SUCCESSFUL APPLICATION OF CONDITIONED CULTURE MEDIUM FOR THE TREATMENT OF A CHRONIC WOUND OF AN AMPUTATION STUMP: A CLINICAL CASE

© **A.I. Cherepanin[1], O.V. Pavlova[1], V.A. Kalsin[1], M.A. Konoplyannikov[1], O.N. Kucherova[2], V.L. Baldin[1], S.V. Deryabin[1]**

[1] Federal Scientific and Clinical Center for Specialized Medical Assistance and Medical Technologies of the Federal Medical Biological Agency, Moscow, Russian Federation

[2] City Clinical Hospital named after V.V. Vinogradov, Moscow, Russian Federation

Background: *Amputation of the lower extremities is a necessary procedure to save a patient with critical arterial and neurotrophic disorders in the lower extremities. The amputation stump-related complications develop in many patients with diabetes mellitus (up to 40% of the total population).* ***Clinical case description:*** *Patient Yu., 64 years old, was admitted on October 19, 21 for an outpatient treatment of purulent-necrotic wounds of the amputation stump of the right lower limb. A high amputation was performed on September 24, 2021 due to thrombosis of the femoral-tibial bypass, installed on September 08, 2021 (bypassing below the knee joint gap with a Vascutek 7 mm synthetic prosthesis on the right) and the development of critical ischemia of the right lower limb with necrosis of the distal phalanges of the right foot toes. The wound was assessed according to the Bates-Jensen scale (BJ) and examined according to the developed protocol. The wound treatment was carried out according to an individual plan using a conditioned culture medium from mesenchymal stem cells (CM-MSCs), which stimulates angiogenesis and improves remodeling and recovery in the wound area. CM-MSC application made it possible to reduce the healing time and achieve a scarless closure of the tissue defect.* ***Conclusion:*** *The use of CM-MSC can be an effective method for healing a purulent-necrotic postoperative wound resulting from amputation of a limb in patients with critical ischemia of the lower extremities.*

Keywords: *lower limb amputation; critical lower limb ischemia; conditioned cell medium; chronic wound; diabetes mellitus.*

For citation: Cherepanin AI, Pavlova OV, Kalsin VA, Konoplyannikov MA, Kucherova ON, Baldin VL, Deryabin SV. Successful Application of Conditioned Culture Medium for the Treatment of a Chronic Wound of an Amputation Stump: A Clinical Case. *Journal of Clinical Practice*. 2022;13(1):92–98. doi: https://doi.org/10.17816/clinpract105089

Submitted 20.02.2022 Revised 22.03.2022 Published 30.03.2022

BACKGROUND

In the foreseeable future, chronic wounds will remain a significant medical and social problem because of frequent disablement, difficult adaptation to society, and major socio-economic losses for both the patient and the state.

Amputation of the lower extremities is a necessary measure to save a patient with critical arterial and neurotrophic disorders in these extremities. However, many patients with diabetes mellitus (up to 40%) develop complications from the amputation stump, which account for up to 50%–70% of all non-traumatic amputations of the lower extremities. Chronic wounds of the stump are caused by an aggravated comorbid background, sepsis, wrong amputation level, hematoma of the stump, macro- and microangiopathies, high virulence, and nosocomial infections [1–4]. These chronic wounds are almost always accompanied by a purulent and necrotic process, including prolonged intoxication, reinfection of the wound surface with keloid scar formation, and sometimes fistulas and superficial wounds in the amputation stump of the lower limb that do not heal for years.

Among methods of regional treatment of chronic wounds (such as enzymatic debridement, necrectomy, hydrosurgical treatment, ultrasonic cavitation, argon-plasma or air-plasma flow therapy, high-energy laser radiation, and pulse jet), the use of a conditioned cellular medium from mesenchymal stem cells (CM-MSCs) demonstrated safety and versatility of

https://doi.org/10.17816/clinpract105089

journal of clinical practice 2022 Vol 13 №1

effects on chronic wounds with different wound stages because of combined anti-inflammatory and regenerative activities [5].

