Cell and Organ Transplantology. 2017; 5(1):14-20.
doi:10.22494/cot.v5i1.65

The effect of human adipose-derived multipotent mesenchymal stromal cells in the fibrin gel on the healing of full-thickness skin excision wounds in mice

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ABSTRACT

Prospects for the widespread use of multipotent mesenchymal stromal cells (MSCs) in regenerative medicine determine the relevance of studying their abilities to affect the reparative process in experimental systems in vivo.

MATERIALS AND METHODS. *The effect of human adipose-derived MSCs on the healing rate and completeness of damaged skin site reconstitution was examined using full-thickness excision wound model in mice. The reparative activity of MSCs was revealed in planimetric and histological studies. Human blood plasma-derived fibrin gel was used as a scaffold for MSCs delivery.*

RESULTS AND CONCLUSIONS. *Compared to the spontaneous healing process, application of fibrin gel on the excisional skin wounds promotes earlier maturation of granulation tissue and further formation of loose scar tissue with skin derivates. MSCs in the fibrin gel contribute to the improve of wound epithelialization, the decrease of the inflammatory response, faster maturation of the granulation tissue, including marks of angiogenesis, as well as promotes complete recovery of the dermal and epidermal layers of the damaged site of skin.*

KEYWORDS: *adipose-derived multipotent mesenchymal stromal cells; fibrin gel; excisional skin wounds; wound healing*

Healing of skin wounds is a complexly organized multifactorial process, which aims to restore the structure and integrity of the injured tissue site [1]. Normally, the skin defect close up due to the active interaction of cells, extracellular matrix and growth factors, while regional multipotent mesenchymal stromal cells (MSCs) play the most important coordinating role [2]. Systemic or local administration of isolated MSCs, obtained with the use of modern *in vitro* methods, contributes to the improvement of repair processes in chronic non-healing wounds of various etiology: for burns, trophic ulcers, bedsores, epidermolysis bullosa [3, 4].

For local application of MSCs in the treatment and/or correction of skin defects, the method of intradermal injection of cellular suspensions into the wound bed or into surrounding area is most often used [5, 6]. However, with this variant of injection, MSCs cannot fully realize their potential for restoring damaged tissues, since the possibilities of their engraftment and functioning in the area of the wound defect in conditions of inflammation and severe hypoxia are minimal, and the positive effect is limited mainly to the short-term paracrine effect [7]. Increase of MSCs reparative potential realization is allowed by the use of biopolymer carriers, which, in addition to targeted delivery of cells, are able to create conditions for their attachment, proliferation, differentiation and functioning [8]. The majority of cellular scaffolds are created based on natural biopolymers, such as collagen, hyaluronic acid, chondroitin-6-sulfate, chitosan and fibrin [3, 7, 8].

The use of fibrin matrices in dermal wounds simulates normal physiological processes occurring in the early stages of healing [9]. Bensaid et al. determined that fibrin gel (FG) possesses high biocompatibility, provides adhesion, proliferation and migration of MSCs, and stimulates secretion of proangiogenic factors *in vitro* [10]. The application of MSC in platelet-rich fibrin (PRF) gel, on burns and chronic wounds promoted the restoration of the normal structure of the skin and accelerated the process of defect replenishment [1, 11, 12]. The researchers concluded that such a beneficial wound healing effect is largely due to the influence of biologically active molecules that are a part of PRF. However, it is known that the content of growth factors in PRF varies significantly depending on

the method of preparation, age and individual characteristics of the donor [13], in addition, the combined use of MSCs with PRF does not allow to detect the contribution of the cellular component to the final biological effect. In contrast, the removal of platelets from the blood plasma before the preparation of PRF reduces, on the one hand, the effect of individual differences between donors, and on the other hand reduces the content of growth factors, which contributes to a more correct assessment of the effect of MSCs on the healing process of skin wounds.

The purpose of this study was to evaluate the wound healing effect of human MSCs in fibrin gel from blood plasma depleted of platelets on the model of excision full-layer skin wounds in mice.

MATERIALS AND METHODS

Isolation, cultivation and identification of MSCs. The source of MSCs was fragments of subcutaneous adipose tissue of the abdominal wall (5-10 g), obtained during planned surgery from adult male volunteers aged 23-54 with the written consent of donors in compliance with bioethical standards. MSCs were isolated according to the method described previously [14]. To do this, fragments of adipose tissue (n = 5) were washed with Hanks solution, after which a solution of 0.1 % collagenase type II (*Sigma*, USA) was added in a ratio of 1:3, and incubated for 1.5 hours at 37 °C with intensive shaking. The cell suspension was centrifuged at 1500-2000 rpm for 20 minutes. The supernatant was discarded and the pellet was resuspended in 10 ml of Hanks' solution and filtered through a blood transfusion system. The purified suspension was centrifuged at 1000 rpm for 10 minutes. The resulting cell pellet was a stromal-vascular fraction of adipose tissue containing MSCs.

