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Peculiarities of Osteogenesis by Periosteal Cells after Experimental Ectopic Transplantation

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We carried out a comparative study of the features of osteogenesis from the progenitor osteogenic periosteal cells in rabbit and human. At the initial stages, high osteogenic potential of both human and rabbit periosteal cells was observed. However, at the later stages, the cell response favors resorption of the new bone tissue formed from periosteal cells in rabbits, but does not affect the bone tissue formed from human progenitor osteogenic periosteal cells. These functional characteristics of rabbit periosteal cells should be considered when planning the experiment.

Key Words: *periosteum; osteogenic cell culture; ectopic transplantation*

Cell therapy is one of the most attractive approaches for recovery of injured organs and tissues. During the last decade, cell-based technologies are actively implemented in various areas of medicine and studied in numerous clinical trials [6]. Mesenchymal stem cells (MSCs) obtained from various sources are most often used [4]. These cells demonstrate considerable differentiation potencies, while the procedure of their isolation is easier and cheaper compared to induced pluripotent stem cells, although these types of stem cells are similar in their properties.

Many cell-based techniques are aimed at the osteogenic differentiation of MSCs, since the problem of restoring both tubular and flat bones is of high social importance [8,9]. However, the need for MSC stimulation and *in vivo* differentiation control limits their widespread use in clinical practice. Unlike broad spectrum of MSC differentiation, periosteal cells have only osteogenic and chondrogenic potential [10]. Harvesting of the periosteum is a minimally invasive procedure that may be carried out during planned surgery. Osteogenic activity of periosteal cells is more pronounced in flat bones compared to that in long bones, which makes them very attractive for the use in bone tissue recovery [7]. Ectopic transplantation, which enables studying the ability of these cells to form a particular tissue in an atypical place is often used to assess the differentiation potential of stem and progenitor cells *in vivo* [2,11]. The use of ectopic transplantation enables more comprehensive assessment of the osteogenic potential of studied cells with allowance for possible differences in osteogenic potential of periosteal cells *in vitro* and *in vivo* [3].

Here we compared peculiarities of bone tissue formation from rabbit and human osteogenic progenitor cells derived from the periosteum in ectopic transplantation.

MATERIAL AND METHODS

Periosteal tissue samples (5×5 mm) from alveolar bones of patients and rabbits were harvested during surgery under sterile conditions and placed in a 50-ml test tube (Sarstedt) with transport medium. The procedure was approved by the local Ethics Committee.

Two hours after sampling, biopsy specimens were subjected to mechanical and enzymatic treatment in a sterile box. After 16-20-h incubation in collagenase II solution (Sigma), tissue homogenate was centrifuged (800 rpm for 5 min at 18-20°C) and the precipitate was resuspended in DMEM-GlutaMAX growth medium

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(Gibco) supplemented with fetal calf serum (Gibco) and antibiotic/antimycotic (Gibco) in standard concentration.

The cell suspension was placed in a plastic culture flask (25 cm^2; Corning) and cultured in a CO_2 incubator at 5% CO_2 for 10-15 days until the formation of a

