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OSTEO-REPLACEMENT PROPERTIES OF SCLERACTINIUM CORAL AQUACULTURE SKELETON (EXPERIMENTAL STUDY)

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Aim: to evaluate the osteo-replacement properties of the coral aquaculture skeleton of *P. verrucosa* and *A. abrotanoides* (CAS) on a model of a fenestral defect in the femur of rats in comparison with the natural coral skeleton of *A. cervicornis* (NCS). **Materials and methods.** CAS grown at a Russian-Vietnamese tropical research and development technology center, as well as NCS were cleaned of organic residues, crushed into 300–600 μm granules, sterilized by γ-radiation (24 kGy) and used to fill bone defects in rat femur. Three groups of animals were formed according to the number of types of coral skeleton samples. Two animals were removed from the experiment every 3, 6, 9, 12 weeks. Tissues excised from implantation zones were fixed, decalcified in EDTA, and their histological slides stained with hematoxylin-eosin were prepared. **Results.** There were no fundamental differences in the dynamics of replacement of bone defects with newly formed bone tissue after implantation of CAS and NCS. NCS, like CAS, were biocompatible and caused no inflammatory reactions in the implantation zone. In the defect area, there was good consolidation of NCS granules with the bone bed. Their bioresorption rates were also similar. Three weeks after implantation, periosteum grew over the defect zone and bone formation began by periosteal osteogenesis. By week 12, the defect area was filled with newly formed cancellous bone tissue with hematopoietic zones between the bone trabeculars. **Conclusion.** The scleractinium coral aquaculture skeleton of *P. verrucosa* and *A. abrotanoides* has osteoplastic properties similar to those of the coral skeleton of *A. cervicornis* from natural settlements.

Keywords: coral aquaculture, osteoplastic properties, marginal excision of rat femur.

INTRODUCTION

In some cases, the use of materials of natural origin to replace bone defects provides for the formation of organotypic structures in the implantation zone. For instance, good osteoplastic properties are demonstrated for coral skeleton from natural settlements (CSNS) [1–4], some chitin derivatives [5], alginates, polyoxyalkanoates [6], and silk fibroin [7]. However, the widespread adoption of some of them in clinical practice is limited by the high cost (silk fibroin), the difficulties of extraction and/or standardization of the composition (CSNS). This forces the development of alternative methods for their obtainment.

Earlier, we showed splendid bone replacement properties of CSNS *A. cervicornis* [8]. Due to the developed surface and through porosity, they were quickly populated by osteogenic predecessors, and the rate of their biodegradation corresponded to the rate of neoosteosis, which ensured organotypic replacement in the area of the bone defect. However, the limitation of CSNS production induced the study of the osteoplastic properties of their aquacultures. The employees of FSRI A.N. Severtsov Institute of Ecology and Evolution Problems of the Russian Academy of Sciences, on the basis of the Russian-Vietnam Tropical Research Technological Centre, have identified the climatic conditions for cultivation of aquaculture of some species *Pocillopora* of *Acropora*, investigated their physicochemical properties, and showed the similarity of architectonics of CSAC and CSNS, as well as significantly higher strength of CSAC [9]. We have further shown the good matrix (for cells) properties of CSAC and their biocompatibility in the subcutaneous test in small laboratory animals [10–12].

The purpose of the present stage of the investigation was to evaluate the osteo-replacing potentials of CSAC as compared with CSNS.

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MATERIALS AND METHODS

Purification of the CSAC samples (*Pocillopora verrucosa* and *Acropora abrotanoides*) and CSNS (*Acropora cervicornis*) from organic residues was carried out in several stages. At the first stage, the branches of the coral skeleton were subjected to rough mechanical cleaning using a suitable size brush with a hard synthetic bristle under running water. To remove organic residues, the skeleton was treated with a 5.0–7.5% sodium hypochlorite solution (24 h), repeatedly washed, first in running, then in distilled water in an ultrasonic cleaner ("Finnsonic", Finland, 40 kHz, 60 °C, 15 min.). Then the coral branches were mechanically crushed in a planetary ball mill ("Retch", Germany) up to a particle size of 300–600 microns. At the second stage, to clean the coral pores from coral dust, the particles were thoroughly washed in several portions of distilled water, retreated with 3% sodium hypochlorite solution (3–5 minutes) and washed again with distilled water. At the final stage of sample preparation, the coral particles were washed with ultrasound in the above-described mode, dried in a thermostat, laid out in penicillin vials, and sterilized by γ-irradiation (24 KGy).