Currently, cell therapy in the treatment of skin wounds represents an active field of research. Multipotent adult stem cells represent an attractive choice for cell therapy because they have great proliferative potential, ability to differentiate into different cell types, and produce various cytokines and growth factors important for wound healing.

Thus, endothelial progenitor cells (EPC) are involved in the revascularization and repair of damaged tissues. Several studies reported that EPC transplantation accelerated wound healing by enhancing neovascularization in the granulation tissue, secreting various growth factors and cytokines associated with wound healing, thereby promoting monocyte/macrophage involvement, and stimulating endogenous angiogenesis during the wound healing process [6].

MSCs are also a promising cell type for repairing or replacing damaged tissues. They are can differentiate into several cell lines, such as adipocytes, chondrocytes, and osteoblasts. Sasaki et al. [7] revealed that MSCs promote wound healing by differentiating into several skin cell types, namely, keratinocytes, endothelial cells, pericytes, and monocytes. Wu et al. [8] reported that MSCs improved significantly wound healing in both diabetic and non-diabetic mice, demonstrating accelerated wound closure because of the release of pro-angiogenic factors.

Analysis of paracrine factors released from MSCs showed that MSCs secrete vascular endothelial growth factor, insulin-like growth factor 1, epidermal growth factor (EGF), keratinocyte growth factor, angiopoietin-1, and stromal cell-derived factor 1 [9]. All these factors contribute greatly to the recruitment of CD14+ monocytes, keratinocytes, and endothelial cells to the wound. The MSC culture medium, known as a conditioned medium, is thus a rich source of paracrine factors [10]. The introduction of these factors to the damaged site increases their metabolic activity, improves oxygen supply, and remodels the extracellular matrix. Moreover, cell-free preparations based on CM-MSCs have several advantages when compared with standard MSC therapy because they are easy to manufacture, package, and transport, and most importantly, they do not bear any risks and side effects associated with cell administration [10]. Compared with cellular technologies (live skin equivalent, allofibroblasts, suspensions of donor leukocytes in a wound, etc.), CM-MSCs can be used on an outpatient basis.

CLINICAL CASE

Patient information

Female patient Yu, aged 64 years, was referred for aftercare on an outpatient basis on October 19, 2021, with complaints of a non-healing wound in the area of the postoperative stump of the right lower limb.

Anamnesis morbi. She was diagnosed with atherosclerosis of the arteries of the lower extremities, occlusion of the femoropopliteal segment. On September 08, 2021, femorotibial shunting below the knee joint cleft was performed with the Vascutek synthetic prosthesis, 7 mm, on the right. She had shunt thrombosis, critical ischemia of the right lower limb, and necrosis of the distal phalanges of the right foot toes. On September 24, 2021, amputation of the right lower limb was performed at the level of the middle third of the thigh, with resection of the alloprosthesis and autoplasty of the common femoral artery on the right, and secondary sutures in the right inguinal region. Postoperatively, a purulent and necrotic wound of the amputation stump of the right lower limb at the level of the middle third of the thigh was observed. Concomitant diseases were insulin-treated type 2 diabetes mellitus, nephropathy of mixed origin, diabetic macroangiopathy, distal-type polyneuropathy, neuroischemic diabetic foot syndrome, grade 2 arterial hypertension, risk four dyslipidemia, and fatty liver disease.

On an outpatient basis, the patient continued complex therapy in accordance with the recommendations of the hospital.

Physical diagnostics

Upon examination, the general condition was satisfactory. Her body type was normosthenic. No visible abnormalities of skin and visible mucous membranes were noted. Her body temperature was 36.8°C. She had no respiratory system abnormalities. Her blood oxygen saturation ($SatO_2$), heart rate, blood pressure, and pulse rate were 96%, 18 per min, 140/80 mm Hg, and 68 beats per minutes (regular), respectively. On palpation, no abdominal abnormalities were noted; peristalsis was auscultated. She had no urination abnormalities.