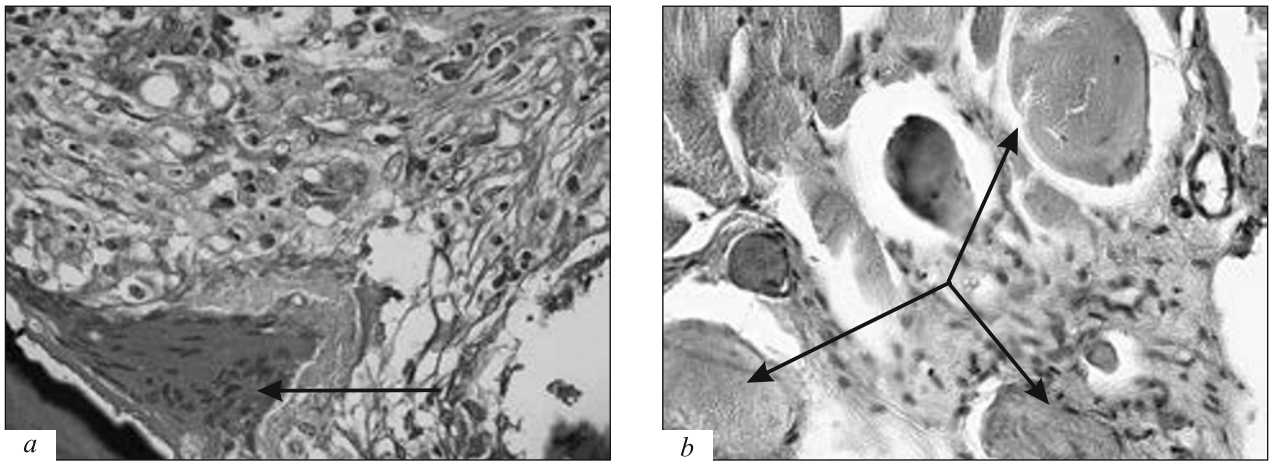

Fig. 1. Rabbit muscle tissue in the ectopic transplantation area, week 4 of the study. Hematoxylin and eosin staining, ×200 (*b*), ×400 (*a*). *a*) Formation of islets of human periosteal bone cells (arrow). *b*) Control group: microcarriers without osteogenic cells in muscle tissue (arrows).