Cell cultivation was performed in an α-MEM medium (*Sigma*, USA) supplemented with 10 % fetal bovine serum (PAA, Austria), 2 mM L-glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin at 37 °C, 5 % CO_2 and 95 % humidity.

The cells of the 4th to 6th passages were used in the work. Immunophenotypic analysis was performed using monoclonal antibodies CD29 (*Serotec*, USA), CD34 (*Dako*, Denmark), CD45 (*Serotec*, USA), CD73 (*BD Biosciences*, USA), CD90 (*Serotec*, USA) and CD105 (*Serotec*, USA) on the flow cytometer FACS Calibur (*BD Biosciences*, USA).

To confirm the multipotent differentiation potential, standard induction media were used to induce adipo-, osteo- and chondrogenesis [15], the cultivation period was 21 days. The directed adipogenic differentiation was evaluated by the accumulation of intracellular neutral lipids positively stained with Oil Red O (*Sigma-Aldrich*, USA); the effectiveness of osteogenic differentiation – by expression of alkaline phosphatase using the Fast Blue RR Salt kit (*Sigma-Aldrich*, USA), according to the manufacturer's instructions. Chondrogenic differentiation was confirmed by the detection of proteoglycans and glycosaminoglycans by staining with Alcian blue and Safranin O according to the standard protocol [15].

Preparation of fibrin gel from platelet-poor plasma. FG was obtained from whole blood of adult donors (30-45 years old, n = 5) tested for infectious diseases, provided by the Kharkiv regional blood service center, in accordance with the described method [16]. To separate of leukocytes and red blood cells, whole blood was centrifuged at 1800 rpm for 15 minutes. The resulting suspension was repeatedly centrifuged at 4,400 rpm for 10 minutes, which led to plasma separation into two fractions – platelet-poor and platelet-rich plasma. A platelet-poor plasma fraction ($5 \cdot 10^4$ platelets/μl) was combined in a 9:1 with a mixture of blood serum and 10 % calcium chloride solution (in a 3:1 ratio). The blood serum, which served as a source of thrombin, was obtained from the coagulated blood of the same donors using a single centrifugation procedure (3000 rpm, 10 min). To obtain MSCs in FG, the blood platelet-poor plasma fraction was supplemented with a cell suspension in concentration $5 \cdot 10^6$ cells per 1 ml of the final mixture.

Experimental groups. The studies were performed on 5-6-month-old male Balb/C mice (n = 27) weighing 25-30 g with full-layer excision skin lesions. The experiments were approved by the Committee on Bioethics of the Institute for Problems of Cryobiology and Cryomedicine of National Academy of Science of Ukraine, regulated according to the «4th European Convection for the Protection of Vertebrate Animals» (ETS 123, Strasbourg, 1986) and corresponded to the «General Principles of Experiments on Animals» adopted by the 5th National Congress on Bioethics (Kyiv, 2013).

Animals were anesthetized with 2 % «Sedazine» (*Biowet*, Poland) and 1 % «Propofol» (*Cleric Lifesence Limited*, India) at 20 mg/kg body weight. Full-thickness skin wounds were performed under aseptic and antiseptic condition using a Dermo Punch (*Stiefel*, Germany) 6 mm in diameter. Two wounds were applied simultaneously to the fascia and hemostasis was performed [17]. To prevent contraction, the edges of the wounds were fixed with a polymer medical adhesive plaster (*Dr. House*, China) and glue BF-6 (*Lubnyfarm*, Ukraine).

All wounds (n = 54) were divided into 3 groups:

- group 1 – control, self-healing wounds;
- group 2 – wounds with FG;
- group 3 – wounds with MSCs in FG.

At the same time, 27 animals (54 wounds) participated in the experiment, which were distributed according to the series as follows:

- 9 animals with wounds of group 1 versus group 2;
- 9 animals with wounds of group 2 versus group 3;
- 9 animals with wounds of group 1 versus group 3.

As a result, 18 control wounds, 18 wounds with FG and 18 wounds with MSCs in FG were studied in each experiment.

The wounds of group No. 1 after injury were covered with a transparent semipermeable film (*Tegaderm Film*, Germany); an elastic bandage (*Coban*, Germany) was used as the outer bandage. In the wounds of group No. 2, 50-60 μl of FG prepared ex tempore were added. In the wounds of group No. 3 – the same amount of gel containing $0.25\text{-}0.3 \cdot 10^6$ MSCs. After gel polymerizing, a semipermeable film and an elastic bandage were applied to the wounds of groups 2 and 3 as described above.