Neurological status. She had clear consciousness and was oriented to person, place, and time. No meningeal symptoms were observed. Neurological examination of the left lower limb revealed monofilament 10 g of 1 (0, normal; 1, weakened; 2, absent). Tactile sensitivity was normal. Pain sensitivity was absent to the level of the middle of the foot. Temperature sensitivity was absent to the level of the ankles, and

reflexes at the level of the Achilles tendon and knee were weakened. The right lower limb was amputated at the level of the middle third of the right thigh.

Laboratory and instrumental diagnostics

The parameters of the general blood and urine tests, biochemical blood tests, and coagulogram correspond to the reference values of the corrected comorbid background of the patient. Her blood glucose was 8.2 mmol/L. A microbiological cultural study of the wound discharge (September 05, 2021) revealed *Staphylococcus haemolyticus* 10^3, linezolid-S. In the hospital, a course of treatment with linezolid at a dose of 600 mg, intravenous drip feed, was performed two times a day, for 5 days, and the wound was cleaned with antiseptics. Repeated microscopic cultural examination of wound contents on an outpatient basis (October 19, 2021) revealed *S. epidermidis* less than 1×10^2, and there was a growth of transient microflora, which had no diagnostic value. Ultrasound duplex scanning (USDS) showed atherosclerosis of the vessels of the left lower extremity with a predominant lesion of the lower leg arteries and the amputation stump on the right at the level of the middle third of the thigh. Occlusions of the posterior and anterior tibial arteries were noted. USDS of the veins of the lower extremities revealed preserved patency of the deep and saphenous veins.

Local status

Initially, the wound was examined in daylight with the patient in the supine position. The wound was washed with saline solution, and its visual and planimetric assessment was performed using the Bates–Jensen (BJ) algorithm [11, 12].

General condition of the wound. Location. The amputation stump was at the level of the middle third of the right thigh. The shape was irregular. The wound size was 23×4.5 cm (average BJ score 5 points). Its depth was >6 cm (BJ 3 points). The wound edges were clearly circumscribed, rising above the bottom of the wound (BJ 3 points). Undermining the edges of the wound was with a depth of 2–4 cm, <50% of the wound perimeter (BJ 3 points). The necrotic tissue was represented by adherent soft black scab, mainly along the lateral edge of the wound (BJ 4 points). The necrotic tissue covers 50% of the wound (BJ 3 points). The discharges were purulent, liquid and thick, yellow, and odorless (BJ 5 points). The amount of discharge was moderate (BJ 4 points). The skin color around the wound was normal (BJ 1 point). Edema around the wound was <4 cm, without formation of a pressure pit (BJ 2 points). Induration around the wound was 2–4 cm, <50% of the perimeter (BJ 3 points). Granulation tissue was absent (BJ 5 points). No epithelialization (BJ >5 points) was observed. The total BJ score was 47 (Fig. 1).

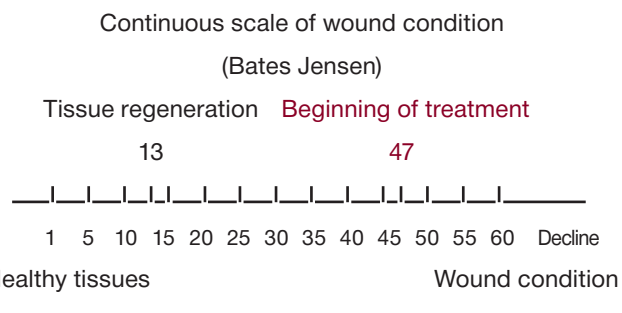

Fig. 1. Patient Yu., 64 years old, diagnosis: "Atherosclerosis of the arteries of the lower extremities, occlusion of the femoral-popliteal segment on the right": the wound condition at the beginning of the treatment (BJ scale).

