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Mesenchymal Bone Marrow Stem Cells More Effectively Stimulate Regeneration of Deep Burn Wounds than Embryonic Fibroblasts

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Regeneration of deep burn wounds after transplantation of allogenic and autogenic fibroblast-like bone marrow mesenchymal stem cells and embryonic fibroblasts on burn surface was studied in 40 Wistar rats. Transplantation of allogenic and autogenic fibroblast-like bone marrow mesenchymal stem cells and transplantation of embryonic fibroblasts decreased cell infiltration of the wound and accelerated the formation of new vessels and granulation tissue in the wound in comparison with the control (burn wounds without cell transplantation). Regeneration processes were most active after transplantation of fibroblast-like bone marrow mesenchymal stem cells, in particular, autogenic cells, which was confirmed by more rapid decrease in burn surface area. Wound healing after transplantation of fibroblast-like bone marrow mesenchymal cells and embryonic fibroblasts was associated with long functioning of transplanted cells (as was shown by staining for β-galactosidase, the cells were transfected with an adenovirus vector carrying the marker gene). It is hypothesized that more rapid regeneration of burn wounds after transplantation of fibroblast-like bone marrow mesenchymal stem cells was due to low differentiation of these cells in comparison with embryonic fibroblasts.

Key Words: *bone marrow; mesenchymal stem cells; fibroblasts; burn*

Previous studies showed that transplantation of embryonic fibroblasts (EF) promoted healing of burn wounds and decreased mortality of victims [2,4]. However, ethical and legal problems concerning the use of embryonic donor material necessitated the search for new approaches to the use of autogenic and allogenic bone marrow as the source of mesenchymal stem cells (MSC) capable of differentiation into fibroblast-like mesenchymal stem cells (FMSC) [6-9].

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We compared the effect of transplantation of allogenic and autogenic FMSC and EF on the surface of deep burn wounds on wound regeneration.

MATERIALS AND METHODS

Deep thermal burns of the skin were induced in 40 adult male Wistar rats (300-350 g). The animals were narcotized with ether (surgical stage) and a metal plate contacting with hot water (97.7°C) was applied to the skin. The duration of plate exposure through wet gauze was 8 sec, the area of the plate was 18-20% total body surface. This mode of exposure ensured involvement of all skin layers. The animals with deep thermal burns

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were divided into 4 groups, 10 per group. In group 1 animals allogenic FMSC were transplanted, group 2 received autogenic FMSC, group 3 (reference group) received allogenic EF, and control group 4 (spontaneous regeneration) received no cells.

Suspensions of FMSC and EF (2×10^6 cells) were applied on the surface of burn wound with a pipette on day 2 after burn modeling and removal of necrotic crusts. Thirty minutes after transplantation (the time requiring for cell adhesion to the burn surface) the wound was dressed with gauze wetted in normal saline with gentamicin. The efficiency of cell therapy was evaluated visually by planimetric and morphological methods.

Bone marrow cells for isolation of MSC were collected under ether narcosis from the femoral bones of rats using a 16G needle. The cavity was washed with buffered saline (0.5 ml).

Embryonic fibroblasts were derived from the lungs of 14-17-day embryos taken from narcotized pregnant rats.

Embryonic fibroblasts and bone marrow cells for isolation of MSC were precultured in Petri dishes at 37°C in a CO_2 incubator at 5% CO_2 and 95% humidity. EF were cultured for 4-6 days. MSC monolayer was grown for 14-17 days. MSC culture was kryopreserved and used as the initial material for preparing FMSC (the procedure included defrosting and culturing for 4 days). The number of FMSC by this term at least 3-fold surpassed the number of EF cultured during the same period.

Visual, planimetric, and histological examinations were carried out on days 1, 3, 7, 15, and 30 after cell transplantation onto burn surface.

For morphological analysis of wound healing process, biopsy specimens were collected from the zone of burn injury on days mentioned above; cryostat sections were prepared and stained with hematoxylin and eosin.

In order to detect transplanted cells in burn wounds they were genetically labeled during culturing using a viral vector based on a V type recombinant adenovirus carrying *Lac-Z* gene encoding *E. coli* β-galactosidase. At different terms after transplantation viable cells were detected immunohistochemically by adding X-Gal substrate (by the formation of blue-green product in the reaction with β-galactosidase) [5,10].

