheless, many relevant problems still remain to be solved in order to prepare materials satisfying all the above-mentioned criteria.

Presented here is a brief overview of the progress achieved by Baikov Institute of Metallurgy and Materials Science, Russian Academy of Sciences, in development of ceramic and composite materials intended for use as matrices for bone tissue regeneration. The emphasis is placed on synthesis of calcium orthophosphates, in particular, in nanocrystalline form; techniques for preparation of nanostructured ceramics and that from thermally unstable orthophosphates; and biopolymer–calcium phosphates composite materials.

State of the Art

The first *in vivo* experiments with calcium phosphates date back to 1920s [6], but biological

compatibility of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ and its ability for osteointegration were proven only three decades ago [7, 8]. Relevant research and development efforts were originally focused on preparation of hydroxyapatite ceramics implants. The intention was to develop ceramic materials to fill the space previously occupied by bone defect so as to replace it and provide high reliability and durability of such construct throughout the patient's lifetime. However, the first attempts to develop ceramics with properties even approaching those of biological bone tissue were unsuccessful because of the difficulties in modeling of its complex organization.

Bone tissue is a composite material possessing unique properties stemming from its complex multilevel structure [9]. It basically consists of collagen fibers with nanocrystals of apatite-like calcium phosphates deposited thereon. In human body bone tissue undergoes permanent reorganization (remodeling) in a cycle including resorption of the apatite-like phase of bone with osteoclasts and mineralization, i.e., deposition onto collagen fibers of nanodisperse calcium phosphate from extracellular liquids. There is a good reason to suggest that gaining insight into this process specifically gave an impetus to development of a novel medical technology, the so-called bone tissue engineering. It aims to regenerate the damaged bone tissue, rather than replace the defect with implant. This technique is underlain by the fundamental concept presupposing that the body itself is able of restoring the damaged tissue under proper conditions. These latter include a matrix whose architecture sustains the living activity of cells to support osteosynthesis and appropriate stimuli.

As mentioned above, of key importance for damaged bone tissue regeneration applications is the choice of the matrix material. It should satisfy the following requirements: be biologically compatible (i.e., do not elicit negative responses from the body); provide living space for osteogenic cells, neovascularization (growth of new blood vessels), and biological flows; be resorbable over time so as to be gradually replaced by newly formed bone tissue; and be able to sustain mechanical loads in the transition period of a new bone tissue formation.

Bone tissue engineering techniques imply that osteogenic cells (osteoblasts or stem cells combined with morphogenetic proteins which induce differentiation of stem cells into osteoblasts) are cultivated into the pore space of the matrix, whereupon such "living" composition is implanted *in situ* into the bone tissue defect. Regeneration proceeds via functioning of collagen-forming osteoblasts, mineralization of the inorganic phase on this collagen matrix, resorption of the initial matrix with osteoclasts, and neovascularization. The period required for complete regeneration is determined by numerous factors (typically, it takes several months).

Numerous materials were tested for matrix applications. Much hope was placed on resorbable polymers such as polylactides and their copolymers with polyglycolides, alginates, and natural biopolymers, in particular, type I collagen. However, many of the above-listed polymers exhibit resorption kinetics incompatible with that of osteogenesis. Also, they have poor mechanical properties insufficient for sustaining the physiological loads. In the case of collagen the situation is further complicated by the fact that, being a zoogenic product, it can exhibit antigene properties under physiological conditions. An alternative to collagen can be found in gelatin, thermally denaturated collagen. Its physicochemical properties can be controlled within certain limits by varying the molecular weight and composition of its constituent amino acids, as well as by cross-linking. In this context, one of the most promising biopolymers for matrix applications is chitosan yielded by chitin deacetylation.

Various techniques are available for preparation of porous matrices from the above-mentioned polymers, e.g., phase separation, foaming, and rapid prototyping (a new technology based on material processing with the use of a computer model). Technologically, the best option can be found in freeze drying (lyophilization) which yields porous structures whose parameters can be varied via controlling liquid phase crystallization in samples.

The mineral component of bone tissue is represented by crystals of apatite-like calcium phosphates containing carbonate groups and physiologically essential magnesium, sodium, chlorine, and fluorine ions at impurity levels. This makes such phosphates especially attractive for porous ceramic matrix applications. As known, ceramic materials are prepared by sintering particles with preset shape and size. In view of the above-said, the relevant tasks can be formulated as follows: provide a system of interconnected pores no larger than 150 μm in size to favor adhesion and functioning of osteoblasts and biological flows, so that the total open porosity of the

material exceed 50–60%; achieve nano- and microporosity to enhance the adsorption of proteins participating in osteosynthesis; and prepare a material with desired bioresorption kinetic and strength characteristics. The dissolution rate can be controlled by varying the component ratio in biphasic hydroxyapatite–tricalcium phosphate materials [5, 10], since at pH ≈ 7 $Ca_3(PO_4)_2$ is better soluble than hydroxyapatite. The problem in preparation of such biphasic materials lies in achieving uniform phase distribution at the microscale.