The wound surface was conditionally divided into three parts: lateral, central, and medial (Fig. 2). The lateral part of the wound (length 8 cm, depth 4 cm) had severe soft tissue necrosis up to 4 cm deep. The central part (length >8 cm, width 6 cm, deepest segment up to 7 cm) had an open area of the beige bone fragment at the depth of the wound, with free areas of resected bright red muscles, actively bleeding when touched with tools. The medial part (length up to 7 cm, width >2 cm) was represented mainly by purulent and inflammatory changes, with a pocket up to 6 cm deep and 3 cm wide in the medial corner of the wound. There were islands of granulation tissue along the wound edges.

Treatment

In the first stage of treatment, enzymatic and autolytic debridement of the wound was performed using hydrogels and enzymes, and the bone fragment was simultaneously coated with collagen, saline solution, and antiseptics. Leakage and pockets were dressed and sanitized daily. The wound surface was treated using the microsurgical method, which was performed once a week for 1.5 months (Fig. 3). Each part of the wound was assessed by the BJ system every 14 days. After 1.5 months, the total scores were 35, 38, and 42 points (BJ) for the medial, central, and lateral parts of the wound, respectively.

In the second stage, a conditioned medium from cultures of MSCs the human placenta (CM-MSC) was included in the complex course of regional wound treatment on the medial part and partially on the central part of the wound.

This study was performed within the clinical study "Open prospective study of the safety and efficacy

https://doi.org/10.17816/clinpract105089

journal of clinical practice 2022 Vol 13 №1

of the use of a conditioned medium from cultures of mesenchymal stem cells for the treatment of chronic wounds of various origins" (code 11.3.21), which was approved by the local ethics committee of the Federal Scientific and Clinical Center of Federal Medical and Biological Agency of Russia (Minutes No. 11 dated October 26, 2021). Cellular material (MSCs) was obtained from the human placenta according to the standard procedure [12]. The growth medium was αMEM (Sigma, USA) containing a mineral saline solution and amino acids with the addition of antibiotics, namely, penicillin (up to 100 U/mL), amphotericin (up to 100 ng/mL), streptomycin (up to 100 μg/mL), L-glutamine (2–4 mM), and 4% human platelet lysate.

Methodology for the use of CM-MSCs. CM-MSCs were used daily in a volume of up to 30 mL, with daily wound treatment. The central part of the wound was treated in combination with CM-MSCs and hydrogel. The application of enzymatic and hydrogel dressings to the lateral part of the wound was continued. Each part of the wound was evaluated according to the BJ scale. At 4 weeks after the initiation of CM-MSC therapy in the medial part of the wound, active granulations over the entire wound surface and pronounced marginal epithelization were observed. In the central part, granulations covered completely the femur, with active growth of granulations on the free ends of the thigh muscles, and necrotic tissues were absent. The lateral part was clean, with scanty serous discharge and sluggish granulations. Changes in the wound surface were shown as 24 points (BJ) after 2 months of treatment, CM-MSC was used on the entire wound surface; 13 points (BJ) after 3 months, transition to the active regeneration phase; and 10 points (BJ) after 3.5 months, complete wound closure without keloid formation (Fig. 4).

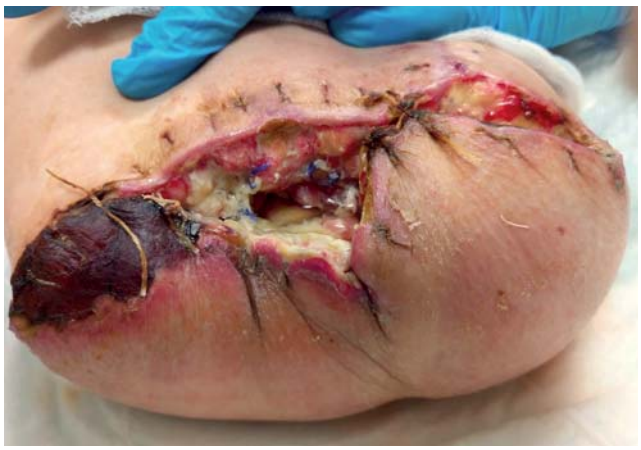

Fig. 2. The same patient: a wound in the area of the right lower limb stump (amputation at the level of the middle third of the thigh), the initial wound appearance.

