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THERMAL INJURY HEALING IN THE CONTEXT OF NEOCOLLAGENOGENESIS INDUCTION: PRECLINICAL RANDOMIZED EXPERIMENTAL STUDY

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ABSTRACT

Background. The healing of skin wounds having various etiologies is known to involve a multistep process characterized by certain intercellular interactions affecting dermal cells, their attachment, migration, and differentiation. Here, recovery is interpreted as the return of dermis to its original state. The fact is, however, that the dermal extracellular matrix (ECM) is structurally impaired, which suppresses the regulatory and repository functions of the dermis, leading to the formation of a scar that inhibits several biological functions in the affected area and causes aesthetic problems associated with mobility.

Objectives. To evaluate the structural features of dermis during wound healing using a calcium-containing biodegradable implant.

Methods. The study used 60 rats that were inflicted with a third-degree burn injury (partially damaged dermis). The selected animals were divided into two groups: experimental and control. On post-burn day 14, a calcium-containing biodegradable implant was administered to rats from the experimental group, while a sterile saline solution was used in the control group. Material was sampled at two months (74 days) and four months (134 days). In order to assess the morphological state of the burn area, its sections were stained with hematoxylin and eosin, according to Mallory and Van-Gieson. For the selective detection of collagens, immunohistochemical tests using antibodies to collagen types I and III (Abcam, England) were employed. To characterize dermal cells, the authors used antibodies to vimentin (LabVision, USA), as well as to CD-68 (cluster of differentiation 68), α-SMA (alpha-smooth muscle actin), CD-105 (cluster of differentiation 105), and VEGF (vascular endothelial growth factor) receptors (Abcam, England). The obtained results were processed using the Statistica 6 software (StatSoft, USA).

Results. The administration of a calcium-containing biodegradable filler during the burn healing process was found to ensure local fibroblast activation with the formation of collagen types I and III. When the implant residence time was prolonged up to four months, an increase in the number of macrophages expressing CD-68 receptors was observed. Of note is that these cells retained their localization, while α-SMA-expressing cells were localized in both the superficial and deep dermal compartments. The number of cells expressing CD-105 and VEGF rose as well.

Conclusion. The use of the biodegradable filler is found to be promising in terms of post-burn dermal regeneration, as well as providing a dermal ECM, whose collagen network composition and assembly are similar to the original. Here, macrophages act as the primary synthesis regulators of the dermal ECM and stimulate fibroblasts, which ensures re-epithelialization and angiogenesis of the inflicted area.

Keywords: dermis**,** burn injury, implant, neocollagenogenesis, calcium hydroxyapatite, filler

Conflict of interest: the authors declare no conflict of interest.

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ОСОБЕННОСТИ ЗАЖИВЛЕНИЯ ТЕРМИЧЕСКОЙ РАНЫ В УСЛОВИЯХ СТИМУЛЯЦИИ НЕОКОЛЛАГЕНОГЕНЕЗА: ДОКЛИНИЧЕСКОЕ ЭКСПЕРИМЕНТАЛЬНОЕ РАНДОМИЗИРОВАННОЕ ИССЛЕДОВАНИЕ

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АННОТАЦИЯ

Введение. Известно, что заживление кожных ран различной этиологии — многоступенчатый процесс, характеризующийся определенными межклеточными взаимодействиями, влияющими на клетки дермы, их прикрепление, миграцию и дифференцировку. Этап восстановления его интерпретируется как возврат дермы к исходному состоянию. Однако на самом деле экстрацеллюлярный матрикс дермы имеет нарушенную организацию, что подавляет регулирующую и репозиторную функцию дермы, приводит к формированию рубца, который лишает участок повреждения ряда биологических функций и вызывает эстетические проблемы, связанные с мобильностью.

Цель исследования — оценить структурные особенности дермы при заживлении кожных ран в условиях использования биодеградируемого кальцийсодержащего импланта.