The evaluation of wound healing in mice was carried out on the basis of the results of macroscopic, planimetric and histological observations in accordance with the view of the stages of wound healing. The wound was analyzed on the 3rd (the peak of the inflammation phase), the 7th (the proliferative phase peak – the formation of granulation tissue and marginal epithelium), the 14th (maturation of the granulation tissue and growth of the epithelial layer) and the 28th (remodeling of the dermal part and final epithelialization of the wound defect) day after the experimental impact. The animals were euthanized by the cervical dislocation under injection of 2 % «Sedazine» (*Biowet*, Poland) at a dose of 20 mg/kg of body weight.

Measurement of wound area during planimetric studies was carried out on photographs obtained using the IXUS 80 IS camera (*Canon*, Japan). The evaluation was carried out using the program ImageJ v. 1.5b (*National Institutes of Health*, USA), according to the instructions of the developers.

The percent wound surface area reduction was estimated by the formula:

$$\frac{S_0 - S_t}{S_0} \times 100\%$$

where So is the initial area of the wound; St is the area of the wound a day t.

For histological examination, skin flaps around the wound were excised, fixed in a buffered 10 % formalin and mounted in TissueTec mounting medium (*O.C.T. Compound*, UK). Serial 5-6-μm-thick sections were obtained with cryotome Slee Cryostat MEV (*Slee Medical GmbH*, Germany). After standard staining with hematoxylin and eosin and dehydration, the sections were mounted in Canada balsam.

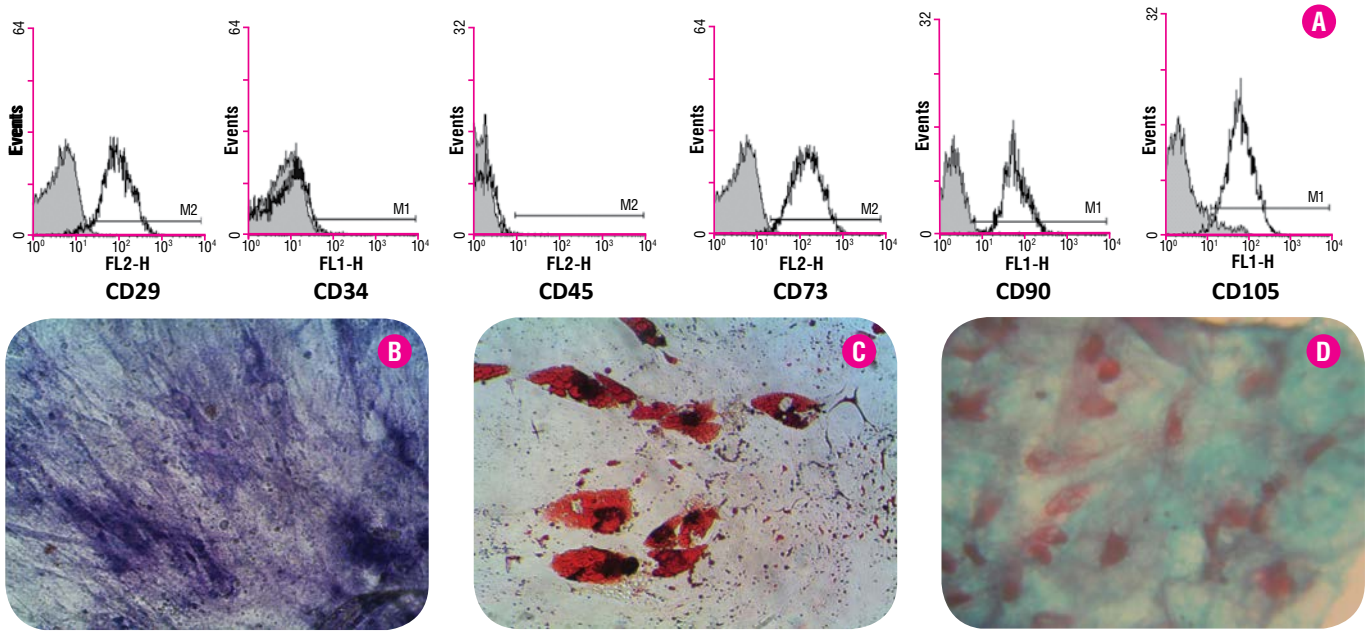

Fig. 1. Characteristics of adipose-derived MSCs of the 4th passage: A – Histograms of the expression of surface markers CD29, CD34, CD45, CD73, CD90, CD105 (flow cytometry, gray contour – isotype control, black contour – incubation of cells with the antibody); B – osteogenic differentiation, positive staining for alkaline phosphatase (blue color); C – adipogenic differentiation, positive staining for neutral lipids by the Oil Red O dye (red color); D – chondrogenic differentiation, positive staining for acid mucopolysaccharides with Alcian blue (blue color) and Safranin O (purple color). Oc. ×10, ob. ×20.