Materials based on carbonated hydroxyapatite are also characterized by accelerated, compared to hydroxyapatite, resorption. The content of carbonate groups in biological bone tissue reaches 8 wt % [11]. Carbonated hydroxyapatite is a thermally unstable compound; its decomposition is characterized by the onset temperature as low as 300–400°C and is accompanied by CO and CO_2 evolution. At temperatures above 1000°C carbonated hydroxyapatite is converted to oxyapatite via losing a significant number of carbonate groups. Therefore, it is essential that the sintering temperature in techniques for preparation of carbonated hydroxyapatite-based ceramics be decreased by at least 400–500°C (against 1250–1300°C for hydroxyapatite ceramics).

Certain promise for ceramic matrix applications is offered by hydroxyapatite-based nanocrystalline materials [12]. Compared to microcrystalline ceramics, they exhibit accelerated biological resorption and also lead to enhanced function of the cells participating in bone remodeling (osteoclasts, osteoblasts) and suppressed function of the competing cells (fibroblasts). These nanocrystalline materials possesses unique surface properties (nanoroughness, energy condition), thereby promoting adsorption of certain proteins [12].

(Bio)Polymer-calcium phosphate composite materials can combine benefits offered by both components, e.g., osteoconductivity of the ceramic phase with elasticity and easily controllable resorption rate. Reinforcement of the polymeric skeleton with inorganic particles can lead to substantial increases in strength and modulus of elasticity. Such composites can be prepared by mixing the components to be followed by pore formation in the polymeric skeleton. An alternative can be found in the biomimetic procedure which implies crystallization of the phosphate particles on the biopolymer molecules. The latter method deserves more serous attention, being a kind of analog to mineralization proceeding in human body. It is possible to deposit biomimetically the apatite nanocrystals on the gelatin template molecules. Research activities along this line are still in infancy.

An essential aspect of bone tissue engineering is preparation of osteoinductive materials, i.e., those able to induce *de novo* bone tissue formation, without exposure to osteogenetic factors [10]. It is commonly accepted that calcium phosphate-based ceramic materials are osteoconductive but not osteoinductive. However, some of the recent data suggest possible osteoinductive behavior for some calcium phosphates. This is apparently associated not only with their chemical composition (this phenomenon was revealed for octacalcium phosphate [13]) but also with their microstructural features. Specifically, this concerns their nanoporosity that favors adsorption and concentration of morphogenetic proteins from the physiological environment. Thus, preparation of osteoinductive calcium phosphate-based ceramics can mark a new stage in development of materials intended for directed regeneration via osteogenesis of missing portions of bone tissues.

Materials with Controllable Bone Resorption Rate

Hydroxyapatite–Tricalcium Phosphate Ceramic Systems

Biphasic hydroxyapatite–tricalcium phosphate materials can be prepared both by mechanically mixing the components and by heat treatment of calcium-deficient apatite (Ca/P = 1.5–1.67) at temperatures above 700°C. The latter method is preferable, since it provides highly uniform spread of components in the product. Calcium-deficient apatite is prepared by "wet" techniques of precipitation from solutions or hydrolysis of dicalcium phosphate.

The influence of the composition of calcium-deficient apatite and heat treatment temperature on phase relationships was examined for the composite materials prepared by thermal decomposition [14]. The reactants were calcium nitrate and diammonium hydrogen phosphate solutions. Synthesis was run at 40°C with pH of the reaction mixture maintained within 7–8.5, followed by drying the precipitate and calcining the resulting powder at 900°C.

The X-ray phase analysis showed that the phase composition of the thermal decomposition product depends on the Ca/P ratio in the synthesis product and on the heat-treatment temperature. For example, the

product with Ca/P = 1.57 after heat treatment at 900°C contains 62% β-tricalcium phosphate and 38% hydroxyapatite. With temperature increased to 1000°C the content of hydroxyapatite increases to 47%, and that of β-tricalcium phosphate, decreases to 53%. At 1100°C hydroxyapatite constitutes the basic phase (61%). After heat treatment above 1200°C, the X-ray pattern contained peaks of α-tricalcium phosphate.

Thus, precipitation of calcium-deficient apatite from solutions with varied component ratio (Ca/P = 1.54–1.64), followed by heat treatment at temperatures within 900–1300°C, yields biphasic phosphates with the hydroxyapatite/β-tricalcium phosphate ratio broadly varying from 12:88 to 89:11, as well as of triphasic phosphate system with the hydroxyapatite/β-/α-tricalcium phosphate ratio ranging from 55:24:21 to 93:3:7.