To assess the osteoplastic potentials of CSAC and CSNS samples, a bone defect (shin bone "fenestration defect") was formed in sexually mature rat males of Wistar line weighing 180–200 g (laboratory animal breeding nursery "Andreevka branch of the Federal State Budgetary Scientific Institution Biomedical Technology Centre of the Federal Medical and Biological Agency"). The operation was performed under anaesthesia: the animals were presedated with 0.25% droperidol solution (0.5 ml, intraperitoneally), and then 0.25% ketamine solution (0.25 ml) was given intramuscularly. Further, in the position of the animal on the back, along the inner medial surface of the right thigh, about 5 mm from the knee joint, a 2–2.5 cm long skin incision was made. The skin was separated, the lower leg muscles were mobilized by moving them to the side, and the body of the shin bone was exposed. To eliminate the physiological regeneration of bone tissue, the bone was cleaned from the periosteum. Then, on the border of the upper and middle third of the bone, a "fenestration" defect was formed using drill (length – 6–8 mm, width – 1.5–2.0 mm, depth – 2.5–3.0 mm). The defect penetrated the bone canal cleaned of bone marrow. The defect area was filled with sterile CSAC or CSNS granules, and then the surgical wound was closed in layers.

Three groups of 10 animals were formed in accordance with three types of implanted materials: CSAC *P. verrucosa* and *A. abrotanoides* and CSNS *A. cervicornis*. 3, 6, 9 and 12 weeks after implantation of materials in the area of the bone defect, the sampling of material for morphological studies was carried out with taking the animals out of the experiment (under ether anaesthesia, two animals per each term). The shim bone was cut, and the bone fragment including the defect zone was removed and placed in 10% buffered formalin for fixation (7 days). Next, the material was decalcified in a 0.3 M EDTA solution (37 °C, 28–30 days). During this stage, the course of decalcification was monitored and the decalcifying fluid was replaced with a fresh portion. When the material became elastic, the residual EDTA was removed by quick rinsing in running water; samples were dehydrated and embedded into paraffin. After preparing the slides, they were stained with haematoxylin-eosin, and the light microscopy was performed using the Nicon Eclipse Ti microscope (Japan).

When evaluating the osteoconductive properties of CSAC and CSNS samples, attention was paid to presence/absence of signs of inflammation in the implantation zone, the evolution of osteoplastic material in the defect area was traced: morphological signs of its biodegradation and the appearance of de novo-formed tissue were noted, as well as the cellular composition of the regenerate, the quality of its consolidation with the parent bed, as well as the timing of formation of organotypically mature bone tissue.

Animal studies have been carried out in compliance with international bioethics rules in accordance with the requirements of the Helsinki Declaration of the World Medical Association and the rules of humane attitude to laboratory animals. During the experiments, the animals were kept in a vivarium equipped with operating and manipulation rooms, with standard food and water rations, under standard lighting and humidity conditions.

RESULTS

The results of histological studies of CSAC *P. verrucosa* and *A. abrotanoides* in comparison with CSNS *A. cervicornis* showed that there were no critical differences in the dynamics of bone defect replacement with these materials.

Thus, 3 weeks after implantation, restoration of the periosteum from dense connective tissue has occurred over the bone defect. Granules of the coral skeleton in this area (represented on the histological preparations as voids after decalcification) were walled up in the periosteum (Fig., a1, b1, c1). In some fields of vision, bone-resorbing cells were visualized at the border of the connective tissue and granules. In some places, the "tongues" of the periosteum penetrated the area of the bone defect with activation of periosteal osteogenesis. On certain preparations, it was evident that the defect areas began to be replaced by spongy bone tissue with the foci of haematopoiesis (Fig., a2, b2, c2).

