Reparative Osteogenesis during Transplantation of Mesenchymal Stem Cells

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Reparative osteogenesis was studied after xenotransplantation of suspension cell graft from human mesenchymal stem cells. A model of experimental damage to rat femoral diaphysis was developed. The state of animals was satisfactory and non-depressed in the early and late postoperation period. We revealed no local pathological reactions and complications. Administration of mesenchymal stem cells into the area of bone defect accelerated and improved regeneration. Unilateral transplantation of the cell graft stimulated regeneration in the contralateral limb due to acceleration of bone tissue maturation. On day 90 after treatment the bone regenerate was completely developed in the area of defect in animals of various groups. The newly formed bone tissue was well integrated into the bone organ.

Key Words: *reparative osteogenesis; mesenchymal stem cell; histomorphometric study*

High incidence of traumas and degenerative disorders of the locomotor apparatus and early employment or house disability reflects the absence of effective methods for pharmacotherapy and surgical treatment [3]. Despite high regeneration activity of the bone tissue, reparative osteogenesis does not necessarily result in structural and functional recovery of the bone organ (even after treatment with osteoplastic and osteoinductive materials). This state is associated with disintegration or insufficiency of cambial cells in the bone tissue and designated as osteogenic deficiency [1]. Taking into account deficiency of functionally active precursors, transplantation of auto- or allogeneic precursor cells holds much promise for the therapy of traumas and diseases [1]. Cultures of stem/progenitor cells are now obtained under laboratory conditions. There are several populations of self-renewing cells that possess high proliferative activity and determine the growth and reconstruction of bone tissue [3,5]. Mesenchymal stem cells (MSC) can be isolated, cultured, and applied to stimulate regeneration during diseases of the bone and cartilaginous tissue [4-6].

MATERIALS AND METHODS

Fresh autopsy specimens were isolated from healthy women 2-3 h after induced abortion (18-20 weeks' pregnancy) and used to obtain the culture of bone marrow stromal MSC. The women were pre-examined for viral and bacterial infections. The culture of MSC was obtained routinely [2]. The cell suspension graft was prepared in physiological saline 2-3 h before transplantation. Cell count in the graft was brought to 1×10^8 cells/ml.

Experiments were performed on male outbred albino rats weighing 190-200 g. The animals had a typical defect of the femoral diaphysis (diameter 1.5 mm, depth 0.5 mm). The cell graft (1×10^7 cells per 0.1 ml physiological saline) or equivalent volume of physiological saline was introduced to the bottom of diaphysial defect using an insulin syringe. A polyester

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sulfonic membrane was applied to fix the cell graft in the damaged area. The total number of operated rats was 36. Each group included 4-6 animals (for each point of observations). The rat had a defect of both limbs. The animals were killed on days 10, 30, 60, and 90. In treated rats the cell graft was administered into the damaged area in one of the limbs; physiological saline was applied to the contralateral limb. In control animals physiological saline was injected into the area of defect in both limbs.

We performed 3 experimental series: MSC-experiment (administration of the MSC suspension, 1×10^7 cells per 0.1 ml physiological saline, into the bone defect); MSC-control (administration of 0.1 ml physiological saline into the contralateral limb); and control (administration of 0.1 ml physiological saline into the bone defect in both limbs).

Bone objects were isolated, fixed with 10% neutral formalin, and decalcified in 25% Trilon B. The sections were stained with hematoxylin and eosin, subjected to Van Gieson staining, and silver-impregnated by the method of Foot.

Histomorphological analysis included evaluation of the following areas: regenerate, bone component of regenerate, bone matrix, connective tissue, and hemopoietic tissue. Area of structural components in the regenerate was expressed relative to the total area of regenerate.

RESULTS

The regenerate consisting of the connective tissue and bone components was formed in animals of different groups on day 10 after treatment. In the control group, fibrocellular connective tissue lay between edges of the bone defect. Immature bone osteoid and fibrous trabeculae were found only in deep layers of the bone wound (at the level of the bony canal, Fig. 1, *c*). During this period newly formed bone structures substituted the connective tissue component of the regenerate in animals of the MSC-experiment and MSC-control groups (Fig. 1, *a*, *b*). Intergroup differences were found in the degree of differentiation of structural components in the regenerate. Newly formed bone structures and intensive maturation of the bone matrix were revealed in animals of the main groups (Fig. 1, *a*, *b*). We studied the development of main components in the regenerate. The area of the bone component increased significantly, while the ratio of connective tissue decreased in MSC-treated animals (Table 1).