Методы. Исследование выполнено на крысах (60 особей). Животным был нанесен ожог IIIa степени, затем животных разделили на 2 группы: опытная и контрольная. На 14-й день после нанесения ожога крысам опытной группы вводили биодеградируемый кальцийсодержащий имплант. В контрольной группе использовали стерильный физиологический раствор. Материал забирали в сроки, соответствующие 2 месяцам (74-й день) и 4 месяцам (134-й день). Для оценки морфологического состояния зоны ожога срезы окрашивали гематоксилином и эозином, по Маллори и Ван-Гизону. Для избирательного выявления коллагенов использованы иммуногистохимические тесты с антителами к коллагену I и III типов (Abcam, Англия). Для характеристики клеток дермы использовали антитела к виментину (LabVision, США), а также рецепторам CD-68 (cluster of differentiation 68), α-SMA (alpha-smooth muscle actin), CD-105 (cluster of differentiation 105), VEGF (vascular endothelial growth factor) (Abcam, Англия). Обработку результатов осуществляли с использованием программы Statistica 6 (StatSoft, США).

Результаты. Установлено, что введение биодеградируемого кальцийсодержащего филлера в срок, соответствующий процессу заживления ожога, обеспечивает локальную активацию фибробластов с образованием коллагена I и III типов. При пролонгировании времени пребывания импланта до 4-х месяцев происходит увеличение числа макрофагов, экспрессирующих CD-68 рецепторы, причем клетки не меняют своей локализации, в то время как клетки, экспрессирующие α-SMA, локализуются на участке как поверхностного, так и глубокого компартментов дермы. Число клеток, экспрессирующих CD-105 и VEGF, также увеличивается.

Заключение. Полученные данные позволяют считать, что использование биодеградируемого филлера представляется перспективным в аспекте регенерации дермы после ожога и может обеспечить получение экстрацеллюлярного матрикса дермы, имеющего состав и сборку коллагеновой сети, приближенной к исходной. Макрофаги выступают в роли главных регулировщиков синтеза экстрацеллюлярного матрикса дермы, стимулируют фибробласты, что обеспечивает реэпителизацию и ангиогенез зоны повреждения.

Ключевые слова: дерма**,** ожоговая рана**,** имплант, неоколлагеногенез, гидроксиапатит кальция, филлер

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INTRODUCTION

The healing of skin wounds having various etiologies is known to involve a multistep process characterized by certain intercellular interactions affecting dermal cells, their attachment, migration, and differentiation [1–8]. In this case, the healing process comprises four successive stages: coagulation and homeostasis, inflammation, proliferation, and remodeling [9–12].

The first stage involves wound surface closure followed by the formation of a fibrin plug and extracellular matrix (ECM) by fibroblast cells. Here, recovery is interpreted as the return of dermis to its original state. The fact is, however, that the dermal ECM is structurally impaired, which suppresses the regulatory and repository functions of the dermis, leading to the formation of a scar that inhibits several biological functions in the affected area and causes aesthetic problems associated with mobility [13–18].

Although current advances in biomaterial science and tissue engineering have yielded various skin substitutes, it remains to solve the specified problems associated with the activation of repair rather than regeneration mechanisms [19–22].

The current strategy of tissue engineering advocates the use of the patient's cells in order to create *in vivo* vascularized ECM characterized by the absence of exogenous material, for it can trigger the process of physiological recovery [24–28].

The article aims to evaluate the structural features of dermis during wound healing using a calcium-containing biodegradable implant.

METHODS

Experimental animals

The experiment used 60 male outbred rats weighing 250 ± 30 g that were provided by the Rappolovo Laboratory Animal Breeding Facility of the National Research Center Kurchatov Institute (NRC Kurchatov Institute).

Accommodation and maintenance of animals

The animals were kept under observation at the vivarium laboratory of the Education Department of the Kuban State Medical University (KubSMU) where a standard food ration was provided with free access to food and water[1].