In six to nine weeks after the implantation, the process of replacing a bone defect by neo-osteogenesis had been continuing: an active spongy bone formation was observed in the operating area, where the foci of bone marrow haematopoiesis were visualized between the trabeculae

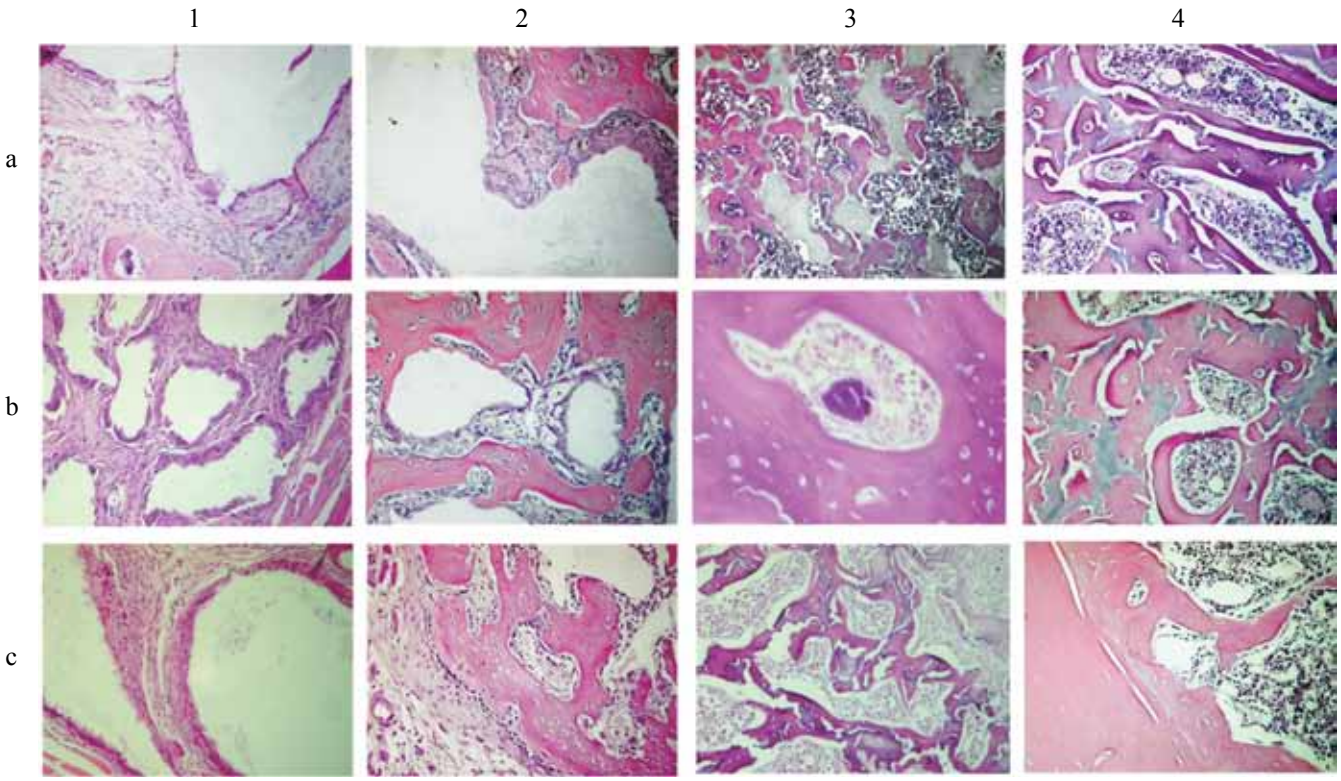

Fig. Dynamics of bone defect substitution by granules of CSAC of *P. verrucosa* (a) and *A. abrotanoides* (b) and CSNS of *A. cervicornis* (c): 1, 2 – 3 weeks; 3 – 6 weeks; 4 – 12 weeks after implantation

(Fig., a3, b3, c3). In separate fields of vision, the formation of compact bone tissue between the periosteum and the spongy bone tissue was noted. Within those timeframes, the defect area was almost completely replaced by newly formed bone tissue. However, osteogenesis cannot be considered complete due to the presence of small calcification zones in the spongy bone tissue.