Thirty days after administration of MSC the regenerate in both limbs was formed by large trabeculae with narrow intertrabecular spaces (Fig. 2, *a*, *b*). Its bone matrix underwent strong differentiation, gained high density (comparable to the maternal bone), and had a lamellar structure (Fig. 2, *a*). Small regions of

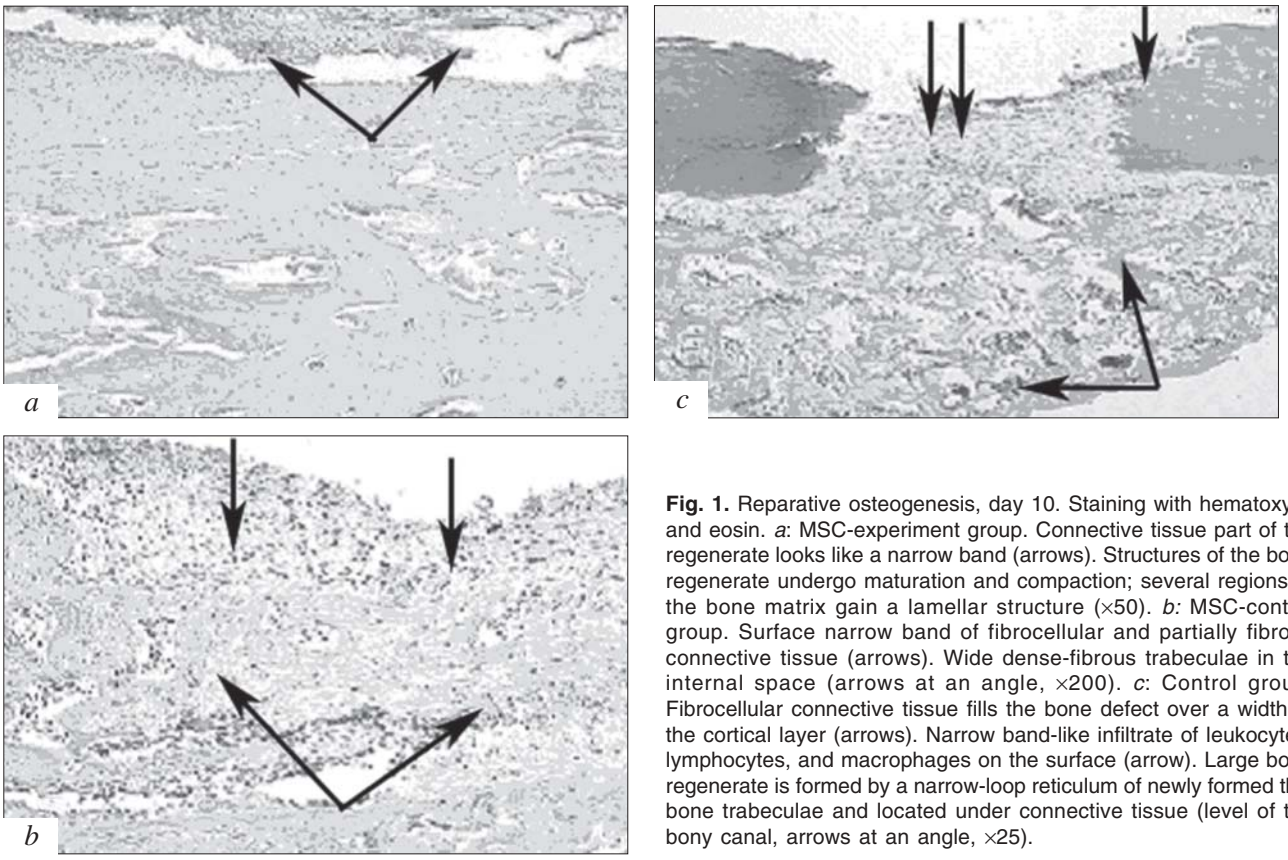

Fig. 1. Reparative osteogenesis, day 10. Staining with hematoxylin and eosin. *a*: MSC-experiment group. Connective tissue part of the regenerate looks like a narrow band (arrows). Structures of the bone regenerate undergo maturation and compaction; several regions of the bone matrix gain a lamellar structure (×50). *b:* MSC-control group. Surface narrow band of fibrocellular and partially fibrous connective tissue (arrows). Wide dense-fibrous trabeculae in the internal space (arrows at an angle, ×200). *c*: Control group. Fibrocellular connective tissue fills the bone defect over a width of the cortical layer (arrows). Narrow band-like infiltrate of leukocytes, lymphocytes, and macrophages on the surface (arrow). Large bone regenerate is formed by a narrow-loop reticulum of newly formed thin bone trabeculae and located under connective tissue (level of the bony canal, arrows at an angle, ×25).

TABLE 1. Histomorphometric Indexes for Reparative Osteogenesis after MSC Transplantation (%, *M*±*m*)

Group		Bone part of regenerate	Bone matrix	Mature bone matrix	Connective tissue	Hemopoietic tissue