The powdered biphasic materials were used for preparation of granules. To this end, phosphate powders were mixed with a gelatin solution (1.8–3.0 ml of solution per gram of powder). The resulting suspension was introduced into the dispersion medium (oil). The precipitate was allowed to settle, filtered off, washed, air-dried, and heat-treated at 1000°C. The average size of the resulting granules and their size distribution within 50–2000 μm was governed by the gelatin concentration, temperature of the dispersion medium, and stirring rate. The 300–500-μm fraction was screened with a set of sieves, and the solubility of the granules was examined in 0.1 M NaCl solution simulating the extracellular body fluid (the granules were kept for 28 days at a constant volume of the liquid phase; such technique is applied in biomaterials examination).

The regression analysis of the kinetic dissolution curves (Origin 6.0 program) showed that dissolution of granules is a multistage process, like in the case of Bioglass® granules [15]. In the initial, 1-day, stage the kinetic dependence can be approximated by a logarithmic or power function. The dissolution is decelerated with the course of time, and the dependence changes to the exponential run

$$c(t) = c_0 + c_m[1 - \exp(-bt)],$$

where c_0 is the conditional initial concentration; c_m, saturation concentration; b, a coefficient; and t, time.

The exponential run corresponds to a first-order kinetic equation: The number of "reactive dissolution centers" in the solute changes at a rate proportional to their number at a given moment.

It was of interest to estimate the rate at which the calcium concentration in solution changes in relation to the hydroxyapatite:β-tricalcium phosphate ratio in the granules. In the exponential stage, the rate of change of the calcium concentration tends to decrease in time as $dc(t)/dt = c_m b\exp(-bt)$. This makes the initial rate, dc/dt (t = 0) = $c_m b$, suitable as a quantitative measure of dissolution. This parameter tends to increase with increasing content of β-tricalcium phosphate in the granules. The granules comprised of α-tricalcium phosphate exhibit the maximal solubility. The initial rate of calcium elimination, reduced to the specific surface area of the granules, tends to increase with increasing content of β-tricalcium phosphate in the granules. This suggests that the dissolution rate depends not only on the specific surface area but on the phase composition as well. A substantial increase in the specific surface area of the granules after keeping in isotonic solution suggests selective dissolution of some structural elements. According to X-ray phase analysis data, the content of β-tricalcium phosphate in the samples during the experiments tends to decrease. An increase in the specific surface area of neat hydroxyapatite granules after the experiments can be associated with its partial dissolution.

Magnesium is a physiologically essential component of bone tissue, for which reason magnesium-containing biphasic calcium phosphates $Ca_{10-x}(MgHPO_4)_x(PO_4)_6(OH)_{2-x}$ were synthesized [16]. The product was precipitated with aqueous ammonia from $Ca(NO_3)_2$, $Mg(NO_3)_2$, and diammonium hydrogen phosphate, $(NH_4)_2HPO_4$, solutions.

Heat treatment led to decomposition of the product into hydroxyapatite and β-tricalcium phosphate. The precipitate was left to settle, filtered off, dried at 120°C, and heat-treated at 900 and 1100°C. The resulting powder was characterized by the particle size of 300–500 nm.

It was found that the content of β-tricalcium phosphate tends to increase with increasing magnesium content in the product. With 1% calcium ions replaced by magnesium, the product contained 16% β-tricalcium phosphate. After 3% replacement, hydroxyapatite and β-tricalcium phosphate phases were in the 56/44 ratio. Introduction of >6% magnesium led to nearly single-phase β-tricalcium phosphate. The phase composition of the powder varies only slightly with heat treatment temperature increasing from 900 to 1100°C.

Carbonated Hydroxyapatite and Ceramics Thereof

Carbonate groups can occupy two sites in the hydroxyapatite structure, specifically, replace OH (substitution type A) and PO_4 (substitution type B) groups [17]. Substitution of PO_4^{3-} by CO_3^{2-} ions leads to decreases in the hydroxyapatite crystallite size and degree of crystallinity. Both the natural bone tissue and synthetic carbonated hydroxyapatite precipitated from solutions are characterized by AB mixed substation type, in which the A to B component ratio is governed by numerous factors, in particular, reaction conditions.

The B or mixed AB type product is prepared by precipitation from aqueous solutions at enhanced pH [18, 19]. This can be achieved via reaction between solutions of calcium nitrate, diammonium hydrogen phosphate, and ammonium carbonate in the presence of concentrated aqueous ammonia. In the case of the B type substitution, preservation of the charge balance is problematic. In this connection, B type products are typically synthesized with the use of sodium or ammonium ions that partly substitute calcium ions [18]. The synthesis temperature and the content of carbonate groups substantially affect the morphology of the crystallizing particles (their shape varies from spherical at high, to needle-like at low, content of CO_3^{2-} groups). The dominating substitution type is also governed by the content of carbonate ions: When in amounts of >4 wt %, the CO_3^{2-} ions preferably occupy B sites in carbonated hydroxyapatite.