Study design

A randomized controlled study was conducted using male rats inflicted with burn wounds as a model. The experiments were carried out at the Department for Histology and Embryology of the Kuban State Medical University. The design of this study is presented in Fig. 1.

Sample size

The animals were divided into two groups: control (n = 30) and experimental (n = 30). All of them were inflicted with third-degree burn injuries (partially damaged dermis)[2]. Test subjects from the experimental group were injected with a calcium-containing biodegradable filler (Radiesse, Germany), while those from the control group received a sterile 0.9% isotonic sodium chloride solution. The preparations were administered once at 14 days post burn, with

[1] GOST 33044-2014 *Principles of Good Laboratory* Practice approved by Order No. 1700-st (November 20, 2014) of the Federal Agency for Technical Regulation and Metrology
[2] Pavlenko S.G., Shablin D.V., Khuranov A.A., Zobenko V.Ya., Evglevskii A.A. *A device for modeling an experimental burn injury in animals*. Utility patent No. 151026, registered on Feb. 11, 2015. Patent holders: Pavlenko S.G. and Shablin D.V.

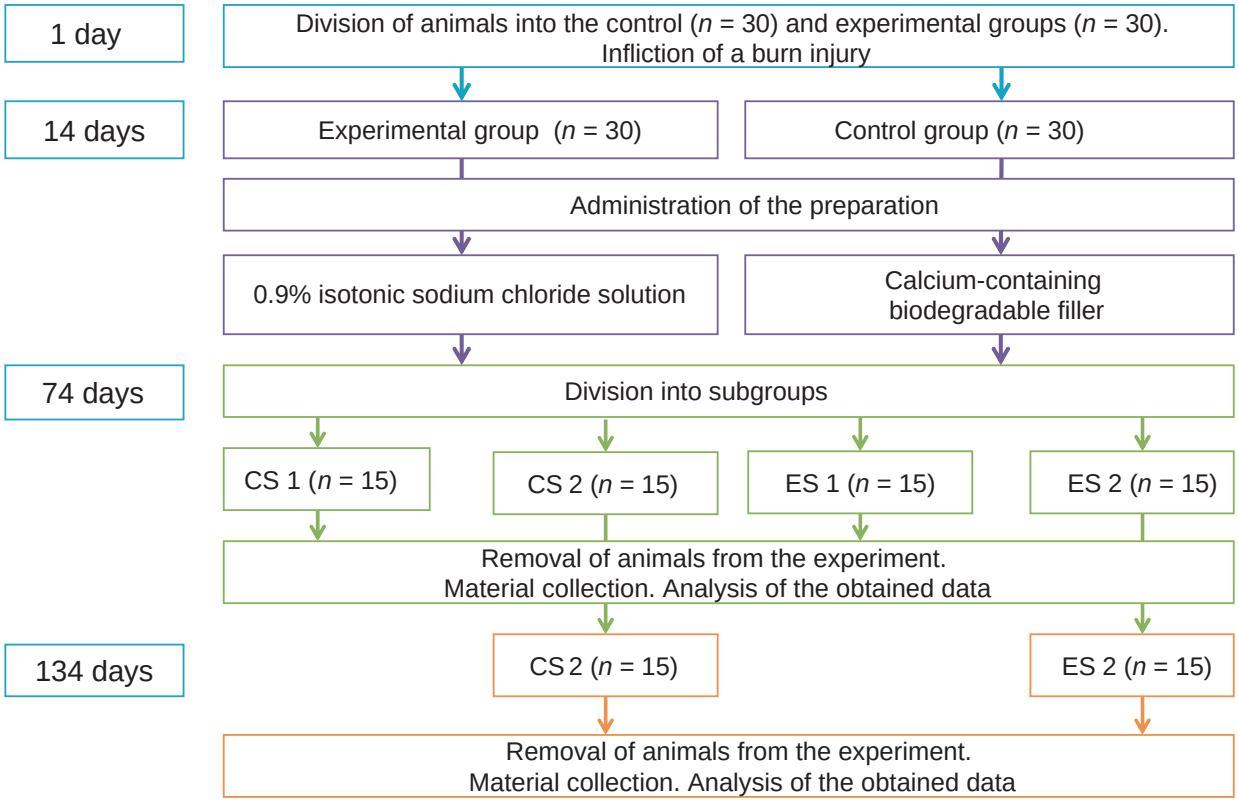

Fig. 1. Schematic diagram of the research design
Note: CS1 — control subgroup 1; CS2 — control subgroup 2; ES1 — experimental subgroup 1; ES2 — experimental subgroup 2.
Рис. 1. Блок-схема дизайна исследования.
Примечание: КГ1 — контрольная подгруппа 1; КГ2 — контрольная подгруппа 2; ОГ1 — опытная подгруппа 1; ОГ2 — опытная подгруппа 2.

material sampled at two (74 days) and four months (134 days). Different residence times of the preparation in the dermis were used as a criterion for dividing the animals into subgroups. The subgroups were formed at two months (74 days) following drug administration, with 15 animals randomly allocated to each group (experimental and control).

Eligibility criteria

Inclusion criteria

Male outbred rats exhibiting no external signs of diseases and anatomical abnormalities were selected for this experiment.

Exclusion criteria

No female subjects or subjects whose weight differed by more than 50 g were entered into the experiment.

Removal criteria

If animals were to injure the implantation area at any stage of the study, wound abscess would also prevent them from further participating in the experiment.

Randomization