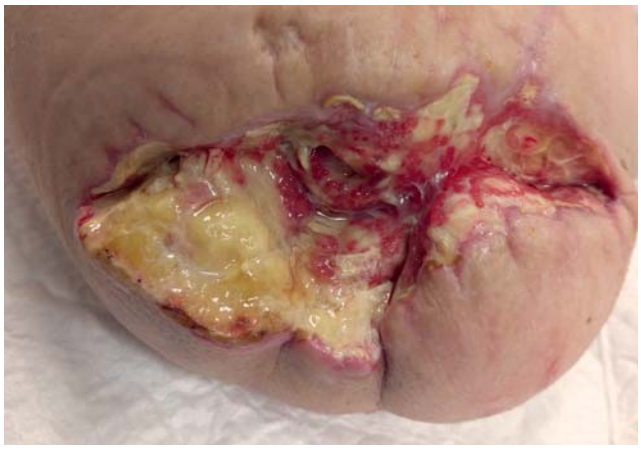

Fig. 3. The same patient: a view of the wound in 1.5 months.

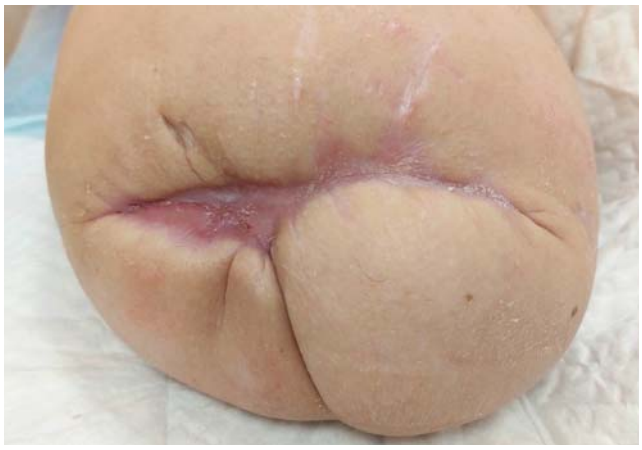

Fig. 4. The same patient: a complete wound closure in 3.5 months, the final result.

DISCUSSION

MSCs are multipotent adult stem cells with the potential to proliferate, self-sustain, and differentiate into three major pathways, namely, fibroblasts, osteoblasts, and adipocytes. MSCs can be isolated from several sources, such as the bone marrow, adipose tissue, umbilical cord, amnion, placenta, and dental pulp. [13]. The beneficial effects of MSCs were due not only to their multipotency but also to the exosomes they secreted that contain cytokines and growth factors [14]. CM-MSCs provide great opportunities for the treatment of many skin diseases, including chronic wounds, and skin rejuvenation.

Saheli et al. [15] studied the effect of human bone marrow-derived CM-MSCs on skin wound healing in diabetic rats and found that significant improvements occurred because of the activation of fibroblast functions. *In vivo* studies have revealed that diabetic wounds treated with CM-MSCs achieved a significantly higher percentage of wound closure, with increased

expression of EGF and basic fibroblast growth factor (*bFGF*) genes. Similarly, Li et al. [16] revealed that CM-MSCs isolated from the human umbilical cord enhance the healing of skin wounds through paracrine activity. CM-MSC-treated wounds showed accelerated healing, with less scarring than in control groups. These findings suggest that the use of CM-MSCs may be a feasible strategy to promote skin repair and a potential tool to achieve scarless healing.

In addition to experimental studies, CM-MSCs have been successfully used in clinical trials. Thus, in the clinic of the A.F. Tsyba National Medical Research Radiological Center of the Ministry of Health of Russia (Obninsk), a method was developed for the treatment of various radiation injuries using a conditioned medium from cultures of MSCs isolated from human bone marrow. Specifically, CM-MSCs were successfully used in the treatment of 55 patients aged 19–70 years with rectal radiation injuries that developed after combined radiation therapy for cervical and prostate cancer [5].

