Effect of Embryonic Bone Tissue on Bone Regeneration

N. P. Omel'yanenko, O. A. Malakhov, G. T. Sukhikh,*
O. V. Kozhevnikov, I. N. Karpov, and I. A. Petrov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 130, No. 10, pp. 469-474, October, 2000
Original article submitted July 24, 2000

Fragmented embryonic bone tissue stimulates bone regeneration. Bone formation starts not from implanted embryonic fragments, but in intact periosteum and endosteum containing cambial cells of the osteodifferon. In rabbits, recovery of damaged radial bone after implantation of fragmented embryonic bone tissue into bone defect was associated with a pronounced periosteal reaction and focal resorption of intact ulnar bone. Consolidation of damaged radial bone without implantation of fragmented embryonic bone tissue was incomplete in all experimental animals.

Key Words: *regeneration; embryonic tissue; bone; pig; rabbit*

Despite numerous methods for the treatment of diseases and injuries of the locomotor system, the incidence of complications due to impaired reparative osteogenesis remains very high. The search for new means improving bone reparation is still in progress. In particular, bone auto-, allo- and xenoimplants prepared from native, partially or completely demineralized bone, including allogenic fetal bone tissue, are now widely used in traumatology and orthopedics. These implants are used for repair of extensive bone defects, false joints, cysts, and residual cavities, for stimulation of slowly maturing distraction regenerates, *etc.* [1-3,5,13]. There are theoretical grounds for the use of fragmented embryonic bone tissue (EBT) for stimulation of reparative osteogenesis. The components of embryonic tissue (immature collagen and amorphous calcium phosphate) are easily resorbed. Osteoinductive properties of EBT are largely determined by the presence of several types of morphogenetic bone protein and growth factors stimulating proliferation and differentiation of the osteodifferon precursors and angiogenesis [14].

We studied the effect of fragmented EBT on bone regeneration.

N. N. Priorov Central Institute of Traumatology and Orthopedics; *Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

Experiments were carried out on 48 Chinchilla rabbits (2.0-2.5 kg) aged 8-10 months. A 1-cm resection was made in the central part of radial bone diaphysis. In controls (n=16) bone defects were not filled. In experimental animals immediately after ostectomy the defect was filled with native fragmented porcine EBT. Particle size in group 1 (n=16) varied from 0.2 to 0.7 mm and in group 2 (n=16) from 0.7 to 2.0 mm. The volume of implanted EBT corresponded to the volume of removed bone segment. Fragmented EBT was prepared by crushing of brachial and femoral bones of 11-week pig embryos under sterile conditions. The size of particles of fragmented EBT and their differential distribution were evaluated by computer-assisted morphometry. The animals were sacrificed 2, 4, 8, and 16 weeks postoperation by intravenous injection of 2-10 ml 4% sodium thiopental.

Operated limbs of experimental and control animals were compared. For morphological analysis the material was fixed in 10% neutral formalin, decalcified in 12% HNO_3, dehydrated in ascending alcohol concentrations, and impregnated with celloidin. After hematoxylin-eosin and van Gieson staining histological sections were examined under a Jenalumar optic microscope (Karl Zess) attached to a computer-assisted image analysis system for morphometry.

0007-4888/00/0010-1017$25.00

For scanning electron microscopy (S-800 microscope, Hitachi), EFT fragments were dehydrated, vacuum dried, and coated with gold.

Control x-ray examination of operated limbs was performed at all stages. Films were processed by computer densitometry.

RESULTS

The cortical part of embryonic bone consists of flat fibroreticular bone trabeculae oriented along the long axis of the bone. The epiphysis and diaphyseal bone marrow cavity (BMC) were filled with fibroreticular bone tissue including the bone marrow. All these components of the embryonic bone were present in fragmented EBT.