The randomization was conducted using a closed envelope method. Taking the inclusion criteria into account, 60 rats were selected to be divided into two groups: experimental (30 animals) and control (30 animals).

Data anonymity assurance

We divided the animals into groups and analyzed the results without the input of other individuals.

Resulting indicators (outcomes) of the study

We primarily intend to assess the activation of dermal cells with them entering neocollagenogenesis, as well as the remodeling of dermal ECM in accordance with the original matrix, as the result of using the calcium-containing biodegradable implant.

The criteria for ascertaining dermal activation which involves its cells entering neocollagenogenesis are as follows: presence of dendritic dermal fibroblasts exhibiting high expression of procollagen, type III collagen, and vimentin; emergence of vimentin-positive structures in the connective tissue

matrix; macrophage migration into the burn area; emergence of cells having CD-68 and α-SMA receptors in the superficial layer of the dermis.

Experimental procedures

Burn injuries were modeled by means of a brass cylinder having a surface area of 706 mm^2 and weighing 300 g, which was heated to 100 degrees in boiling water. The cylinder was applied to the depilated skin of animals at the top of shoulders for 15 s, resulting in the formation of a third-degree burn (partially damaged dermis). In this case, Sevorane inhalation anesthetic (Abbott, England) was used for anesthesia. The test subjects were then divided into two groups: experimental (30 animals) and control (30 animals). As part of the experiment protocol, Radiesse was administered to rats in the experimental group at 14 days post burn. The filler was injected subdermally at a volume of 0.05 mL per experimental unit [29] at the border of visually defined intact skin. This day was considered the starting point of the experiment. In order to standardize preparation administration in the control group, a sterile saline solution was used; it was injected subdermally at a volume of 0.05 mL per experimental unit.

Skin fragments collected from the center of the wound, as well as from intact skin areas at the wound periphery, were used as the subject matter in the study. The specified biological material was sampled at two and two months. Tissue processing and embedding were performed in Tissue-Tek VIP-5Jr. processors (Japan); the resulting paraffin blocks were used to make 4–5 μm thick sections using an HM 340 E Rotary Microtome (MICROM Laborgerate GmbH, Germany).

