Study of *In Vivo* Biocompatibility and Dynamics of Replacement of Rat Shin Defect with Porous Granulated Bioceramic Materials

V. I. Chissov, I. K. Sviridova, N. S. Sergeeva, G. A. Frank, V. A. Kirsanova, S. A. Achmedova, I. V. Reshetov, M. M. Filjushin, S. M. Barinov*, I. V. Fadeeva*, and V. S. Komlev*

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Biocompatibility of porous granulated bioceramic materials (hydroxyapatite, β-tricalcium phosphate, hydroxyapatite-β-tricalcium phosphate complex (80:20 wt%), carbonate-containing hydroxyapatite, and silicon-containing hydroxyapatite) was shown in a subcutaneous test on BDF_1 mice. Dynamic (up to 8 months) observation showed gradual replacement of the granular substance with *de novo* forming bone tissue with hemopoiesis foci on a model of fenestral defect in the shin bone in Wistar rats. By the rate of resorption, the materials rank as follows: silicon-containing hydroxyapatite<hydroxyapatite<hydroxyapatite-β-tricalcium phosphate<β-tricalcium phosphate<carbonate-containing hydroxyapatite. The rate of resorption in bone tissue defect was significantly higher than in the subcutaneous test, but lagged behind (even for tricalcium phosphate and carbonate-containing hydroxyapatite) bone tissue formation *de novo*.

Key Words: *bioceramics; biocompatibility; bone tissue defect*

Materials used for filling bone defects in surgical interventions (including reconstructive plastic surgery) in oncology, traumatology and orthopedics, vertebrology and cosmetology, have flaws. For example, the use of specially treated allogenic implants involves the transfer of undetected infectious agents and is fraught with allergic reactions and probability of rejection. The use of autotransplants involves an additional intervention, which is also fraught with complications and involves deterioration of the quality of life. Moreover, the formation of autotransplants is impossible in large bone defects. Polymeric synthetic and metal materials allowed for use are insufficiently biocompatible, while special coatings can improve their compatibility only for a short time.

These facts necessitate the development of biocompatible materials for replacing bone defects and stimulation of osteogenesis in the defect. Ideally, these materials should be biocompatible, in other words, should not be rejected or suppress the morphogenetic potential of the adjacent tissues, maintain differentiation of the host periosteal stromal stem cells or of auto/allogenic cell cultures (serve as the matrix for them), have open interrelated pores for providing biological streams and population by cells. In addition, it is desirable that these materials were biologically active, which suggests utilization of their chemical components in the for-

P. A. Hertzen Moscow Oncological Institute, Rosmedtechnologies; *Institute of Physico-Chemical Problems of Ceramic Materials, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** prognoz.06@mail.ru. N. S. Sergeeva

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mation of the bone tissue. And (the last but not the least) the rate of resorption of these materials should be comparable with the rate of the formation of new bone at the site of defect.

Bioceramics containing the main components of the mineral constituent of bone tissue traditionally attracts the attention of material technologists. A series of materials on the basis of calcium phosphates with different Ca/P proportion and structure of crystal lattice was developed [3,6,12-14], including the two-component materials in the hydroxyapatite—β-tricalcium phosphate system (HA—TCP; biphasic ceramics) [7,8,10] and a variety of materials based of HA with cationic and anionic isomorphic substitutes, essentially modifying the characteristics of ceramics [4,9]. Attempts at creation of the material maximally meeting the above requirements are made by modifying the technology of formation of ceramic materials on the base of calcium phosphates and by modulating their chemical and crystal structure. However, by the present time none of the materials permitted for clinical use fully meets practical requirements: cases of graft rejection, fractures at the site of implantation, and other complications are frequent and their causes are not always clear.

We studied the biocompatibility and dynamics of rat shin bone defect replacement *in vivo* by bioceramic materials on the basis of a new form of HA: granules with well-developed surface and high content of open interrelated pores.

MATERIALS AND METHODS

Bioceramic materials, developed at Institute of Problems of Ceramics, were studied: HA, two-component HA—β-TCP ceramics in 80:20 proportion, β-TCP, silicon-containing hydroxyapatite (Si-HA) with 0.79 wt% silicon, and carbonate-containing hydroxyapatite (CHA) with 5.9 wt% carbonate groups. The carbonate groups were introduced in HA structure in order to create defects in HA crystal lattice for accelerating resorption and approximating the composition of implanted material to that of the mineral constituent of bone tissue. Biphasic HA—β-TCP ceramics was studied with the same purpose, because β-TCP is characterized by more rapid dissolution in water solutions than HA. Silicate groups were introduced in HA for studies of silicon effects on the biological behavior of HA ceramics. Original elements of technology of bioceramic materials creation are protected by patents or presented in published reports [1,4,5].