This product can also be prepared by a solid-liquid reaction involving calcium oxide, diammonium hydrogen phosphate, and ammonium carbonate [20, 21]. After mixing the solid reactants, water is added to the resulting mixture, which is further stirred for 30 min. The reaction mixture is placed into a microwave furnace for acceleration of the reaction [22]. The resulting product is heat-treated in air at 300°C.

Thermal instability of carbonated hydroxyapatite strongly complicates fabrication of ceramics thereof. The decomposition onset is at 300–400°C with CO_2 evolution; above 1200–1300°C the product loses all the carbonate groups [20–23]. The carbonated hydroxyapatite synthesized by a heterophase reaction exhibits a higher thermal stability because of a higher crystallinity of this product. Chemical analysis showed that, under heat treatment (1100°C), it loses >50% carbonate groups. The IR and ^{1}H NMR spectroscopic examinations showed that heat treatment strongly modifies the structure of the compounds: Hydroxyapatite is converted to oxyapatite [24]. An attempt was made to achieve stabilization of the product via compensation of the charge imbalance (associated with incorporation of carbonate anion into the hydroxyapatite structure [25]), via partially substituting Ca^{2+} by a univalent cation, preferably sodium. It was found that sodium occupies the site adjacent to that of the carbonate ion in the calcium sublattice, thereby promoting formation of B type defects which are thermodynamically less stable than AB type defects [26].

Thus, the sintering temperature for powdered carbonated hydroxyapatite used for ceramics preparation should be reduced by at least 450–500°C relative to that for nonsubstituted hydroxyapatite (1250–1300°C). This can be achieved via introducing additions (double carbonates of calcium and alkaline metals) forming the liquid phase. Introduction into the initial blend of up to 8 wt % such additions allowed the sintering temperature to be decreased to 750–800°C, thereby preventing substantial loss of carbonate groups by sintering. This involves secondary crystallization, apparently, via dissolution of the basic phase in the molten addition and subsequent crystallization of the melt in the course of cooling. These results were validated by the X-ray energy dispersive microanalysis data on the elemental composition of the crystals. Figure 1 shows examples of the resulting microstructures. Formation of secondary rod-like crystals can lead to ceramics hardening: The bending strength of such materials exceeds that of densely-sintered hydroxyapatite-based ceramics (125 against ca. 75 MPa).

Hydroxyapatite-Based Nanocrystalline Powders and Nanostructured Ceramics

The size of hydroxyapatite particles precipitated from aqueous solutions can be decreased by decreasing both the synthesis temperature and precipitate aging time [27]. It was shown [28] that a decrease from 80 to 0°C in temperature of precipitation of the product with ammonia from calcium nitrate and ammonium hydrogen phosphate solution causes a decrease in the particle size of the product from 37 to 12 nm (as estimated by the Scherrer technique) [28].

A promising method for preparation of nanocrystalline hydroxyapatite powders was found in precipitation from solutions of calcium nitrate and ammonium hydrogen phosphate in solution of a biopolymer, in particular, gelatin [29]. The maximal achieved specific surface area of the powder was

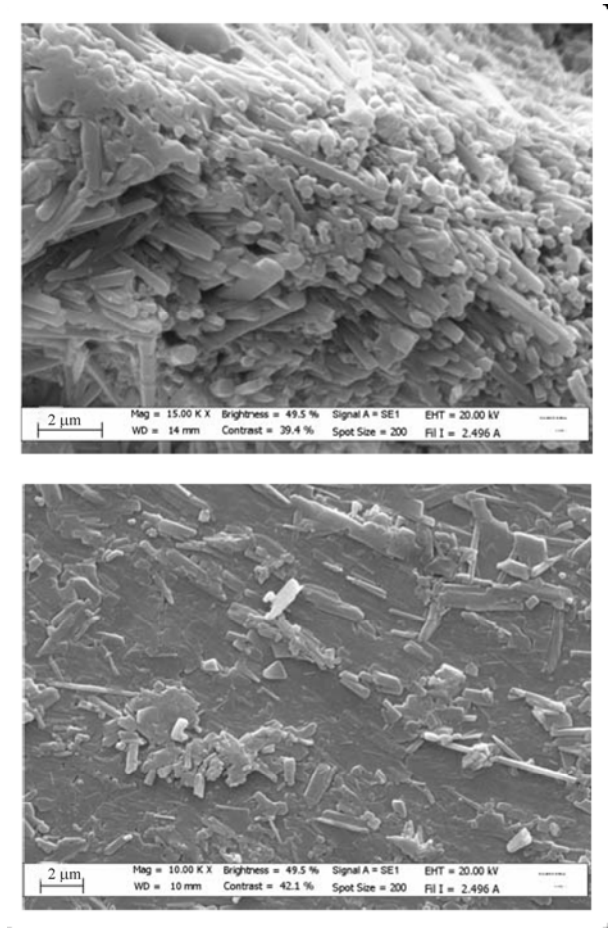

Fig. 1. Examples of microstructures of self-reinforced materials yielded by liquid-phase sintering of carbonated hydroxyapatite powders.