The results of wound planimetry were statistically processed using Student's *t* test.

RESULTS

Differences in burn wound regeneration in different groups were seen as early as on day 3 after cell transplantation. On day 7 these differences were most pronounced. In animals of groups 1 and 2 the wounds looked evenly bright pink, granulation tissue grew over the entire area of the wound and attained the epidermis, burn surface markedly decreased. Collagen

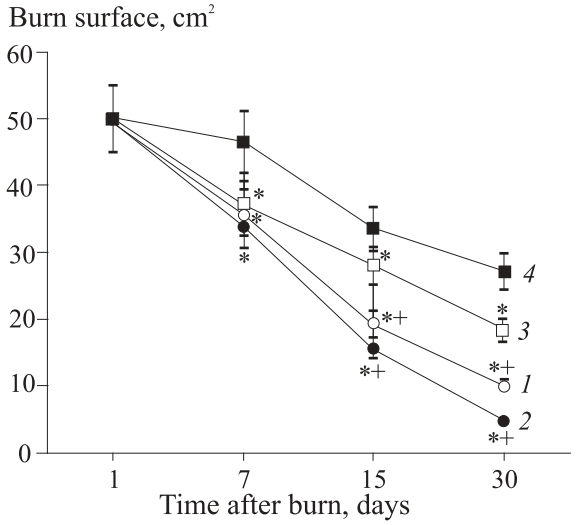

Fig. 1. Time course of burn wound area shrinking after transplantation of different types of cells. *1*) group 1 (allogenic FMSC); *2*) group 2 (autogenic FMSC); *3*) group 3 (embryonic fibroblasts — EF); *4*) control. $p<0.05$ *compared to the control; +compared to EF.

film formed on the wound surface was slightly thinned, but still covered the entire burned area.

In group 3 granulation tissue somewhere grew up to the level of wound edge epidermis, plasorrhea from the wound was more intensive than in groups 1 and 2, while the initially formed collagen film virtually disappeared.

By days 15 and 30 these differences in wound healing in the groups became even more pronounced (Fig. 1). The area of burns notably decreased in groups 1, 2, and 3 ($p<0.05$) in comparison with the control group, which confirmed the important role of transplanted cells in regulation of burn wound regeneration. Comparison of the rates of wound healing in experimental groups receiving cells from different sources showed that regeneration was more rapid in groups 1 and 2 compared to group 3 ($p<0.05$). Although in group 2 the burns healed more rapidly than in group 1, these differences were insignificant.

Histologically accelerated healing of burn wounds under the effect of transplanted cells manifested in more rapid change of phases of the regeneration process: the phase of cell infiltration was shorter, vascularization and formation of the granulation tissue were more rapid. In groups 1 and 2 the newly formed vessels formed a more ramified network, but it was more dense in group 2 animals receiving autogenic FMSC (Fig. 2). In group 3 the number of new vessels was also higher than in the control, but this group was inferior to groups 1 and 2 by the density of vascular network.

Histochemical analysis showed that transplanted cells remained viable on the surface and in the depth of regenerating wounds (which was seen by staining for β-galactosidase) after transplantation of allogenic and autogenic FMSC and EF throughout the obser-