All materials were used in the form of porous granules with well-developed surface. The size of granules was 300-600 μ, pores 10-30 μ, specific surface 0.32-0.58 m^2/g. Initial HA, HA—β-TCP, β-TCP, CHA, and Si-HA powders were prepared by precipitation from water solutions according to methods described previously [1,4]. The size of powder particles was less than 1 μ. Powder suspension was prepared in water solution of P-11 gelatin (GOST 11293-89), which was dispersed in mineral oil of 0.585 Pa×sec viscosity at 25°C during mixing by a blade mixer at 200-1000 rpm. Granules of a shape close to spherical formed within no more than 1 min, precipitated on a Buchner's funnel, washed, dried, and subjected to thermal processing (1000-1200°C during 1 h). the 300-600 μ fraction for further experiments was isolated by filtration through a set of sieves. All materials were sterilized before work.

Biocompatibility of bioceramics was studied in male BDF_1 mice. All manipulations on animals were carried out under total anesthesia under sterile conditions. The animals were divided into 5 groups (a group for each material), 15 animals per group. A small subcutaneous pouch was created with scissors in the thoracic area laterally from the spine in each animal, and granulated bioceramics (50 mg) of one of the 5 types was placed there, after which the wound was sutured.

Over 3 months 3 animals from each group were sacrificed every 2-3 weeks. The implantation zone with adjacent tissues was fixed in 10% formalin, decalcified by the standard method [2], paraffin blocks and histological preparations were made, which were stained by hematoxylin and eosin.

The dynamics of the crural bone defect replacement with bioceramic materials was studied on male Wistar rats, in which a fenestral defect (5-7 mm long) was formed in the upper cortical layer of the shin. The contents of the bone channel under the defect was removed up to the cortical layer. The defect was completely filled with granulated bioceramics. The periosteum at the defect edges was cut off. The operation was completed by layer-by-layer wound suturing. A total of 6 groups, 18 animals in each, were formed: 5 groups for studies of 5 types of materials and group 6 (control) for studies of spontaneous healing of the defect. Three animals from each group were sacrificed after 3, 6, 9, 12 weeks, 6 and 9 months. The defect area with adjacent tissues was sawed out and used for making histological preparations as described above.

RESULTS

Histological analysis of the results of subcutaneous implantation of porous granulated biomaterials to mice showed that all materials were biocompatible