Day 10	control	80.1±2.3	64.4±6.9	0	21.30±2.01	11.7±6.9
	MSC-control	88.4±4.7*	58.9±20.2	0	11.2±5.8*	18.4±6.8*
	MSC-experiment	98.4±4.7*+	72.96±8.00*+	0.82±1.80	2.2±2.0*+	28.1±14.5*+
Day 30	control	83.2±4.3	75.40±8.85	10.70±4.85	16.1±8.6	13.7±5.16*
	MSC-control	89.8±5.7*	77.6±7.1	15.4±5.9*	0	0.52±1.30*
	MSC-experiment	100*+	81.5±8.7*+	24.70±5.03*+	0	5.5±2.8*+
Day 60	control	96.4±4.5	84.5±4.9	21.5±9.6	3.0±4.1	9.6±6.2
	MSC-control	96.3±5.3	75.7±12.5	17.9±5.7	2.8±3.2	8.9±8.7
	MSC-experiment	98.2±2.4	90.2±3.7*+	23.4±13.9*	0	2.9±3.4
Day 90	control	100	93.5±4.5	49.9±12.7	0	2.6±3.0
	MSC-control	94.1±6.2	98.7±3.3	51.2±18.2	3.2±4.8	2.9±7.6
	MSC-experiment	100	89.70±8.95	56.7±11.8	0	0

Note. $p<0.05$: *compared to the control; +compared to MSC-control.

the connective tissue were present in the regenerate of control animals (Fig. 2, *b*, *c*). The indexes for strong compaction (increase in the bone matrix ratio) and maturation of the bone matrix remained high on day 30 (Table 1).

On day 60 after treatment bone structures of the regenerate continued to develop and differentiate in animals of different groups. In control animals the bone regenerate had a spongy structure. The intercellular matrix was mainly dense and lamellar (Fig. 3, *c*). A similar tendency to maturation of the bone regenerate was found in MSC-treated animals (Fig. 3). No significant intergroup differences were revealed in this period of observations (Table 1).