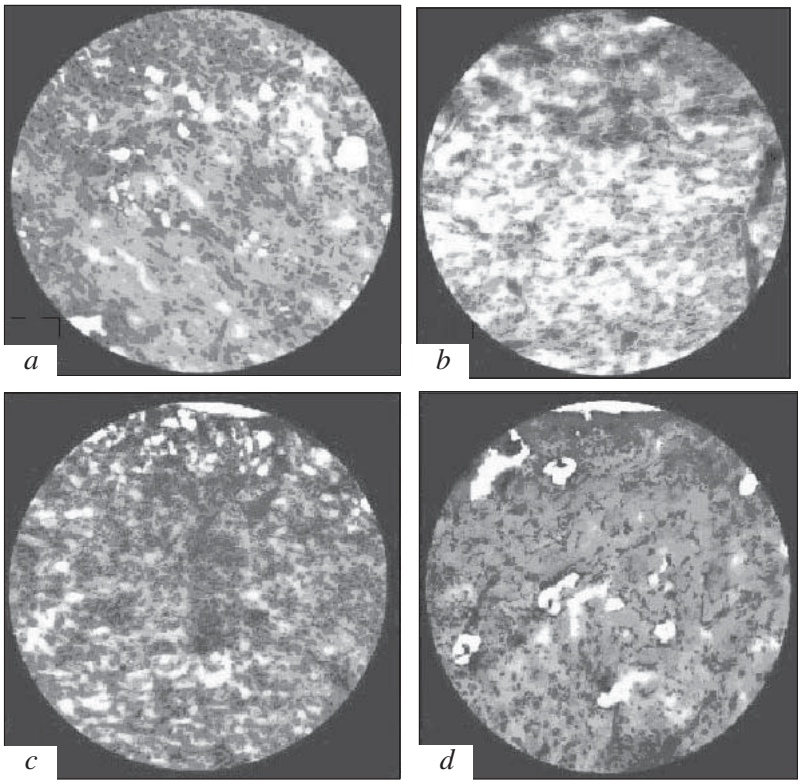

Fig. 2. Cryostat section of burn wound biopsy specimen on day 15 after cell transplantation. Hematoxylin and eosin staining, ×200. *a*) after transplantation of allogenic FMSC precultured in differentiation medium; *b*) after transplantation of autogenic FMSC pre-cultured in differentiation medium; *c*) after transplantation of EF pre-cultured in differentiation medium; *d*) control (no cell transplantation).

vation period (30 days). This means that these calls produce substances accelerating wound regeneration (growth-stimulating factors, tissue-specific peptides, *etc.*) [1,3] (Fig. 3).

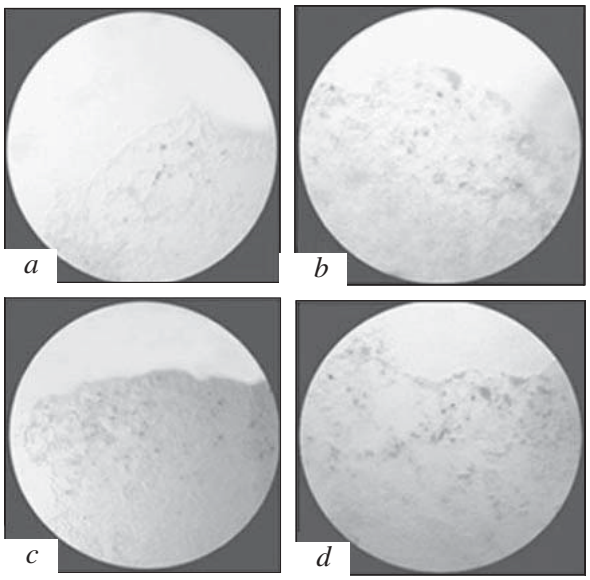

Fig. 3. Cryostat section of burn wound biopsy specimen with detected viable transplanted cells transfected with recombinant adenovirus carrying *Lac-Z* gene. Immunohistochemical detection of β-galactosidase activity by adding X-Gal substrate. *a*) day 15 after EF transplantation, ×200; *b*) day 15 after trans-plantation of autogenic FMSC, ×200; *c*) day 30 after EF transplantation, ×200; *d*) day 30 after transplantation of autogenic FMSC, ×200.

Since in contrast to EF, FMSC are undifferentiated cells, rapid regeneration of burn wounds after transplantation of allogenic and autogenic FMSC can be explained by the release of more active growth-stimulating factors during maturation.

Hence, allogenic and autogenic FMSC, similarly to EF, stimulate regeneration of deep burn wounds due to long maintenance of their viability after transplantation and release of bioactive substances into the wound. Acceleration of wound healing process under the effect of transplanted cells is associated with considerable acceleration of vascularization and granulation tissue formation on the burn surface. When choosing cells for transplantation, FMSC should be preferred, because burn wounds regenerate more rapidly after transplantation of these cells and preparing cell material for transplantation (*i. e.* culturing of MSC) takes less time due to more rapid accumulation of these cells in comparison with EF. The absence of significant differences in the regeneration activity of autogenic and allogenic FMSC indicates the possibility of using these latter cells for stimulation of burn wound healing.

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