The obtained histological specimens were studied using a light microscope with a DCM300 digital camera with a photo output XSP-139TP (*JNOEC*, Japan-China).

Statistical analysis of the data was carried out using the Past 3.0 software [18]. The quantitative data distribution was evaluated using the Shapiro-Wilk test. The data were represented as the mean ± standard deviation. For comparison of two independent samples, a nonparametric Mann-Whitney test was used, for paired comparisons – the Wilcoxon signed rank test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The impact of MSCs on the reparative processes in tissues depends on the source of cells, the age of the donor, the cell dose, the method of MSCs delivery into the body, the properties and composition of the vehicle, the type of the wound, the characteristics of the recipient, the neurohumoral effect of the organism and other factors. Comparing MSCs obtained from different sources, the wound healing effect of adipose-derived stromal cells was more pronounced, compared to MSCs from the amniotic membrane and bone marrow [19]. At the same time, the acceleration of migration of dermal fibroblasts and re-epithelialization was observed, as well as the formation of a more pronounced layer of granulation tissue. The minimal invasiveness of the obtaining procedure, relative availability and high content of mesenchymal progenitor cells can also be included to the undoubted advantages of the use of adipose tissue as a stem cell source. [15].

In our work, we used human MSCs, isolated by the enzymatic method from adipose tissue of adult donors. The freshly isolated suspension of the stromal-vascular fraction from adipose tissue was heterogeneous. With subsequent cultivation, the adhesive cells gradually unified, and by the 3rd and 4th passage most of them had a fibroblast-like shape. They expressed immunophenotype $CD29^+$, $CD73^+$, $CD90^+$, $CD105^+$, $CD34^-$, $CD45^-$ (**Fig. 1A**) and were capable of induced differentiation in adipogenic, osteogenic and chondrogenic directions. After induction of osteogenic differentiation, most cells expressed alkaline phosphatase (**Fig. 1B**). When cultured in the adipogenic medium, the cells acquired a rounded shape and accumulated intracellular lipids that were positively stained with Oil Red O (**Fig. 1B**). Directed differentiation of MSCs in the chondrogenic direction was performed after the formation of dense cellular aggregates. Histochemical evaluation of aggregates revealed a significant accumulation of extracellular matrix, represented mainly by acid mucopolysaccharides, which was confirmed by positive staining with Alcian blue. Cell nuclei, stained with safranin, were located diffusely in the extracellular matrix (**Fig. 1D**).

Thus, the cells used in this study in terms of their properties meet the minimal criteria for defining multipotent mesenchymal stromal cells [20].

For realization of reparative potential of MSCs *in vivo*, the method of cells delivery, as well characteristics and properties of a possible scaffold are significant. In our work, fibrin gel from poor-platelet plasma was used to target cells to the damage zone. The chemical properties of the fibrin hydrogel ideally correspond to the characteristics of the natural matrix, and its ability to polymerize within a few minutes after mixing the main components ensures uniform filling of skin defects [21].

Two key ingredients are needed to prepare FG – thrombin and fibrinogen. The source of fibrinogen is often the donor's blood plasma, for the activation of which the commercial preparations of bovine thrombin are used [12, 22, 23]. The xenogeneic origin of bovine thrombin causes certain limitation for application of the gel in medicine. The approach used in our study made it possible to obtain the main components for FG via a simple step-by-step procedure of the centrifugation of the whole blood from one donor, wherein the initiation of plasma gelation was performed with a mixture of calcium chloride and serum.

Investigation of wound healing processes in experiments with laboratory animals for the purpose of subsequent translation of the results to the human requires a correct approach to the model choice, taking into account physiological differences. To date, 65 % of the studies on the healing of skin wounds using MSCs are performed on an excision wound model and the most common animals for this type of research are laboratory rats and mice [24]. However, the healing of a wound defect occurs primarily due to contraction, rather than re-epithelialization and

the formation of granulation tissue, as is typical for humans [24]. The approach to the modelling of a full-thickness excision wound model in mice with the fixation of a silicone ring to the edges of the wound prevents contraction and allows the maximum possible approximation of experiments in mice to the features of skin repair in humans [17]. In our work, the edges of the wound were fixed with the help of medical glue and a polymer patch, which made it possible to reduce traumatism, painfulness and time of the surgery. The modified model of excision wounds in mice thus proved to be easily reproducible and informative in studying the features of the wound healing effect of MSCs.