In recent years, several researchers have demonstrated that in the healing of skin wounds using CM-MSCs, the main therapeutic effect is due to paracrine action, namely, the exosomes contained in CM-MSCs that carry functional molecules (growth factors, cytokines, microRNA, etc.) to target cells, thereby affecting the biological processes of recipient skin cells (migration and proliferation) and the secretion of extracellular matrix components (e.g., collagen) [17–20].

CONCLUSION

In this case, we have demonstrated that CM-MSC therapy is an effective method for healing a purulent and necrotic postoperative wound resulting from limb amputation in patients with critical lower limb ischemia. Further clinical studies are required to elaborate the protocol in detail and create a new medical technology based on it.

ADDITIONAL INFORMATION

Author contribution. *A.I. Cherepanin, O.V. Pavlova, O.N. Kucherova* — collection and processing of the material; *A.I. Cherepanin, O.V. Pavlova, V.A. Kalsin, M.A. Konoplyannikov, O.N. Kucherova, V.L. Baldin, S.V. Deryabin* — study concept and design; manuscript writing, editing. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work

Funding source. This study was supported by Federal Medical and Biological Agency of Russia, the project title “Adoptive immunotherapy”.

Competing interests. The authors declare that they have no competing interests.

REFERENSES

1. Бенсман В.М. Хирургия гнойно-некротических осложнений диабетической стопы: руководство для врачей. Москва: Медпрактика-М, 2010. 495 с. [Bensman VM. Surgery of purulent-necrotic complications of diabetic foot: a guide for doctors. Moscow: Medpraktika-M; 2010. 495 p. (In Russ).]
2. Царев О.А., Прокин Ф.Г., Захаров Н.Н., и др. Ампутация конечности у больных с атеросклеротической гангреной // *Саратовский научно-медицинский журнал*. 2011. Т. 7, № 4. С. 947–953. [Tsarev OA, Prokin FG, Zakharov NN, et al. Limb amputation in patients with atherosclerotic gangrene. *Saratov Scientific Medical Journal*. 2011;7(4):947–953. (In Russ).]
3. Ozdemir S. Untersuchung zur Effektivität von offenen und geschlossenen Minoramputationen bei diabetischer Gangrän [Open or closed minor amputation for diabetic gangrene?]. *Vasa*. 2009;38(Suppl 74):54–61. doi: 10.1024/0301-1526.38.S74.54
4. Coulston JE, Tuff V, Twine CP, et al. Surgical factors in the prevention of infection following major lower limb amputation. *Eur J Vasc Endovasc Surg*. 2012;43(5):556–560. doi: 10.1016/j.ejvs.2012.01.029
5. Патент RU 2292212 C1. Коноплянников А.Г., Колесникова А.И., Саенко А.С., и др. Кондиционная среда, обладающая лечебным эффектом. 2007. [Patent RU 2292212 C1. Konoplyannikov AG, Kolesnikova AI, Saenko AS, et al. An air-conditioned environment with a therapeutic effect. 2007. (In Russ).]
6. Suh W, Kim KL, Kim JM, et al. Transplantation of endothelial progenitor cells accelerates dermal wound healing with increased recruitment of monocytes/macrophages and neovascularization. *Stem Cells*. 2005;23:1571–1578.
7. Sasaki M, Abe R, Fujita Y, et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol*. 2008; 180:2581–2587.
8. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*. 2007;25:2648–2659.
9. Ratajczak MZ, Kucia M, Jadczyk T, et al. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? *Leukemia*. 2012;26:1166–1173. doi: 10.1038/leu.2011.389
10. Pawitan JA. Prospect of stem cell conditioned medium in regenerative medicine. *Biomed Res Int*. 2014;2014:965849. doi: 10.1155/2014/965849
11. Harris C, Bates-Jensen B, Parslow N, et al. Bates-Jensen wound assessment tool: pictorial guide validation project. *J Wound Ostomy Continence Nurs*. 2010;37(3):253–259. doi: 10.1097/WON.0b013e3181d73aab
12. Bates-Jensen BM, McCreath HE, Harputlu D, Patlan A. Reliability of the Bates-Jensen wound assessment tool for pressure injury assessment: the pressure ulcer detection study. *Wound Repair Regen*. 2019;27(4):386–395. doi: 10.1111/wrr.12714
13. Bogatcheva NV, Coleman ME. Conditioned medium of mesenchymal stromal cells: a new class of therapeutics. *Biochemistry (Mosc)*. 2019;84(11):1375–1389. doi: 10.1134/S0006297919110129
14. Pittenger MF, Discher DE, Péault BM, et al. Mesenchymal stem cell perspective: cell biology to clinical progress. *NPJ Regen Med*. 2019;4:22. doi: 10.1038/s41536-019-0083-6