Two weeks after implantation x-ray examination revealed a weak shadow at the site of radial bone defect. Histological analysis showed that bone defect was filled round or elongated EBT fragments distributed more or less evenly among bone fragments and surrounded by connective tissue (Fig. 1, *a*). EBT bone structure was homogeneous with cells between bone plates. In rabbit bone fragments BMC were partially or completely closed with fibrous connective tissue which filled the defect and even grew beyond the anatomical borders of a distant diaphyseal segment. This fibrocellular complex was surrounded with compact connective tissue. The rabbit bone fragments had irregular notched contours and basophil borders, somewhere at their edges osteocytes were absent. Bone regenerates (BR) with spongy structure were seen on the

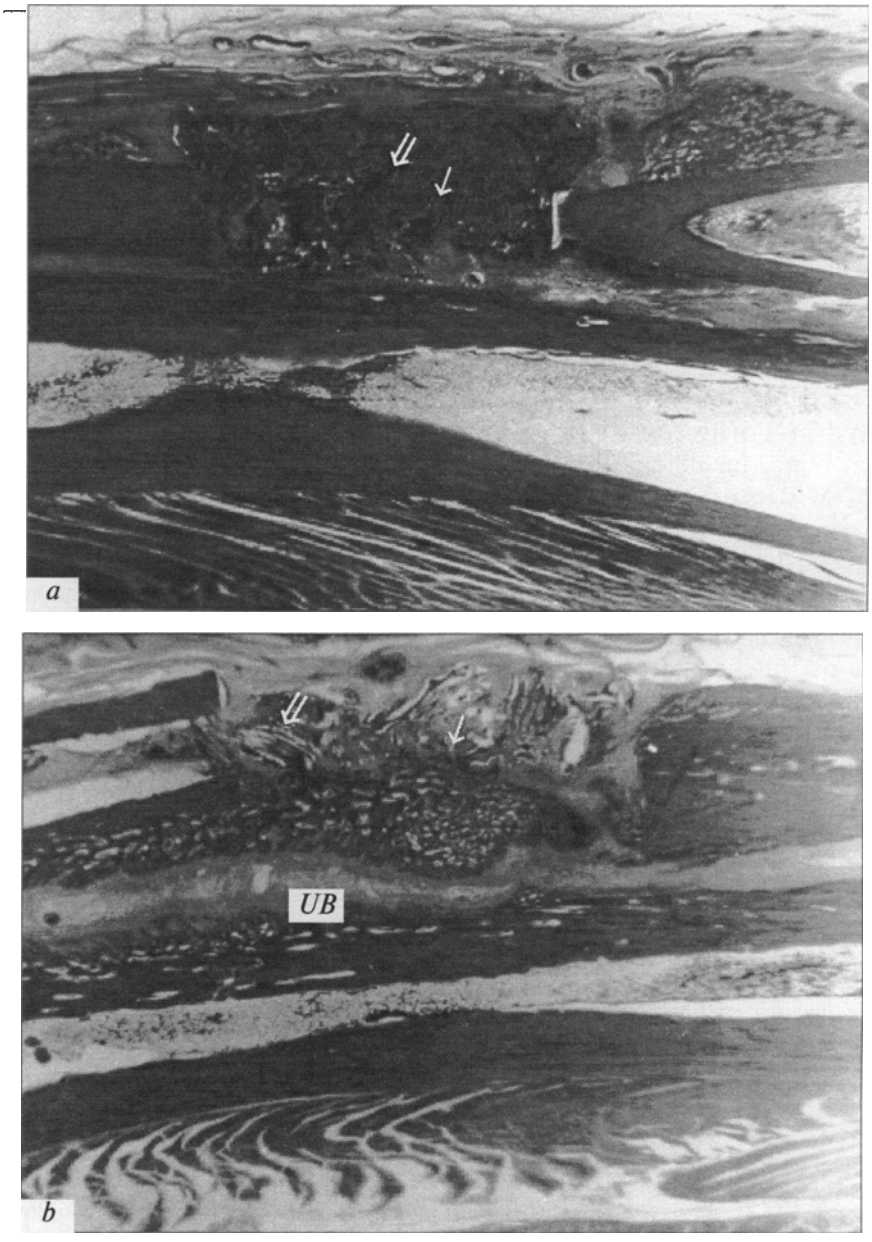

Fig. 1. Rabbit antebrachium (experimental group 1) 2 (*a*) and 4 (*b*) weeks after ostectomy. *a*) radial bone defect filled with fragmented embryonic bone tissue (EBT) (arrow) and connective tissue (double arrow); *b*) osseocartilaginous regenerate (arrow) and EBT fragments (double arrow). Ulnar bone (UB). Here and in Figs. 2 and 3: histological preparations, hematoxylin-eosin staining, ×10.