The general condition of mice with control and experimental excision wounds for all periods of observation was satisfactory; the animals had a good appetite and high motor activity.

Skin wounds of all study groups on the 3rd day of observation looked similar (**Fig. 2**). They had clearly contoured, slightly uneven edges, the bottom of the wounds was pinkish, more intense in group 3, where MSCs were applied into FG. Sometimes there was a small amount of exudate.

On the 7th day of healing along the uneven inner edge of the wounds of all groups, a bright rim of marginal epithelialization of various widths was observed. A thin pinkish-brown dry scab was found on the bottom of self-healing wounds, the bottom of wounds of the 2nd and 3rd groups had a translucent coating without damages and ruptures.

By the 14th day, the wounds of all study groups were almost completely healed. They had a smooth surface of a whitish-pink color, in some cases there were layered remains of a scab on it, with epithelium under it. The growth of fur in place of epithelial defects in all wound groups was absent.

On the 28th day of observation, a restored fur was observed in all the study groups at the wound site.

To quantify the effect of PG and MSCs on the epithelialization of the surface of excision wounds in mice, a planimetric study was performed. **Fig. 3** shows that the closing rates of the wounds of the control group and the group with the FG application did not differ significantly during the entire observation period. Thus, on the 3rd day of the experiment, the percentage of closure of the wound surface in these groups was 16.9 ± 6.5 % and 14.3 ± 6.3 %, and on the 7th day – 38.5 ± 5.5 % and 36.3 ± 7.1 %, respectively.

At the same time, after insertion of MSCs in FG into the cavity of the wound, the area of the open wound surface by the 3rd day decreased by 29.5 ± 6.9 %, which significantly differed from the values for the other groups. The difference was also observed on the 7th day, the percentage of closure of the wound surface for the group 3 at that time was 52.2 ± 5.1 % (**Fig. 3**).

On the 14th day of the experiment, almost complete epithelialization of the wound surface was observed in animals of all study groups.

Thus, planimetric studies allowed to reveal the stimulating effect of MSCs in the composition of FG on the rate of closure of uncomplicated excision wounds at least in the first 7 days after the introduction of cells. The obtained results are in line with the data of [21, 22], in which the effectiveness of combined use of MSCs and FG was demonstrated in the treatment of burns and chronic non-healing skin wounds. At the same time, according to the data published by Blanton [12] and Radwa A. Mehanna [25], the use of MSCs did not affect the rate of epithelialization of uncomplicated wound defects in healthy animals, but contributed to the full tissue restoration.

It is known that healing of the cutaneous wound, in addition to restoring the epithelial component, involves repair of the connective tissue dermal part, which ensures the completeness of repair of the skin defect as a whole. We studied features of the epidermis and underlying tissues regeneration on histological sections (**Fig. 4**).

Self-healing of excision wounds (group 1) in mice proceeded in accordance with existing concepts of the phases of uncomplicated wound process [24]. On the 3rd day after excision, filling of the wound defect with fibrin was observed, necrotic changes were noted in the wound bottom. Intensive diffuse inflammatory infiltration with polymorphonuclear