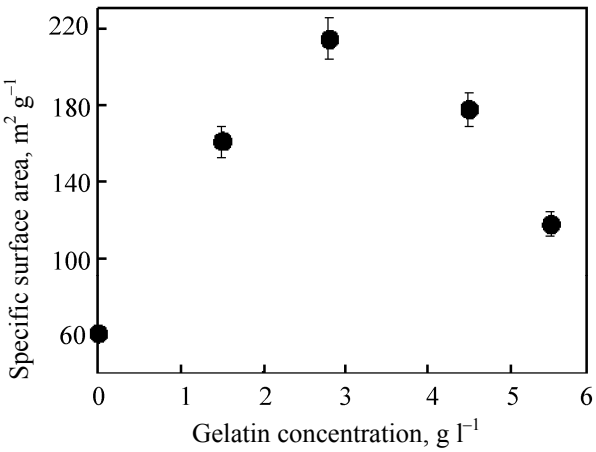

Fig. 2. Specific surface area (BET) of the hydroxyapatite nanopowder vs. gelatin concentration in the reaction solution.

~220 m^2 g^{-1} (Fig. 2), which corresponds to the crystallite size <15 nm [30]. An electron transmission microscopic examination validated those data (Fig. 3). In hydroxyapatite precipitation, the gelatin concentration is the deciding factor for crystal nucleation [29]. When synthesis is run in an aqueous solution without gelatin, the Ca^{2+} and PO_4^{3-} ions enter into homogeneous reaction. In a gelatin solution, the calcium ions first react with carboxy groups of gelatin, and the new phase nucleation is initiated by the reactive centers of gelatin via the heterogeneous reaction mechanism. This is followed by attachment of the phosphate ions to the calcium complexes being formed, thereby leading to formation of new phase nuclei with the critical size, from which hydroxyapatite nanocrystals originate. Apparently, the density of the crystallization centers, dependent on the gelatin concentration, decides the particle size of the segregating phase. At high densities of the crystallization sites the interaction of the growing particles leads to coalescence and coarsening. The $CO_3 \rightarrow (PO_4,OH)$ and $SiO_4 \rightarrow PO_4$ anionic substitutions in hydroxyapatite, which result in lattice deformations because of the difference in the sizes of the anions, also cause the crystallite size to decrease [31].

To preserve the grain size of the ceramics sintered from hydroxyapatite nanopowders, raw billets are pressed at high pressures [32]. This allows decreasing the sintering temperature by ca. 550°C relative to the case of billets prepared at 100 MPa and, thereby, keeping the grain size of the ceramic material within 35–50 nm. The microhardness of the ceramics reaches 5.8 GPa, which substantially (1.6 times) exceeds that of the ceramics sintered from micropowders.

Nanocrystalline calcium phosphate powders and granules were used for reinforcing composite materials based on a chitosan matrix. Such materials were prepared by foaming, freeze drying, and phase separation in the biopolymer with gradual solvent substitution.

Composite Materials Based on Calcium Phosphate-Reinforced Chitosan Matrix

Chitosan-based materials are biocompatible and bioresorbable. Compared to porous ceramics, composites with chitosan matrix possess higher porosity and elasticity. This allows them to fill bone defects of any shape without leaving gaps between the bone and the implant. Introduction of hydroxyapatite as filler into porous chitosan structures leads to enhanced adsorption of proteins and calcium ions, thereby