In order to assess the morphological status of the burn area, the sections were stained with hematoxylin and eosin, according to Mallory, Van-Gieson, and Masson. For the selective detection of collagens, immunohistochemical tests using antibodies to collagen types I and III (Abcam, England) were used. To characterize dermal cells, we used antibodies to vimentin (LabVision, USA), as well as CD-68 (cluster of differentiation 68), α-SMA (alpha-smooth muscle actin), CD-105 (cluster of differentiation 105), VEGF (vascular endothelial growth factor) receptors (Abcam, England). The immunohistochemical tests were performed according to the protocol using positive and negative controls to adjust the staining.

The obtained micropreparations were subjected to visual examination in hematoxylin and eosin stains according to Mallory, Van-Gieson, and Masson.

Care and monitoring of the animals

The animals were kept under observation while receiving a standard food ration with free access to food and water. No adverse effects were noted during the study. The test subjects were removed from the experiment using the most humane method according to World Animal Protection, i.e., by administering an anesthetic overdose (a dose three times the usual amount of the drug).

Statistical procedures

Principles behind sample size determination

The sample size was not determined in advance.

Statistical methods

A descriptive medical study was conducted for a series of cases, i.e., we used the calcium-containing biodegradable implant for several rats, achieving the activation of dermal cells with them entering neocollagenogenesis, which resulted in the remodeling of the dermal ECM in accordance with the original matrix. The sample size was determined according to the number of animals required for an adequate immunohistochemical study. In order to assess the statistical significance of the difference between experimental parameters factoring in the effect produced by the calcium-containing biodegradable implant on the connective tissue in the context of thermal injury healing, we used the fourfold contingency table method. In this case, the difference was considered significant at $p < 0.05$. The obtained results were processed using the Statistica 6 software (StatSoft, USA).

RESULTS

The study of micropreparations sampled from the control group of animals at the first day of eschar detachment, i.e., at 14 days post burn, indicates that the inflicted area is epithelialized; the epidermis has from three to five cellular lines (even though poorly contoured); cell nuclei are vacuolated. The dermis comprises short, irregularly arranged collagen bundles.

When stained using a standard set of conventional histochemical reactions according to Mallory and Van-Gieson, the burn area of experimental animals containing filler in the dermis is typable as an implant with microspheres surrounded by a capsule at two months of the filler residence. In the area above and below the implant, the dermis is organized as dense connective tissue. In the immunohistochemical detection of collagen type I (Fig. 2), the reaction reveals dermal fibroblasts in the form of dendritic cells exhibiting a high level of procollagen expression (indicated by the arrow in

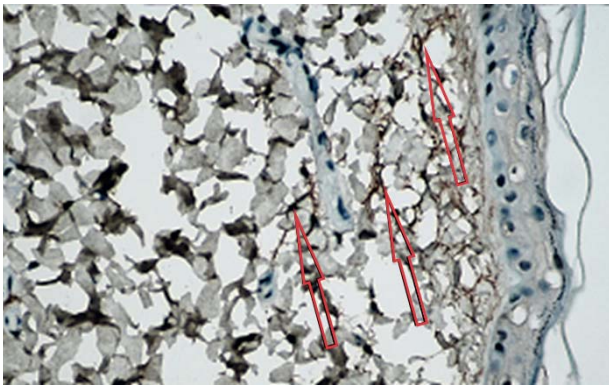

Fig 2. Dermis in the burn area two months following the filler administration. Immunohistochemical staining for type I collagen (×400 magnification).
Рис. 2. Участок дермы в зоне ожога через 2 месяца после введения филлера Иммуногистохимическая окраска на коллаген I типа. Увеличение ×400.