periosteal and endosteal surface. Their thickness was maximum near the edges of the defects, decreases with the distance from the defect and eventually merge with the periosteum. At the same time, there were zones of intact periosteal and endosteal surface closer to the edges of the bone fragments. Widened central channels and vessels with erythrocyte aggregates were seen in the cortical plates of the bone fragments. Fibrocellular complex with EBT fragments filling the bone defect directly contacted with the surface of the ulnar bone in some cases. The surface of the ulnar bone at the site of defect was uneven, with numerous resorption lacunal. The periosteum was absent, but there were BR (spongy bone) on the surface of ulnar bone plate. Periosteal bone formation on the ulnar bone was observed in the projection of bone defect and at some distance on both sides from it on intact sites of the radial bone. Sometimes BR were cartilaginous. Vessels inside the cortical plate of the ulnar bone were dilated and filled with aggregated erythrocytes. The central channels were enlarged due to resorption of the compact bone substance around vessels.

X-ray examination after 4 weeks showed a weakly contrast shadow in bone defects, which indicated the formation of BR partially filling the bone defect (Fig. 1, *b*). The interface between BR and bone fragments was still discernible. Histologically BR looked like a bridge connecting bone fragments and filling the diastasis between them by a half of its width (Fig. 1, *b*). The greater part of BR looked like spongy bone:

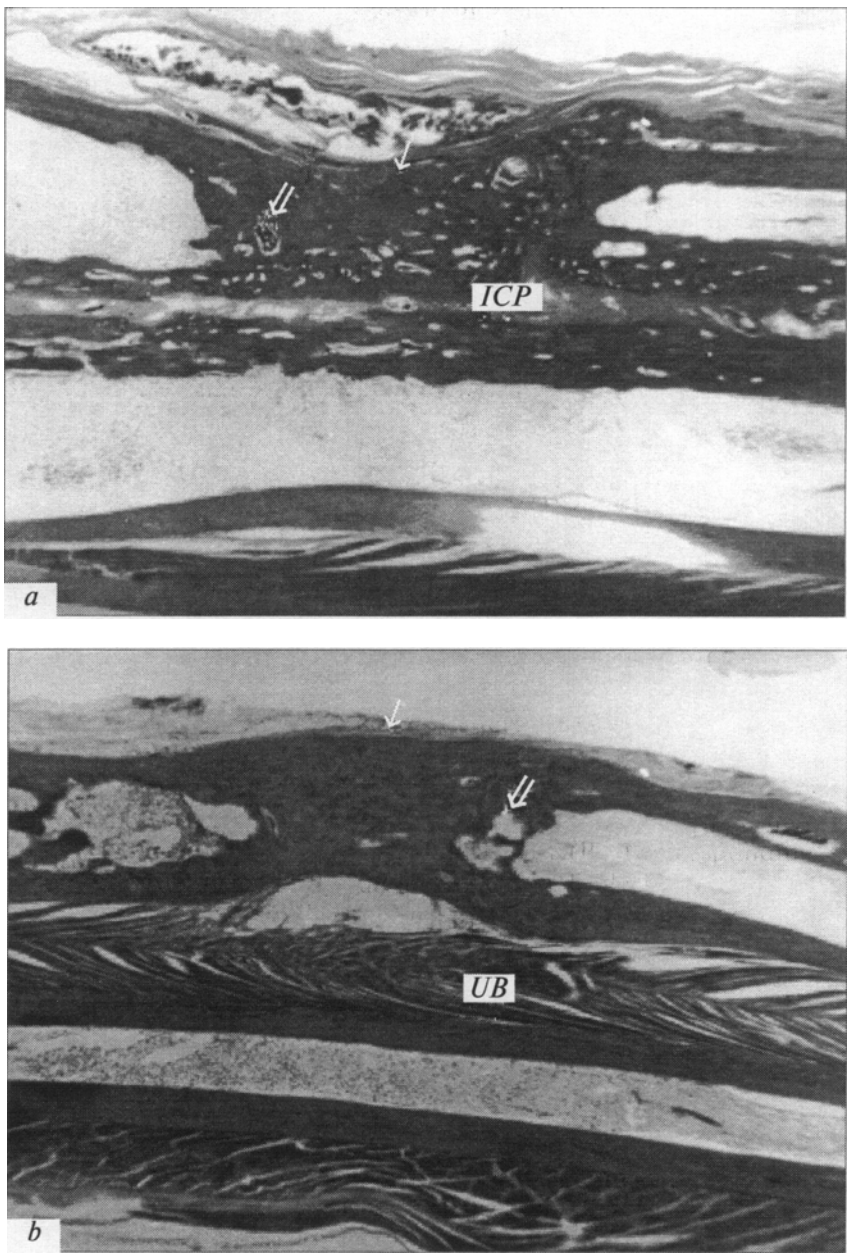

Fig. 2. Rabbit antebrachium (experimental group 1) 8 (*a*) and 16 (*b*) weeks after ostectomy. *a*) radial bone regenerate (arrow), EBT fragments in the regenerate (double arrow). Internal cortical plate (*ICP*) of the ulnar bone; *b*) restored radial bone. Arrow shows new bone, double arrow shows resorption cavity, *UB:* ulnar bone.

bone structures (primary bone trabeculal) were separated with spaces filled with reticular tissue containing numerous connective tissue cells. Some of them could belong to the osteodifferon. Osteoblasts were situated on the surface of bone trabecular. In other zones of reticular tissue (with larger cells) there were vessels and spaces free of cell elements. The lesser portion of BR consisted of cartilage. Periosteal regenerate at the level of the radial bone defect and on both sides of it was seen on the ulnar bone surface facing the radial bone. In some animals BR partially filling the radial bone defect grew into the ulnar bone or to its periosteal regenerate, forming a sort of interosseous bridge. Vessels were dilated in resorption zones in the cortical plate around some vessels. The outer cortical plate of the ulnar bone remained intact. Bone marrow vessels in the fragments of radial and ulnar bones were dilated and filled with blood.

After 8 weeks x-ray revealed more contrast BR shadow occupying the entire defect (Fig. 2, *a*); the cortical plate contour was clearly seen. Histological analysis showed that BR filled the defect (space) between fragments of the radial bone and slightly penetrated into BMC, that is, the anatomical intactness of damaged bone was restored (Fig. 2, *a*). BR consisted of compact bone tissue but the orientation of vessels, central osteon channels, and the osteons in the regenerate was disorderly, in contrast to the cortical part of the proximal and distal bone fragments. That is why the borders of BR and bone fragments were clearly