In 12 weeks after the implantation, almost full completion of the osteogenesis process was observed, with organotypic replacement of the defect area with spongy bone tissue with bone marrow islets between the trabeculae of bone tissue, the primordia of osteons and the rim of compact bone tissue adjacent to the periosteum (Fig., a4, b4, c4).

DISCUSSION

The use of CSNS to replace bone defects is justified by their suitable chemical composition (calcium carbonate), strength properties superior to those of ceramic calcium-phosphate materials due to the aragonite crystal lattice [13, 14], and impurity composition close to the microelement composition of bone tissue [15]. In addition, the pronounced dependence of the rate of their passive pH degradation, as well as the ability of the bone-resorbing cells to destroy aragonite, i. e., to ensure its active biodegradation, provide the rate of utilization of the coral skeleton in the bone defect, coordinated with the rate of neo-osteogenesis. And the through porosity of corals ensures their rapid colonization by the cells and osteogenesis throughout the entire volume of the implant. It was shown that the absence of phosphorus compounds in their structure doesn't limit their use as osteo-replacing material [16–19]. ATP is probably the source of phosphorus in this case. Nowadays, some of commercial organizations offer CSNS-based products for the replacement of bone defects of various sizes and configurations ("Silorif" (Russia), "Biocoral" (France), "BoneMedik" (South Korea)). However, technogenic (tests of nuclear and other types of weapons in the oceans) and environmental (underwater volcanic eruptions) impacts can result in undesirable shifts in the microelement composition of CSNS from different water areas (an increase in the content of radioactive isotopes, sulphur, arsenic, etc). Together with the prohibition of many countries on the production of CSNS on the coastal continental shelves, this creates certain difficulties and limitations in the production of CSNS and trigger the need of elemental composition control for all the samples of this raw material.

An alternative to CSNS is the skeleton of their aquaculture used to replace bone defects in laboratory animals in the present work. Coral aquaculture was obtained on the base of Russian-Vietnam Tropical Research Technological Centre by the employees of FSRI Institute of Ecology and Evolution Problems named after A.N. Severtsov of Russian Academy of Sciences (project manager T.A. Britaev).

The cultivation of CSAC on carriers in the water column in the coastal zone, in the sector protected from waves, in the natural microenvironment, made it possible to obtain the CSAC similar to CSNS in microelement composition but superior in strength [14].

In the present study, granular CSAC samples of two families were used to replace fenestration bone defects in the femur of rats – *P. verrucosa* and *A. abrotanoides*. Previously studied CSNS *A. Cervicornis* were used as reference samples [20, 21]. In accordance with the number of sample types, 3 groups of animals were formed. We examined histological specimens from the defect area in the timeframes up to 12 weeks. No significant differences were detected in the dynamics of the replacement with newly formed bone tissue of a defect filled with CSNS and CSAC. Both CSAC and CSNS were biocompatible and did not cause inflammatory reactions in the implantation area. The speed of their bio-resorption was also similar. A good consolidation of CSAC granules with the parent bed in the area of the defect was established. The periosteum grew over the defect area and bone formation began by way of the periosteal osteosis 3 weeks after implantation. By week 12, the defect area was filled with newly formed spongy bone tissue with hematopoietic zones between the bone trabeculae.

CONCLUSION

Skeleton of the aquacultures of corals *P. verrucosa* and *A. abrotanoides* has the osteoplastic properties similar to those of the skeleton of corals *A. cervicornis* from natural settlements.

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The authors declare no conflict of interest.

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