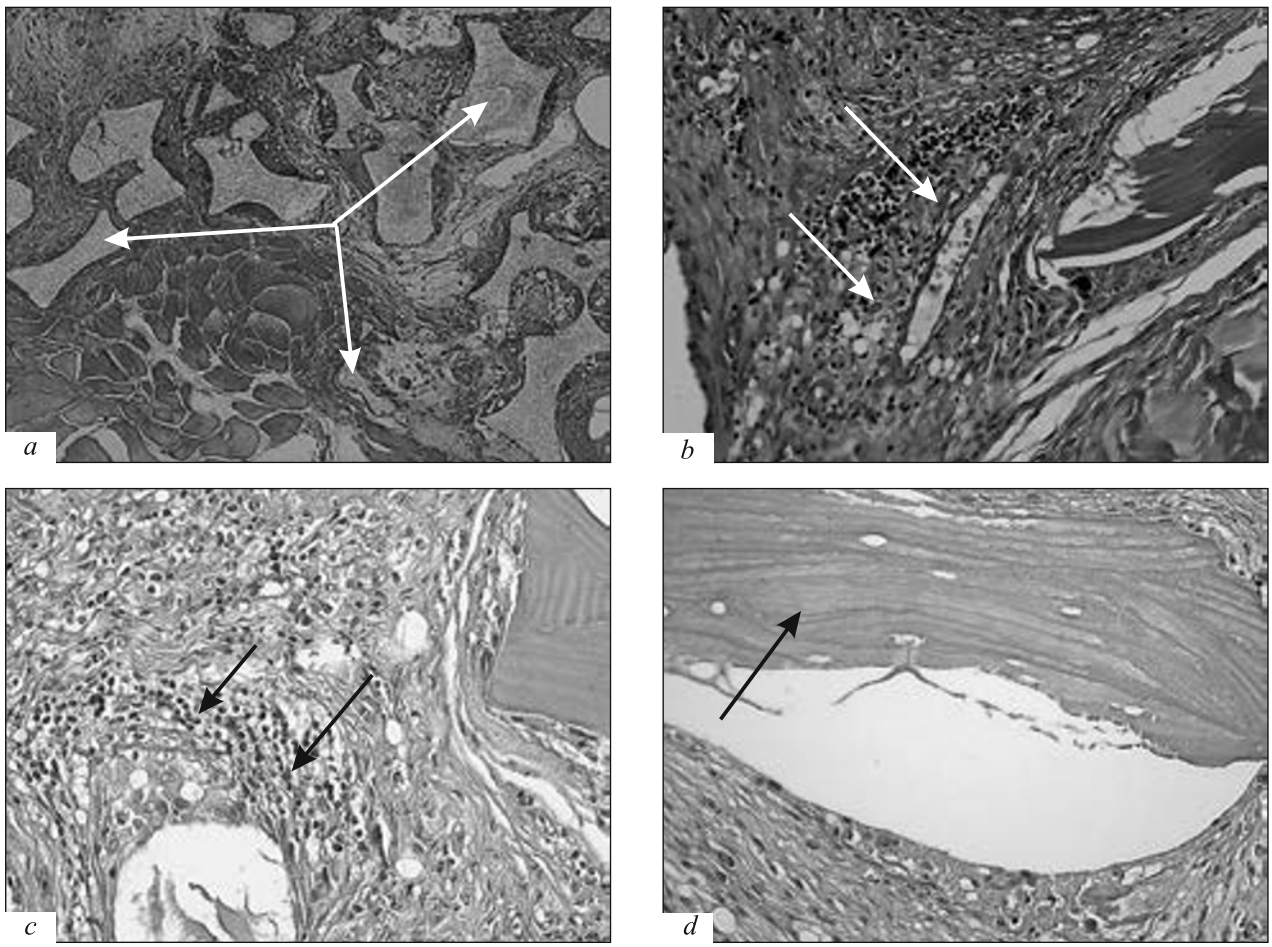

Fig. 2. Rabbit muscle tissue from ectopic transplantation area, weeks 8 (*a-c*) and 12 (*d*) of the study. Hematoxylin and eosin staining, ×100 (*a*), ×400 (*b-d*). *a*) Control group: small fragments of the microcarrier (arrows) with weak mononuclear infiltration in the muscle tissue. *b*, *c*) Osteogenic rabbit (*b*) and human (*a*) cells: mononuclear infiltration area (arrows) around the newly formed bone tissue presented by macrophages and solitary multinuclear cells. *d*) Bone tissue (arrow) formed after transplantation of human periosteal cells.

subconfluent monolayer. Then, an aliquot of cells was then used for standard immunohistochemical analysis and the other portion was cultured with microcarriers at a 2:1 ratio [1]. Osteon-2 microcarriers were used in the experiment. Carriers were washed twice with sterile Hank's solution with phenol red immediately before cell plating. After washing, 1 ml complete growth medium was placed to the wells.

Rabbits weighing 3 kg (n=36) underwent ectopic transplantation of cell suspension on microcarriers in a volume of 1 ml into the longissimus dorsi muscle under local anesthesia in order to evaluate the osteoinductive capability of the bioengineering construct. The animals were divided into 3 groups, 12 animals each: controls (group 1) received transplantation of microcarriers without cells, group 2 animals received Osteon-2 microcarriers with osteogenic human cells, and group 3 rabbits received Osteon-2 microcarriers with osteogenic rabbit cells. The results of ectopic transplantation were evaluated in 4, 8, 12, and 16 weeks. Muscle tissue from the area of ectopic transplantation was fixed in 10% neutral formalin, decalcified, and embedded in paraffin. Paraffin sections were routinely stained with hematoxylin and eosin.

RESULTS

Formation of bone islets began on the 4th week after transplantation of human and rabbit osteogenic periosteal cells of the alveolar bone on the Osteon-2 microcarriers into the longissimus dorsi muscle (Fig. 1). On the 8th week, areas of pronounced osteogenesis were observed in the muscle tissue of group 2 and 3 rabbits. Mononuclear infiltration presented mainly by macrophages, lymphocytes, and isolated multinucleated cells was observed along the contact between the bone and muscle tissue (Fig. 2). There were no plasma cells in the infiltration. Mononuclear infiltration was more abundant around the bone formed by human cells in comparison with rabbit cells. In the control group, small pieces of microcarriers slightly infiltrated with mononuclear leukocytes and isolated multinucle-