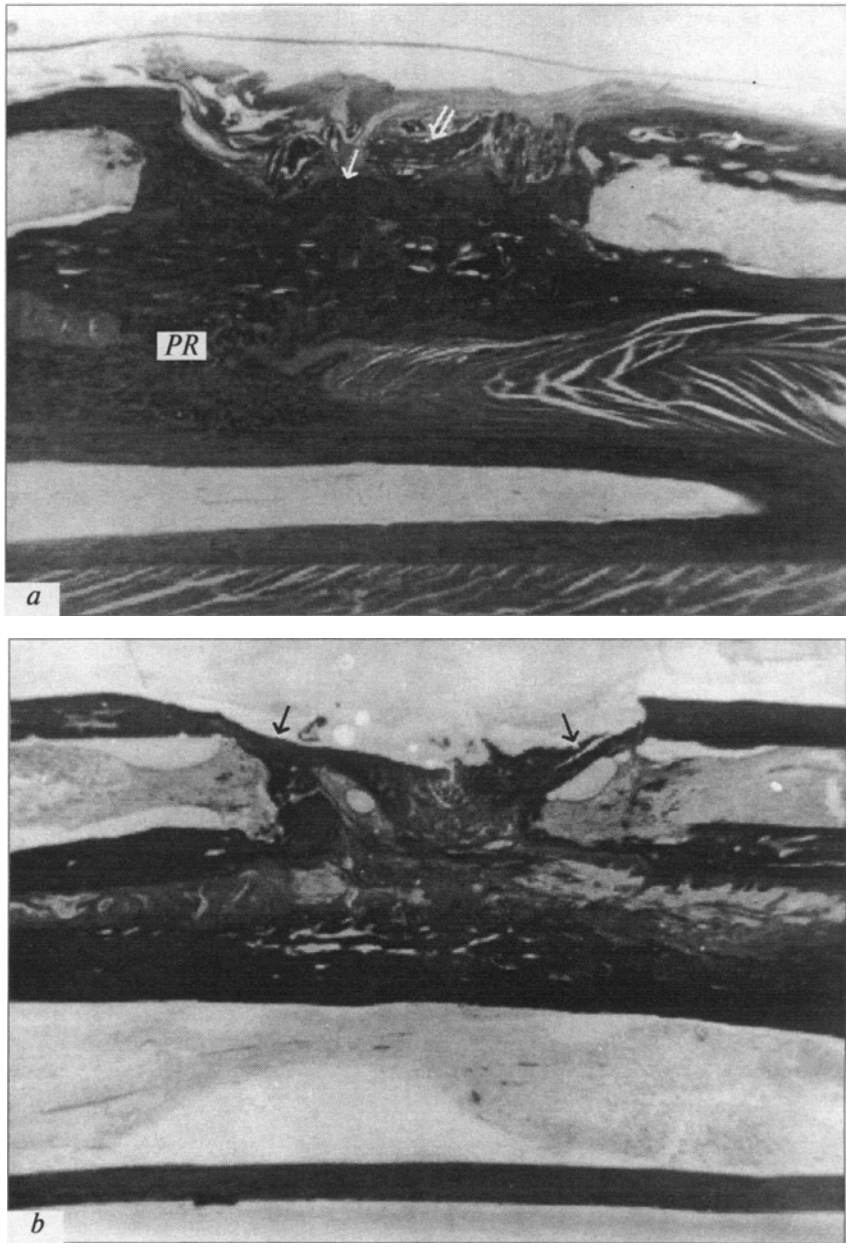

Fig. 3. Rabbit antebrachium in experimental group 2 (*a*) and control (*b*) 16 weeks after ostectomy. *a*) radial bone with dislocated bone regenerate (arrow). EBT fragment (double arrow). *PR*: periosteal regenerate of the ulnar bone; *b*) proximal and distal bone fragments (shown with arrows), radial bone defect.

lined with a basophil line. No BMC were seen. BR contained cavities with residual fragments of EBT and resorption lacunal around the central channels. The vessels in these channels were filled with erythrocytes. From the outer side, the regenerate was covered with a thin layer of compact and loose connective tissue containing residual EBT. From the inner side the new bone was separated from the ulnar bone by an interosseous space containing a connective-tissue membrane. There was a compact bone tissue periosteal regenerate in the outer surface of cortical plates of the radial bone fragments. Resorption lacunal were seen around the vessels in the periosteal regenerate facing the ulnar bone. Zones of resorption around vessels were seen in the cortical plate of the ulnar bone facing the radial bone injury, both in the projection of the injury and at a distance from it on both sides. The shape of resorption lacunal was different (round, elongated, irregular). Some previously formed resorption lacunal were filled with new bone. Accumulations of osteoblasts were observed in these lacunal, and the structure of BR was disorderly. No periosteal or endosteal regenerates of the ulnar bone were seen by this term.