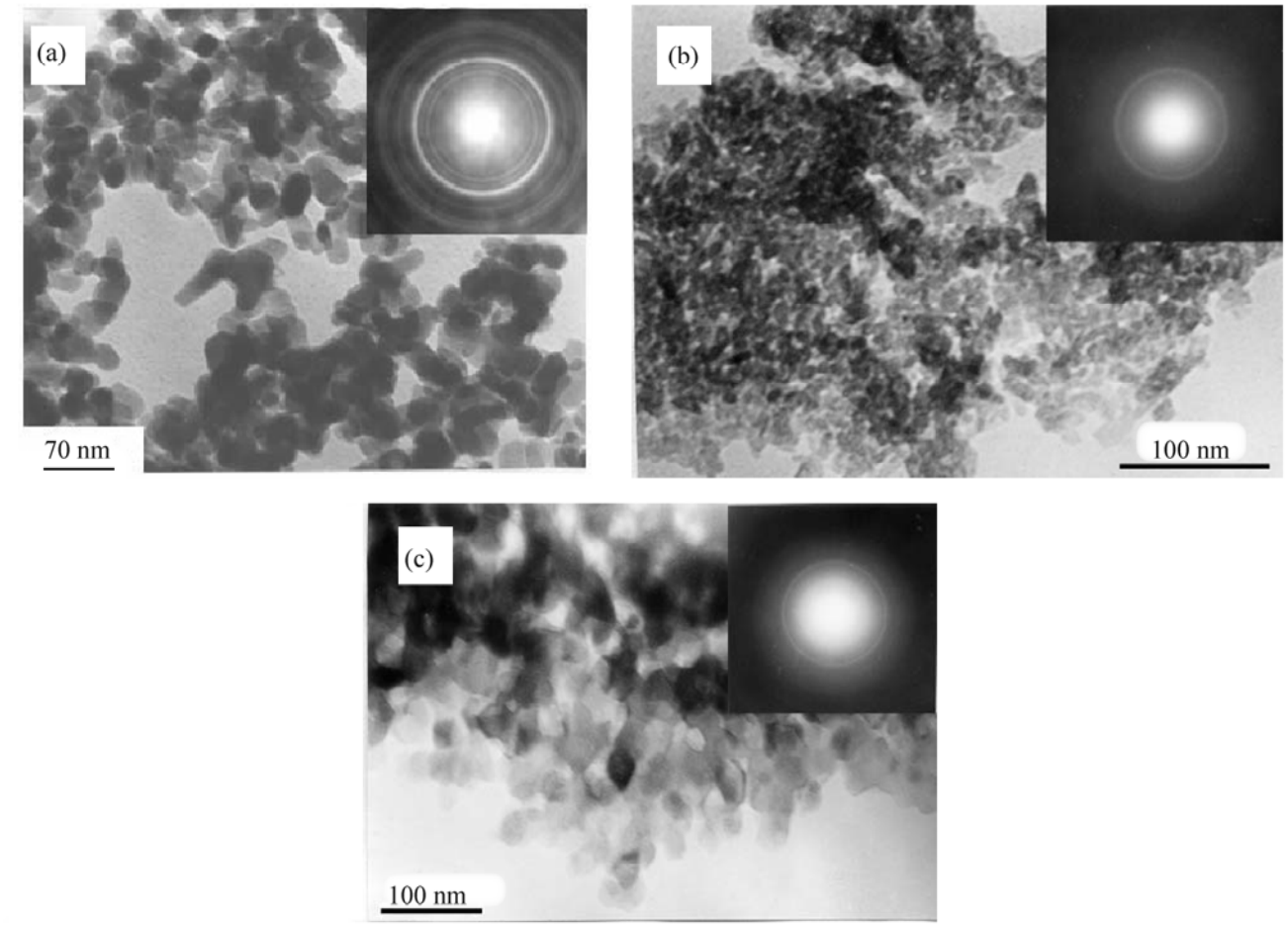

Fig. 3. (a)–(c) Electron images of (a, b) (a) initial and (b) pressed (3.5 GPa) powders and (c) ceramics sintered at 640°C, comprised of hydroxyapatite nanoparticles.

improving adhesion and enhancing osteogenic differentiation of stem cells [33]. Porous chitosan sponges are typically prepared by freeze drying. Glutaraldehyde is often used for chitosan cross linking.

The Baikov Institute of Metallurgy and Materials Science, Russian Academy of Sciences, developed a simple technique for preparation of chitosan-based composite porous sponges comprising dispersed particles of physiologically essential calcium and phosphorus elements [34, 35]. The technique includes the following stages: dissolution of chitosan; introduction of filler; foaming; freezing; replacement of water by a liquid in which chitosan is insoluble; and drying. This technique yielded sponges with >90% porosity, containing up to 50 wt % filler.

Suitable fillers were found in nanodispersed hydroxyapatite powders (specific surface area 110–120 $m^2 g^{-1}$), carbonated hydroxyapatite (80–90 $m^2 g^{-1}$), and calcium carbonate (18–20 $m^2 g^{-1}$) prepared by chemical precipitation from aqueous solutions. A medium-molecular-weight chitosan (50–190 kDa) powder was dissolved in acetic acid, after which a filler [chitosan:filler 1:1 (w/w) ratio] and ammonium carbonate (foaming agent) were added. The resulting suspension was frozen to below –10°C; the frozen sample was placed into ethanol and subsequently dried at 60°C.

The solubility of the composites was estimated from the mass lost by the sample when kept in distilled water at 37°C for 1, 3, 7, 14, and 28 days, which was followed by drying at 60°C.

The structure and properties of the prepared composite materials were compared with those of the samples of unfilled porous chitosan sponges.