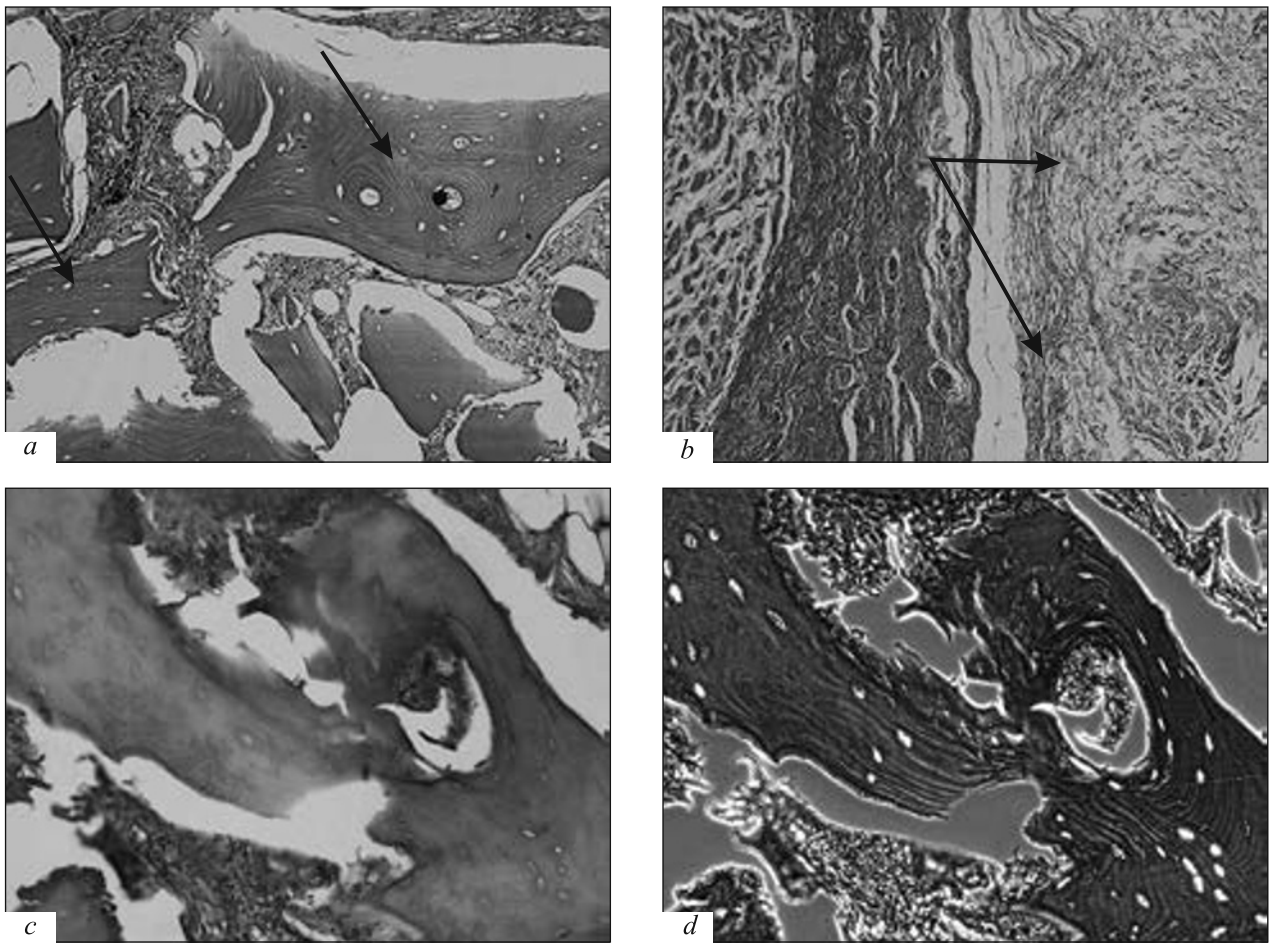

Fig. 3. Rabbit muscle tissue from the ectopic transplantation area, week 16. Hematoxylin and eosin staining, ×100 (*a*), ×200 (*b-d*). *a*, *c*) General view (*a*) and structure (*c*) of human osteogenic cells forming mature bone tissue. *b*) Replacement of bone tissue in the area of transplantation of rabbit osteogenic cells with loose connective tissue (arrow). *d*) Bone tissue structure (dark field).

ated cells were observed in the muscle tissue by the 8th week of the study. On the 12th week, there was no mononuclear infiltration in the muscle tissue around the formed bone in the experimental groups. There was well-developed trabecular bone structure in the group with human cells (Fig. 3). No further osteogenesis was observed in the group with rabbit cells. On the 12th week, no traces of microcarriers were seen in the muscle tissue of rabbits in the control group. By the 16th week, there was almost no bone tissue and microcarrier fragments in group 3 as opposed to group 2, where human osteogenic cells formed adequate bone tissue.

Thus, the results of ectopic transplantation demonstrated pronounced functional difference between human and rabbit periosteal cells. Both rabbit and human periosteal cells have osteogenic activity. However, rabbit cells activate formation of osteoclasts, which destroy the newly formed bone. It is likely that the development of local aseptic response activates rabbit periosteal cells to secrete biological factors triggering osteoclastogenesis process [5]. It is known that the periosteal tissue contains resident macrophages, which however disappear during long-term cultivation and the culture becomes monomorphic. Therefore, the secretory profile of rabbit periosteal cells significantly differs from the secretory profile of human periosteal cells, stimulates resident macrophages and immature dendritic cells in the ectopic transplantation, area and thus initiates bone tissue degradation process. Human periosteal cells do not initiate osteoclastogenesis process and contribute to the formation of adequate bone tissue. These functional characteristics of rabbit periosteal cells should be taken into account when planning the experiment.

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