Sixteen weeks after ostectomy the regenerate shadow filled the entire defect, being darker in the center. Histological analysis showed that the central part of BR consisted of compact bone tissue and the periphery was remodeled into tubular bone and consolidated with the proximal and distal bone fragments (Fig. 2, *b*). The space between the new cortical plates was filled with the bone marrow and in fact was a continuation of BMC. Despite the absence of a clear border, the new bone could be discerned by disorientated vessels, central channels, and osteons. Zones of resorption were seen in the central part of BR. "Counter" resorption of the regenerate was observed from BMC of the proximal and distal bone fragments, that is, a new BMC was forming. No changes in the cortical plates or bone marrow of the ulnar bone were seen, *i. e.* bone structure was completely restored in foci of resorption.

Fragmented EBT with large fragments (0.7-2.0 mm equivalent diameter) also possessed osteogenic activity, but its fragments were resorbed for a longer time and, while in the bone defect, impeded the formation of BR. Nonetheless the regenerate did form and connected the bone fragments, thus restoring the intactness of damaged bone, though with a notable dislocation from the anatomical border of intact bone (Fig. 3, *a*).

In the control, no restoration of damaged bone was incomplete (Fig. 3, *b*).

Hence, EBT stimulated bone regeneration. Its fragments did not become the immediate osteogenesis centers; bone formation started in the intact periosteum and endosteum, where cambial cells of the osteodifferon were located.

Several stages were distinguished in restoration of regular structure of damaged bone after filling the defect with EBT: filling of the defect with fibrous connective tissue surrounding EBT elements; formation of fibroreticular BR with EBT fragments; remodeling and construction of lamellar BR; restoration of BMC with the bone marrow.

Restoration of the structure of damaged radial bone after filling the defect with EBT was associated with pronounced periosteal reaction and focal resorption of intact ulnar bone at the level of the defect and beyond it.

Based on published data [14], we can hypothesize that some growth factors including morphogenetic proteins within bone tissue stimulates reparative (and hence physiological) regeneration. Evidently long presence of these factors in the bone defect within EBT stimulates osteogenesis and formation of bone tissue filling the defect.

This allows us to consider fragmented EBT a promising material for traumatology and orthopedics.

REFERENCES

1. V. P. Bersnev, V. I. Savel'ev, and V. Yu. Zotov, *Demineralized Bone Transplants and Their Use in Repair Surgery* [in Russian], St. Petersburg (1996), pp. 40-43.
2. V. N. Gorbachevskii, A. K. Pokotilenko, V. E. Makashev, and Yu. V. Minin, *Demineralized Bone Transplant and Its Use* [in Russian], St. Petersburg (1993), pp. 79-86.
3. J. G. Andrew, J. Hoyland, S. M. Andrew, *et al., Calcif. Tissue Int.*, **52,** 74-78 (1993).
4. P. Aspenberg, J. Wittbjer, and K. G. Thorngren, *Clin. Orthop.*, **206,** 261-269 (1986).
5. L. S. Beck, E. P. Amento, Y. Xu, *et al., J. Bone Miner. Res.*, **6,** 1257-1265 (1993).
6. M. E. Bolander, *Proc. Soc. Exp. Biol. Med.*, **200,** 165-170 (1992).
7. L. F. Bonewald and G. R. Mundy, *Clin. Orthop.*, **250,** 261-276 (1990).
8. S. D. Cook, M. W. Wolfe, S. L. Salkeld, and D. C. Rueger, *J. Bone Joint Surg.* [Am], **77,** 734-750 (1995).
9. T. Einhorn, *Ibid.*, 940-956.
10. M. Lind, *Acta Orthop. Scand.*, **67,** 407-417 (1996).
11. N. Omelyanenko, O. A. Malakhov, Y. U. Shaposhnikov, *et al., SIROT* [Am], New York (1996), P. 253.
12. R. S. Sellers, D. Peluso, and E. A. Morris, *J. Bone Joint Surg.* [Am], **79,** 1452-1463 (1997).
13. E. Solheim, E. M. Pinholt, G. Bang, and E. Sudmann, *J. Neurosurg.*, **76,** 275-279 (1992).
14. E. Solheim, *Int. Orthop.*, **22,** 410-416 (1998).
15. A. W. Yasko, J. M. Lane, E. J. Fellinger, *et al., J. Bone Joint Surg.* [Am], **74,** 659-670 (1992).