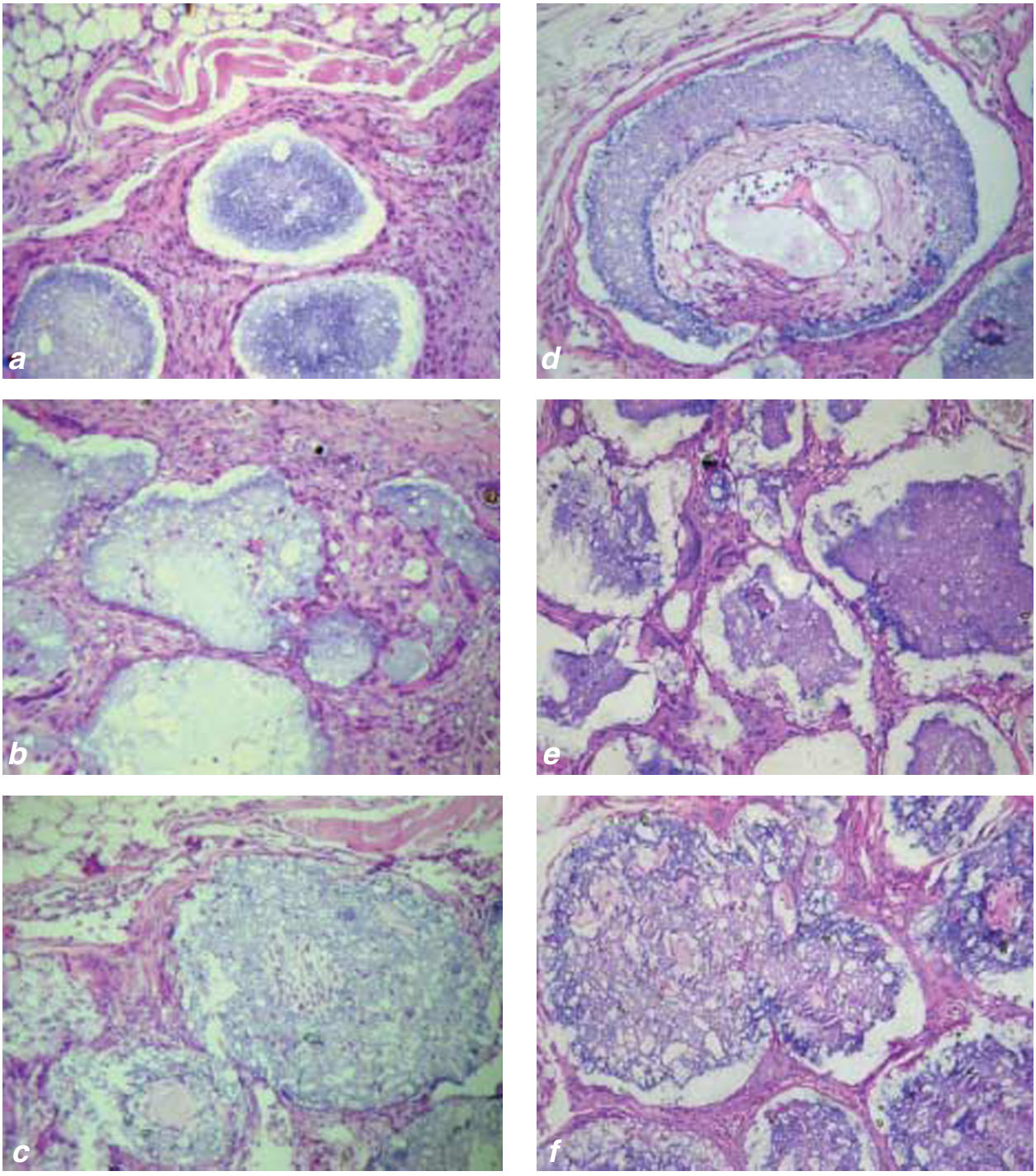

Fig. 1. Porous bioceramic granules under mouse skin 3 weeks (*a-c*) and 3 months after implantation (*d-f*). *a*, *d*) HA—β-TCP; (80:20); *b*, *e*) CHA; *c*, *f*) β-TCP. Here and in Figs. 2, 3: hematoxylin and eosin staining (×100).

over 3 months (minor inflammatory reaction with neutrophilic leukocytes was detected in just solitary cases). In the rest cases, the reaction was lymphoid histiocytic, presenting by small granulomas with a trend to fibrosing. Rejection was seen in none cases. Staring from week 2 after implantation, the connective tissue with oriented collagen fibers (the granules were as if "embedded" in fibrous granulation tissue) formed in spaces between the granules and active neovascularization was in progress (Fig. 1, *a-c*).

This was paralleled by slow resorption of the granules. The sites of resorption inside the granules were also replaced by growing connective tissue