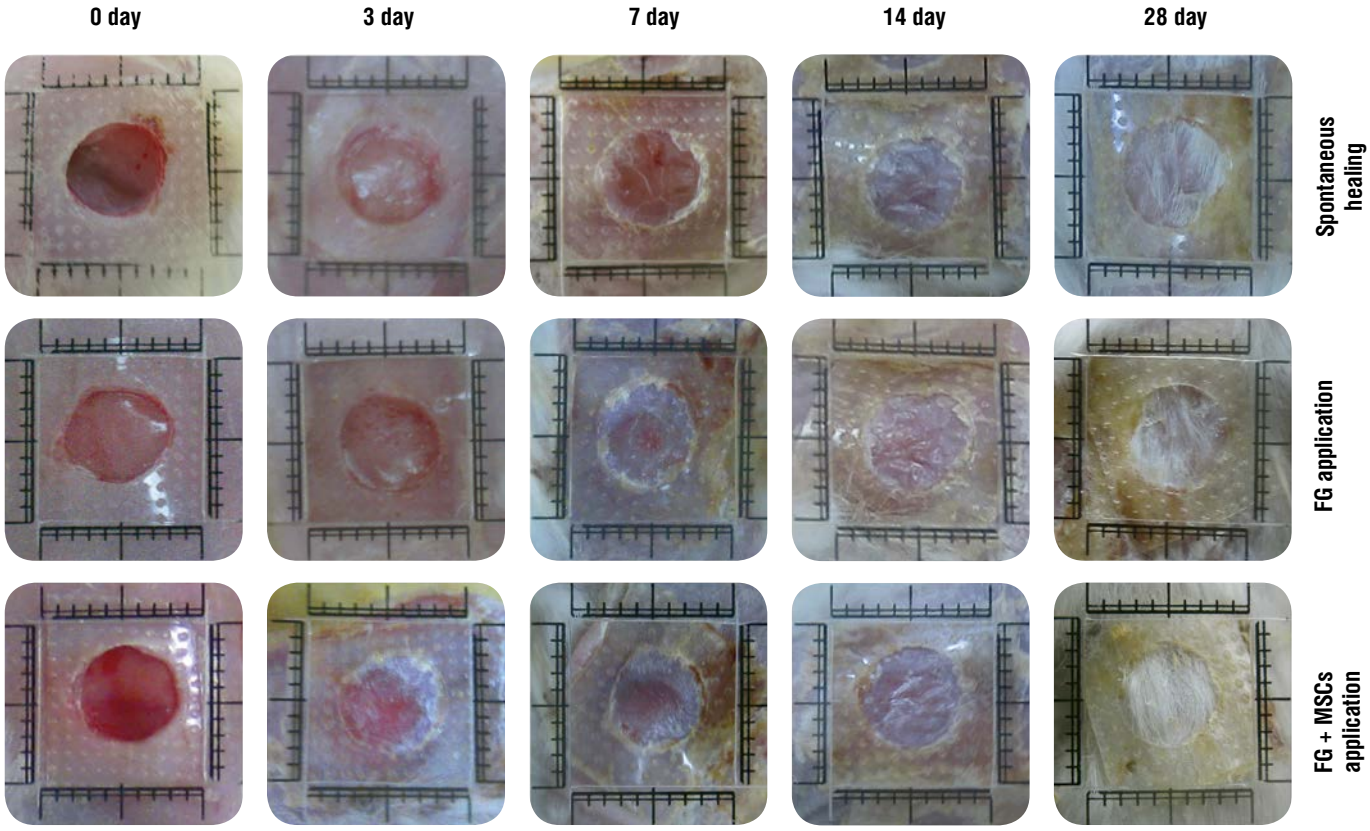

Fig. 2. Excision wounds in mice. To prevent contraction, the edge of the wound was fixed using a polymer patch and medical glue (FG – fibrin gel, MSCs – multipotent mesenchymal stromal cells).

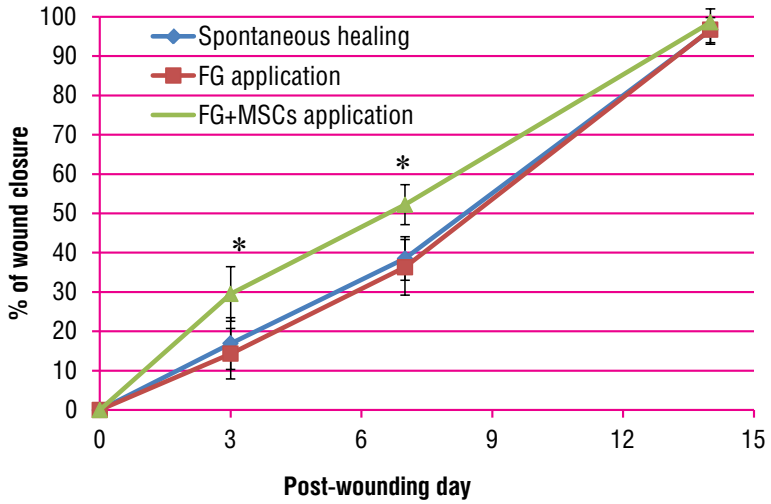

Fig. 3. Closure of the wound surface of excisional skin wounds in mice with independent healing and with the introduction of a fibrin gel (FG) or mesenchymal stromal cells (MSCs) in FG into the wound.

Note: * – significant differences in comparison with the corresponding observation period in self-healing groups and after application of FG.

leukocytes was revealed in the tissues and surrounding tissues, the vessels were expanded and full-blooded, which is typical for the normal course of the wound process in the phase of inflammation.

Histological observations on the 7th day indicated an early period of development of granulation tissue, based on newly formed vessels of the capillary type. Among the leukocytes of the wound zone, there were young connective tissue cells of various shapes and sizes, located mainly near the vessels. The formation of a marginal epithelial layer, growing up under a scab, was also observed.

On the 14th day of wounds self-healing in maturing granulation tissue, there was an increase in the number of connective tissue cell elements, which displaced fibrinoid and infiltrating leukocytes. The predominant cellular elements of the fibroblastic series formed bundles, had an elongated shape, a process cytoplasm, and synthesized collagen. At this stage, complete epithelialization of the wound surface was noted, areas with partial desquamation of the incomplete layer epithelial layer were a sign of immaturity of the granulation tissue at this observation period.