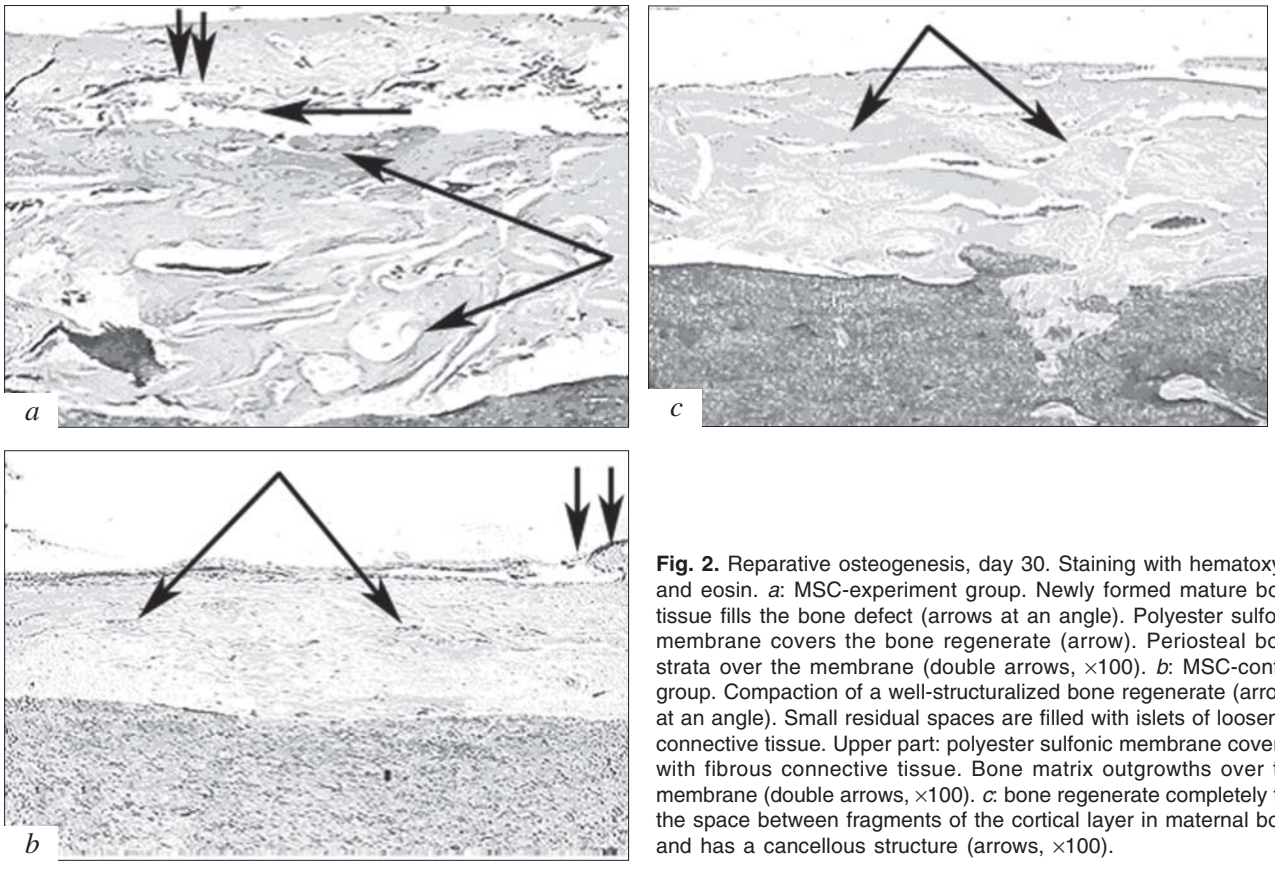

Fig. 2. Reparative osteogenesis, day 30. Staining with hematoxylin and eosin. *a*: MSC-experiment group. Newly formed mature bone tissue fills the bone defect (arrows at an angle). Polyester sulfonic membrane covers the bone regenerate (arrow). Periosteal bone strata over the membrane (double arrows, ×100). *b*: MSC-control group. Compaction of a well-structuralized bone regenerate (arrows at an angle). Small residual spaces are filled with islets of loosened connective tissue. Upper part: polyester sulfonic membrane covered with fibrous connective tissue. Bone matrix outgrowths over the membrane (double arrows, ×100). *c*: bone regenerate completely fills the space between fragments of the cortical layer in maternal bone and has a cancellous structure (arrows, ×100).