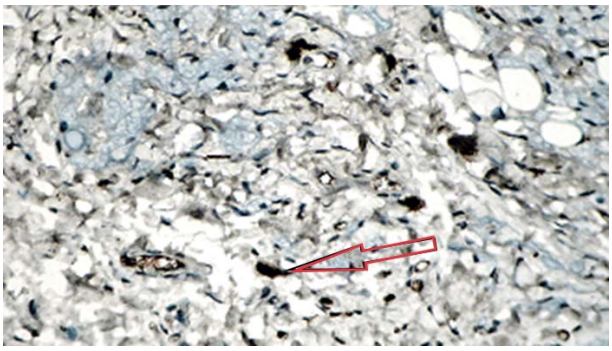

Fig 3. Dermis in the burn area two months following the filler administration. Immunohistochemical staining for type III collagen (×400 magnification).
Рис. 3. Участок дермы в зоне ожога через 2 месяца после введения филлера. Иммуногистохимическая окраска на коллаген III типа. Увеличение ×400.

the figure). These cells are localized in the deep dermal compartment.

When detecting type III collagen, a thin fibrillar network is typable in ECM with predominant localization in the superficial compartment. Fibroblasts showing high expression of type III collagen are also observed here (Fig. 3, *indicated by the arrow*).

An analysis of the cellular composition of dermis in experimental animals as affected by the use of a biodegradable scaffold revealed that its residence in the dermis at two and four months can be interpreted as the most active period of fibroblast stimulation in collagen synthesis. By this time, the proliferative stage is induced in the burn area involving the migration of macrophages that produce cytokines stimulating fibroblast proliferation. This phase occurring in the context of contact between macrophages and fibroblasts can be regarded as a transduction signal between these cells.

At two months, immunohistochemical detection of vimentin revealed that the implant area is surrounded by a well-defined connective tissue capsule. Structures exhibiting a high level of vimentin expression are as follows: the wall of microspheres formed by thin vimentin-positive fibrils; large fibroblasts exhibiting a high level of vimentin expression, as well as groups of cells located between microspheres; some of these cells (possibly macrophages) have a moderately active vacuolated cytoplasm (Fig. 4).

Cells exhibiting high vimentin expression can also be detected in the dermis outside the implant area (papillary layer, i.e., the superficial compartment of the dermis); these cells are small in size and spindle-shaped.

The vimentin level increases at four months of the scaffold residing in the dermis; this phenomenon is observed for cells localized in the superficial compartment of the dermis, i.e., in the papillary layer, with some of these cells migrating to the epidermis. Typable cells grow in size, specifically cells bordering the epidermis, while the level of vimentin expression remains high.

A distribution study of cells expressing CD-68 receptors revealed that two months following Radiesse administration, CD-68-positive cells accumulate in large numbers in the superficial layer of the dermis. These cells are small and dendritic, exhibiting a moderate or even high level of CD-68 expression. In the implant area, a small number of CD-68-positive cells are detected, while a homogeneous mass exhibiting a high level of CD-68 expression can be observed inside the microspheres (Fig. 5).

When the implant residence time is prolonged up to four months, the migration of macrophages expressing CD-68 receptors is observed. However, the cells retain their localization, colonizing an area of the superficial dermal compartment. The appearance of CD-68-positive macrophages may be associated with an increase in the height of dermal papillae. In the deep compartment, they are organized individually, retaining a high level of CD-68 expression.

When identifying α-SMA two months following Radiesse administration, α-SMA-positive cells are detected in the area of the preserved implant: between the microspheres and in the area of the capsule surrounding the implant (Fig. 6).

When the filler residence is prolonged up to four months, the implant is no longer present, with the cells expressing α-SMA localized in both the superficial and deep dermal compartments. Stress fibers

exhibiting high α-SMA levels can also be observed (Fig. 7).

A distribution analysis of the CD-105 marker, which acts as a cell proliferation regulator, revealed that a small number of CD-105-positive cells emerge in the superficial compartment of the dermis two months following Radiesse administration. CD-105-positive cells are small and dendritic, exhibiting low-to-medium levels of expression. With the prolonged residence of the filler in the dermis, an increase is observed in the density of this receptor on the membrane of some cells located in the deep dermal compartment. It is possible that these large cells exhibiting a high level of CD-105 expression can regulate the functional activity of tissue macrophages.