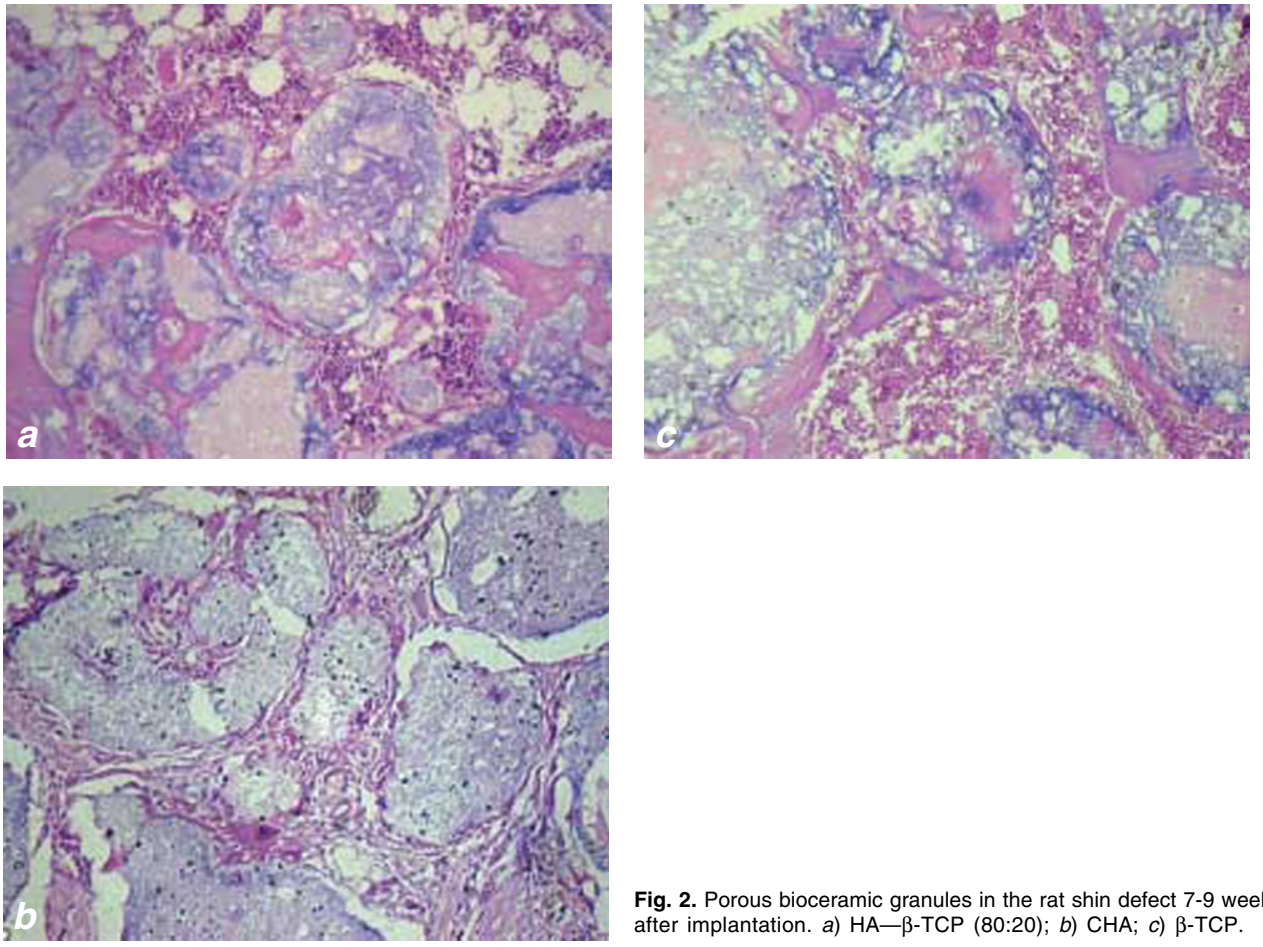

Fig. 2. Porous bioceramic granules in the rat shin defect 7-9 weeks after implantation. *a*) HA—β-TCP (80:20); *b*) CHA; *c*) β-TCP.

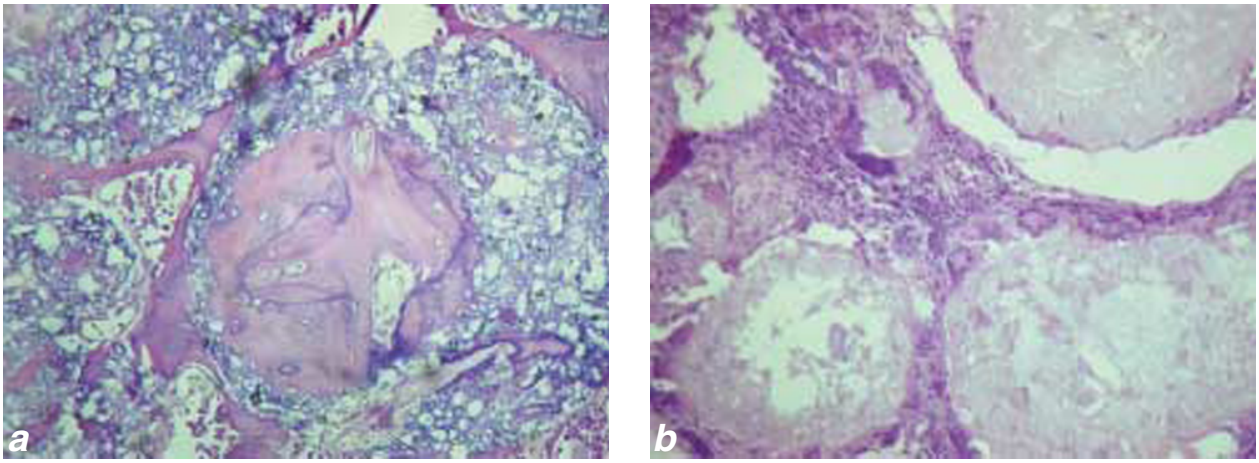

Fig. 3. Porous β-TCP granules in the rat shin defect 8 months after implantation. *a*) remnants of granules embedded in bone tissue; *b*) remnants of granules in the periosteum, surrounded by osteoclasts.