On the 28th day of wounds healing of the group 1, the newly formed connective tissue that filled the wound defect was a dense scar tissue with coarse, chaotically arranged bundles of collagen fibers. Skin derivatives – hair follicles and sebaceous glands – were formed in the dermis. The formed full-layer epidermis was thickened, had a smoothed microrelief, which can be considered a sign of incompleteness of the remodeling phase.

The histological pattern observed on the 3rd day of healing of skin lesions on which FG was applied (group 2) had characteristics of the inflammation phase, as in group 1, but with a slightly more pronounced diffuse infiltration of necrotic wound sites with polymorphonuclear leukocytes. On the 7th day of the experiment maturation of granulation tissue was revealed. Budding of capillaries and fibroblast content among young connective tissue cells increased in comparison with the control group, while the total number of leukocytes decreased.

On the 14th day of healing of cutaneous wounds with FG in the wound defect zone, mature granulation tissue was detected, which turned into a young connective tissue with a large number of fibroblasts in the stage of active collagen formation. In the vast majority of observations, the wound surface was completely epithelialized, but the epidermis was incomplete, and its microrelief was not detected.

By the end of the experiment, the wound defects of the skin of group 2 were filled with connective tissue, which was transformed into a scar tissue less dense than in case of spontaneous healing. A picture of the orderly oriented tender collagen fibers of the dermis and the proliferation of epithelial cells that form the skin derivatives confirmed this. In all the observations, complete epithelialization of the wound defect was detected, with the thickness and microrelief of the epidermis corresponding to normal skin. In addition, the regenerating fibers of the skin's own muscle attracted attention.

Thus, the histological study of wound healing with FG revealed positive differences in comparison with the control group of self-healing. Despite the absence of differences in the rate of closure of the wound defect and a slightly increased inflammatory reaction during the first three days after trauma with the introduction of FG, the structural and functional features of the newly formed dermal part ensured the formation, by the end of the observation period, of practically complete epidermis and skin derivatives. Similar results were obtained by Rodriguez [6] using a carrier based on hyaluronic acid: the use of a hydrogel favored the formation of fibrils mainly from collagen type 1, characteristic of normal skin, and spontaneous healing was associated with increased synthesis of type 3 collagen and the formation of scar tissue.

In the histological study of the healing of cutaneous wounds of group 3 with the use of MSCs in FG on the 3rd day, a moderate inflammatory reaction to the lesion was observed in the wound defect zone, polymorphonuclear leukocytes, macrophages and thin-walled capillary vessels were detected, which can essentially be regarded as the onset of granulation tissue formation.

By the 7th day in the wound defect zone with MSCs in FG, granulation tissue was more mature than in groups 1 and 2, passing into the connective tissue with a number of capillary vessels filled with erythrocytes. In its deep layers, a large number of fibroblasts, which form bundles and are in the stage of active collagen formation, were found. By this time, a significant part of the wound has already been closed by an epithelial layer, which was not always closely interlaced with the underlying tissue.

On the 14th day, a large number of fibroblasts actively synthesizing collagen were found in the papillary layer of the dermis proper, and the formation of skin derivatives against the background of complete epithelialization of the wound defect was noted. The microrelief of the newly formed multilayer epidermis was smoothed.

On the 28th day, at the site of the wound defect in group 3, the transformation of connective tissue into a loose scar tissue was revealed: the delicate collagen fibers were aligned parallel to the surface of the dermis proper, and a number of fibroblasts were still detected in the papilla layer. The formed full-layer epidermis had a normal thickness and a typical microrelief.

Thus, the histological studies we conducted revealed the acceleration of maturation of the granulation tissue, including earlier angiogenesis, in the treatment of MSCs wounds in FG. The initial signs of formation of granulation tissue were detected already on the 3rd day of the observation. By the 7th day such wounds formed sufficiently mature granulation tissue, transforming into the connective, with a large number of capillary vessels.