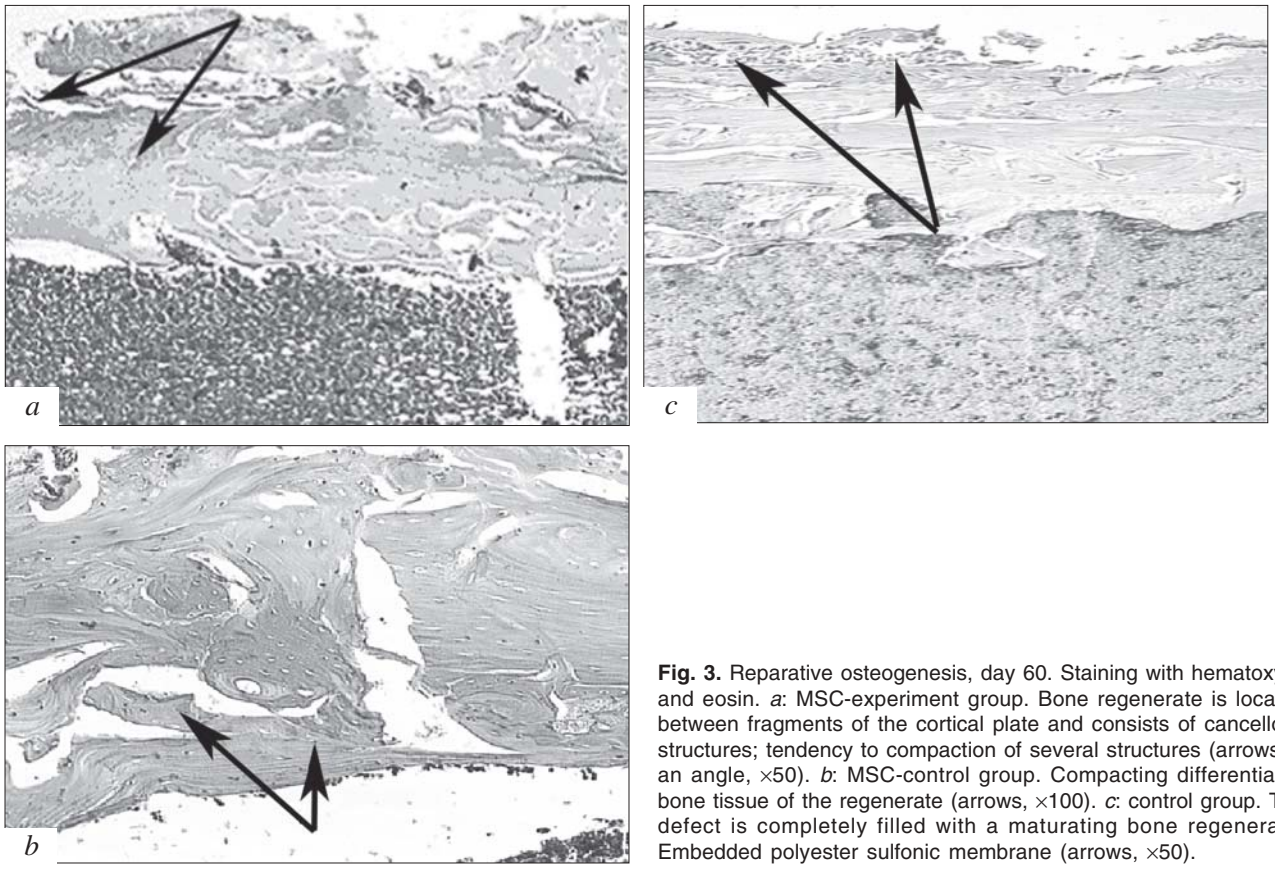

Fig. 3. Reparative osteogenesis, day 60. Staining with hematoxylin and eosin. *a*: MSC-experiment group. Bone regenerate is located between fragments of the cortical plate and consists of cancellous structures; tendency to compaction of several structures (arrows at an angle, ×50). *b*: MSC-control group. Compacting differentiated bone tissue of the regenerate (arrows, ×100). *c*: control group. The defect is completely filled with a maturating bone regenerate. Embedded polyester sulfonic membrane (arrows, ×50).

Histological study showed that the intensity of reparative osteogenesis and maturation of the bone regenerate remained high in animals of various groups on day 90 after treatment. Intergroup differences were insignificant in this period (Table 1).

The proposed experimental model of bone injury allowed us to study reparative osteogenesis under conditions of treatment with the cell suspension graft. Administration of the cell graft from MSC into the bone defect stimulated reparative osteogenesis, which was most pronounced on day 10 of the experiment. After unilateral administration of the cell graft, this effect was revealed in both limbs at the site of treatment. On day 90 the bone regenerate was completely developed in the area of the defect in animals of different groups. The newly formed bone tissue was well integrated into bone organ. Xenotransplantation of prenatal MSC without immunosuppression was not associated with early and delayed complications or local reaction of graft rejection. These data experimentally support the rationale for the therapy of extensive bone damage with allogeneic implantation of prenatal MSC.

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