(Fig. 1, *a-c*). By the rate of resorption in subcutaneous implantation, the studied materials ranked as follows: Si-HA<HA<HA—β-TCP<β-TCP<CHA. Really, Si-HA and HA granules retained the compact structure after 3 months, while the structure of β-TCP and CHA became rarefied, reticular, their contours in connective tissue were virtually not visualized, fibroblasts actively migrated to sites of resorption, and the formation of young granulation tissue at the site of the granules transplantation was completed (Fig. 1, *d-f*).

The dynamics of the replacement of shin bone defect with the same granulated porous ceramic materials was observed similarly. As soon as 3 weeks

after implantation, active ossification in spaces between the granules with foci of hemopoiesis was seen (in other words, spongy bone tissue was forming). This process progressed with time, foci of bone tissue formation were visualized inside resorbed granules by weeks 7-9 (Fig. 2). It is noteworthy that resorption of bioceramic materials in the bone defect was significantly more rapid than after subcutaneous transplantation. For example, the degree of β-TCP resorption in the bone defect after 3 weeks corresponded to those 3 months after subcutaneous implantation. The comparative rate of resorption of different materials remained the same: the lowest for Si-HA and the highest for CHA. By week 9 after implantation, the remnants of the granules were embedded in *de novo* formed bone tissue with retained hemopoiesis foci in all implants. Eight months after implantation, the picture differed from that observed 7-9 weeks postimplantation only by accumulations of multinuclear osteoclasts and solitary macrophages around the remnants of the granules in the inner layer of hyperplastic periosteum (Fig. 3).

Biocompatibility (subcutaneous implantation to mice) and dynamics of the rat shin bone defect replacement by porous granulated bioceramic materials (HA, Si-HA, HA—β-TCP, β-TCP, and CHA), differing by chemical composition, crystal lattice structure, and surface architecture, were studied *in vivo*.

All bioceramic materials used in the study were biocompatible, causing no rejection reaction after subcutaneous transplantation to laboratory animals; properly organized connective tissue formed around the implants, and neovascularization was in progress.

Filling of rat shin defect with these porous granules was paralleled by gradual resorprion of bioceramicsand *de novo* formation of bone tissue, growing through and around ceramic granules and/or their fragments. Foci of bone marrow hemopoiesis appeared between forming bone rods. By the rate of resorption (in subcutaneous test and in bone defect filling) these materials ranked as follows: Si-HA<HA<HA—β-TCP<β-TCP<CHA. The rate of their resorption was significantly higher in the bone defect than in subcutaneous implantation, this indirectly indicating their biological activity (utilization of the granule's substance during *de novo* formation of the bone tissue). On the other hand, the rate of resorption of even β-TCP and CHA in the bone defect lagged behind reparative regeneration of bone tissue. The remnants of bioceramic granules were detected in the bone defect 3-8 months after its filling, their volume being maximum after filling with the least resorbed materials (Si-HA and HA) and the least volume (small fragments of granules) was left after the defect filling with the materials resorbed most rapidly (β-TCP and CHA). Reparative ossification and bone tissue mineralization were presumably over by the end of month 3 after the injury, as the histological picture was virtually the same after 3 and 8 months. The only exclusion was accumulation of active osteoclasts and solitary macrophages, presumably utilizing unresorbed and unused bioceramics, seen around the remnants of granules embedded in *de novo* formed periosteum, 8 months after the injury.

It is noteworthy that the tested materials did not suppress, but supported the morphogenetic potentials of the microenvironment in the bone defect model: the periosteum formed around the granules in the upper layer of the defect and spongy bone tissue formed in the middle layer.

Hence, all porous granulated bioceramic materials were biocompatible and bioactive in replacement of bone defect in the tubular bone of laboratory animals. Bioresorption of these materials indicates their good prospects as matrix for cell cultures.

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