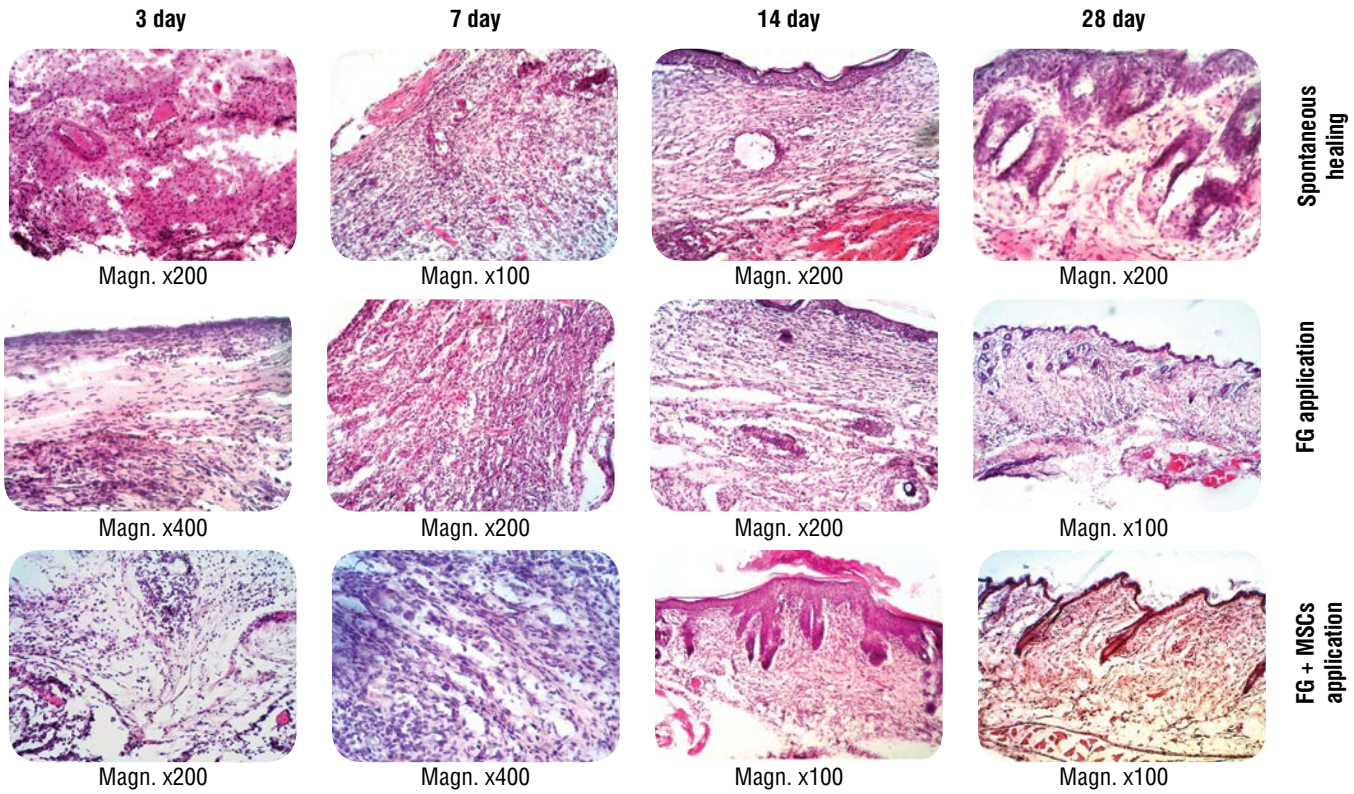

Fig. 4. Histological pictures of healing of excisional skin wounds in mice. Staining with hematoxylin and eosin. FG – fibrin gel, MSCs – mesenchymal stromal cells.

Further reparative processes in the defect zone led to the formation of a full-fledged epidermis with the underlying loose scar connective tissue and newly formed skin derivatives.

At present, it has been shown that the ability of MSCs to stimulate reparative processes is realized both by direct structural replacement of damaged cells and by paracrine mechanisms [5]. Due to the secretion of biologically active trophic factors, MSCs promote the formation of new vessels, activate endogenous progenitor cells and regulate the processes of inflammation and the formation of granulation tissue during wound healing [7]. The wound healing effect of MSCs is manifested in correcting the processes of destruction, enhancing angiogenesis and restoring the architectonics of the skin in the defect zone [25]. The main attention of researchers is paid to the effects that appear at the stage of formation and development of granulation tissue. Our experiments, confirming the positive effect of MSCs on the dermal part of the connective tissue, also revealed the effectiveness of MSCs action on wound closure in the initial wound healing period (the first 7 days). At the same time, MSCs showed the ability to realize their reparative potential with minimal support from exogenous growth factors, with local application at uncomplicated wound (when there are no restrictions to involve their own regional MSCs in the reparative process), which expands the horizons of clinical application of MSCs.

CONCLUSIONS

1. ***Fibrin gel obtained from poor-platelet plasma and applied into excisional skin wounds in mice promotes earlier maturation of the granulation tissue than with independent healing, followed by the formation of a loose scar tissue with skin derivatives.***
2. ***Human adipose-derived MSCs in fibrin gel promote the epithelialization of excisional wounds, earlier than in wounds without MSCs, improve maturation of granulation tissue, including angiogenesis, and complete restoration of the dermal and epidermal layers of the damaged skin.***

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The authors indicate no potential conflicts of interest.

Received: March 05, 2017
Accepted: May 15, 2017