The porosity of the unfilled sponges is represented by interpenetrating spherically shaped large pores (~10%) measuring 200–300 μm in size, nonuniformly distributed in the bulk of the sample, and small pores (~90%) with extended lamellar shape, measuring from several tens to 200–300 μm in length and up to several tens of micrometers in breadth. The pores are separated by scaly or fibrous chitosan walls. This material structure is favorable for osteoblast attachment and functioning.

The filled materials have a more uniform microstructure. Large pores (~80%) in them are characterized by predominantly spherical shape and a size of 100–300 μm; small pores are nonuniformly distributed and have the shape corresponding to pores in the unfilled materials. The filler nanoparticles are spread in the chitosan matrix as large agglomerates with a medium size of 2–5 μm. Like in the case of unfilled sponges, the composite sponges have a developed pore structure with interpenetrating pores.

The solubility of the sponges in distilled water tends to increase as chitosan/hydroxyapatite < chitosan/calcium carbonate < chitosan/carbonated hydroxyapatite < chitosan. The difference in solubilities of the materials is associated with that in solubilities of the fillers, which increases in changing from hydroxyapatite to a carbonated product. It was found that the solubility of all the materials increases during 14-day keeping, after which dissolution is negligible.

Unfilled porous chitosan sponge has the largest among the examined sponges compression coefficient of 0.85. The next largest compression coefficient of 0.8 is exhibited by chitosan sponges filled with hydroxyapatite, and it is followed by carbonated hydroxyapatite and calcium carbonate with compression coefficients of 0.75 and 0.7, respectively. A decrease in elasticity is associated with the reinforcing action of the filler. The lowest compression coefficient for the sponge with calcium carbonate is, apparently, associated with the cross-linking action of the alkaline component.

The biological *in vitro* and *in vivo* tests (carried out by N.S. Sergeeva and colleagues, Gertsen Moscow Research Oncology Institute, Federal State Enterprise) demonstrated good adhesion, viability of cells, and lack of cytotoxicity for the developed materials [36, 37]. Some of them have successfully passed limited clinical tests (over 100 patients). These materials are planned for manufacture.

ACKNOWLEDGMENTS

This study was financially supported by the Federal Agency for Science and Innovations (state contract no. 02.513.12.3008).

The author is grateful to staff-members of Baikov Institute of Metallurgy and Materials Sciences, Russian Academy of Sciences, for providing the research data that underlay this study.

REFERENCES

1. Hench, L.L. and Polak, J.M., *Science*, 2002, vol. 295, pp. 1014–1118.
2. Cancedda, R., Dozin, P, Giannoni, P., and Quarto, R., *Matrix Biol*., 2003, vol. 22, pp. 8–17.
3. Barinov, S.M. and Komlev, V.S., *Calcium Phosphate Based Bioceramics for Bone Tissue Engineering*, Zuerich: Trans Tech. Publ., 2008.
4. Veresov, A.G., Putlyaev, V.I., and Tret'yakov, Yu.D., *Ross. Khim. Zh. (Zh. Ross. Khim. O&3va. im. D. I. Mendeleeva)*, 2000, vol. 44, no. 6, pp. 32–42.
5. Barinov, S.M. and Komlev, V.S., *Biokeramika na osnove fosfatov kal'tsiya* (Calcium Phosphate-Based Bioceramics), Moscow: Nauka, 2005.
6. Albee, F. and Morrison, H., *Ann. Surg*., 1920, vol. 71, pp. 32–38.
7. Aoki, H., *Science and Medical Applications of Hydroxyapatite*, Tokyo: JAAS, 1991.
8. Jarcho, M., Kay, J., Gumaer, K., et al., *J. Bioeng*., 1977, vol. 1, pp. 79–92.
9. Martin, R.B., *Mater. Sci. Forum*, 1999, vol. 7, no. 1, pp. 5–16.
10. LeGeros, R.Z., *Chem. Rev*., 2008, vol. 108, pp. 4742–4753.
11. Suchanek, W. and Yoshimura, M., *J. Mater. Res. Soc*., 1998, vol. 13, no. 1, pp. 94–103.
12. Liu, H. and Webster, T.J., *Biomaterials,* 2007, vol. 28, p. 354.
13. Kamakura, S., Sasano, Y., Nakajo, S., Shimizu, T., Suzuki, O., Katou, F., Kagayama, M., and Motegi, K., *J. Biomed. Mater. Res*., 2001, vol. 57, no. 2, pp. 175–182.
14. Kubarev, O.L., *Cand. (Tech.) Sci. Dissertation*, Moscow, 2007.
15. Cerutti, M.G. and Greenspan, D., and Powers, K., *Biomaterials*, 2005, vol. 26, pp. 4903–4909.
16. Kubarev, O.L., Barinov, S.M., and Komlev, V.S., *Dokl. Ross. Akad. Nauk*, 2008, vol. 417, no. 4, pp. 497–499.
17. Elliott, J.C., *Structure and Chemistry of the Apatites and Other Calcium Phosphates*, Amsterdam: Elsevier, 1994.