https://doi.org/10.17816/clinpract105089

15. Saheli M, Bayat M, Ganji R, et al. Human mesenchymal stem cells-conditioned medium improves diabetic wound healing mainly through modulating fibroblast behaviors. *Arch Dermatol Res*. 2020;312(5):325–336. doi: 10.1007/s00403-019-02016-6
16. Li M, Luan F, Zhao Y, et al. Mesenchymal stem cell-conditioned medium accelerates wound healing with fewer scars. *Int Wound J*. 2017;14(1):64–73. doi: 10.1111/iwj.12551
17. Zhang Y, Pan Y, Liu Y, et al. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. *Stem Cell Res Ther*. 2021;12(1):434. doi: 10.1186/s13287-021-02517-0
18. Hu J, Chen Y, Huang Y, Su Y. Human umbilical cord mesenchymal stem cell-derived exosomes suppress dermal fibroblasts-myofibroblasts transition via inhibiting the TGF-β1/Smad 2/3 signaling pathway. *Exp Mol Pathol*. 2020;115:104468. doi: 10.1016/j.yexmp.2020.104468
19. De Gregorio C, Contador D, Díaz D, et al. Human adipose-derived mesenchymal stem cell-conditioned medium ameliorates polyneuropathy and foot ulceration in diabetic BKS db/db mice. *Stem Cell Res Ther*. 2020;11(1):168. doi: 10.1186/s13287-020-01680-0
20. Sun J, Zhang Y, Song X, et al. The healing effects of conditioned medium derived from mesenchymal stem cells on radiation-induced skin wounds in rats. *Cell Transplant*. 2019; 28(1):105–115. doi: 10.1177/0963689718807410

AUTHORS' INFO

The author responsible for the correspondence:
Andrey I. Cherepanin, MD, PhD; Professor
address: 28, Orekhovy blvd, Moscow,
115682, Russia;
e-mail: surgdoccher@yandex.ru; eLibrary SPIN: 8652-7153;
ORCID: https://orcid.org/0000-0002-6254-8966

Co-authors:
Olga V. Pavlova,
e-mail: vasa-vasorum@mail.ru;
ORCID: https://orcid.org/0000-0002-9459-7391

Vladimir A. Kalsin,
e-mail: vkalsin@mail.ru; eLibrary SPIN: 1046-8801;
ORCID: https://orcid.org/0000-0003-2705-3578

Mikhail A. Konoplyannikov, PhD;
e-mail: mkonopl@mail.ru; eLibrary SPIN: 9211-6391;
ORCID: https://orcid.org/0000-0003-1180-2343

Olga N. Kucherova, MD, Ph.D.;
e-mail: ola-kucherova@mail.ru;
ORCID: https://orcid.org/0000-0003-3773-9629

Victor L. Baldin, MD, PhD,
e-mail: baldinvl@gmail.com

Sergey V. Deryabin;
e-mail: deryabin@mail.ru; eLibrary SPIN: 4929-0910