18. Barralet, J.E., Best, S., and Bonfield, W., *J. Biomed. Mater. Res*., 1998, vol. 41, pp. 79–85.
19. Gibson, I. and Bonfield, W., *J. Biomed. Mater. Res*., 2002, vol. 59, pp. 697–705.
20. Barinov, S.M., Rau, J.V., Nunziante Cesaro, S., Durisin, J., Fadeeva, I.V., Ferro, D., Medvecky, L., and Trionfetti, G., *J. Mater. Sci. Mater. Med*., 2006, vol. 17, pp. 597–603.
21. Rau, J.V., Nunziante Cesaro, S., Ferro, D., Barinov, S.M., and Fadeeva, I.V., *J. Biomed. Mater. Res*., 2004, vol. 71B, pp. 441–448.
22. Sampath Kumar, T.S., Manjubala, I., and Gunasekaran, J., *Biomaterials*, 2000, vol. 21, pp. 1623–1626.
23. Barinov, S.M., Rau, J.V., Fadeeva, I.V., Nunziante Cesaro, S., Ferro, D., Trionfetti, G., Komlev, V.S., and Bibikov, V.Yu., *Mater. Res. Bull*., 2006, vol. 41, pp. 485–490.
24. Buznik, V.M., Kozlova, S.G., Gabuda, S.P., Barinov, S.M., Bibikov, V.Yu., Fadeeva, I.V., and Komlev, V.S., *Dokl. Ross. Akad. Nauk*, 2007, vol. 413, no. 2, pp. 64–68.
25. Barinov, S.M., Fadeeva, I.V., Ferro, D., Rau, J.V., Nunziante Cesaro, S., Komlev V.S., and Fomin, A.S., *Zh. Neorg. Khim*., 2008, vol. 53, no. 2, pp. 204–208.
26. Peroos, S., Du, Z., and Leeuw, N.H. de, *Biomaterials*, 2006, vol. 27, pp. 2150–2158.
27. Pang, Y.X. and Bao, X., *J. Eur. Ceram. Soc*., 2003, vol. 23, pp. 1697–1702.
28. Bakunova, N.V., Barinov, S.M., and Shvorneva, L.I., *Ross. Nanotekhnol*., 2007, vol. 2, no. 10, pp. 102–106.
29. Chang, M.C. and Douglas, W.H., *J. Mater. Sci. Mater. Med*., 2006, vol. 17, pp. 387–392.
30. Fomin, A.S., Barinov S.M., Ievlev, V.M., Fadeeva, I.V., Komlev, V.S., Belonogov, E.K., and Turaeva, T.L., *Dokl. Ross. Akad. Nauk*, 2006, vol. 411, pp. 219–222.
31. Bakunova, N.V., Fomin, A.S., Fadeeva, I.V., Barinov, S.M., and Shvorneva, L.I., *Zh. Neorg. Khim*., 2007, vol. 52, no. 10, pp. 1594–1599.
32. Fomin, A.S., Barinov, S.M., Ievlev, V.M., Smirnov, V.V., Mikhailov, B.P., Belonogov, E.K., and Drozdova, N.A., *Dokl. Ross. Akad. Nauk*, 2008, vol. 418, no. 3, pp. 22–25.
33. Zhao, F., Grayson, W.L., Ma, T., Bunnell, B., and Lu, W.W., *Biomaterials*, 2006, vol. 27, no. 9, pp. 1859–1867.
34. Fedotov, A.Yu., *Perspekt. Mater*., 2008, no. 5 (spec. issue), pp. 363–364.
35. Fedotov, A.Yu., Smirnov, V.V., Fomin, A.S., Fadeeva, I.V., and Barinov, S.M., *Dokl. Ross. Akad. Nauk*, 2008, vol. 423, no. 6.
36. Chissov, V.I., Sviridova, I.K., Sergeeva, N.S., Kirsanova, V.A., Akhmedova, S.A., Filyushin, M.M., Barinov, S.M., Fadeeva, I.V., Komlev, V.S., and Smirnov, V.V., *Klet. Tekhnol. Biol. Med*., 2008, no. 2, pp. 68–75.
37. Chissov, V.I., Sviridova, I.K., Sergeeva, N.S., Frank, G.A., Kirsanova, V.A., Akhmedova, S.A., Reshetov, I.V., Filyushin, M.M., Barinov, S.M., Fadeeva, I.V., and Komlev, V.S., *Klet. Tekhnol. Biol. Med*., 2008, no. 3, pp. 151–156.