When identifying cells expressing VEGF receptors during the first two months of implant residence, the number of these cells rises in all areas of the deep and superficial compartments. In some cases, such cells are primarily localized in the superficial compartment. At four months, the number of VEGF-expressing cells increases dramatically in both the superficial and deep dermal layers. In the papillary layer, the cells are small, whereas in the deep compartment area, the cells are large and dendritic. The level of VEGF expression is moderate in the cells of the superficial compartment and intensive in the deep compartment.

Tables 1 and 2 present the results of analyzing the primary outcomes of the experiment at two and four months in terms of how the calcium-containing biodegradable implant affects the connective tissue in the context of thermal injury healing.

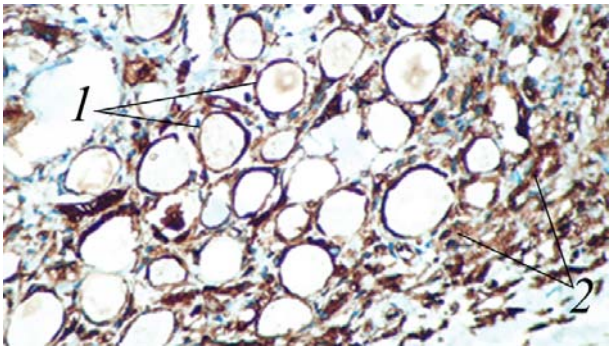

Fig. 4. Dermis in the burn area two months following the filler administration. 1 — microsphere wall formed by thin vimentin-positive fibrils; 2 — vimentin-positive cells. Immunohistochemical reaction (x400 magnification).
Рис. 4. Участок дермы в зоне ожога через 2 месяца после введения филлера. 1 — стенка микросфер, сформированная тонкими виментин-положительными фибриллами; 2 — виментинположительные клетки. Иммуногистохимическая реакция. Увеличение ×400.

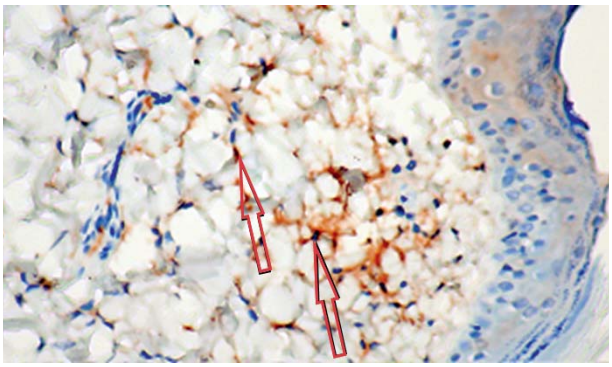

Fig. 5. Dermis in the burn area. CD-68+ macrophages (indicated by the arrows) in dermal cells two months following the filler administration. Immunohistochemical reaction (x400 magnification).
Рис. 5. Участок дермы в зоне ожога. CD-68+ макрофаги (обозначено стрелками) в клетках дермы через 2 месяца после введения филлера. Иммуногистохимическая реакция. Увеличение ×400.

DISCUSSION

Interpretation/scientific merit

The current strategy of tissue engineering using the patient's endogenous cells to create an *in vivo* ECM similar to the original matrix could provide an alternative to skin repair in the post-burn healing process. This approach can reduce the difference between reparative and healthy tissue.

Research limitations

Not identified.

Generalizability/extrapolation

Thus, the obtained data indicate that the administration of a biodegradable filler during the burn healing process, i.e., at 14 days following the wound infliction, ensures local fibroblast activation. This phenomenon is related to the well-known neocollagenogenesis-inducing properties of a calcium-containing filler. The filler is characterized by biodegradation involving the formation of calcium and phosphorus ions followed by the utilization of these substances; of note is that the prolonged effect of neocollagenogenesis for dermal cells (specifically for fibroblasts) is maintained with the formation of both collagen type I and III. The synthesized collagen serves as the foundation for dermal cells recreating the effect of a stiff and porous frame, which ensures an assembly of the *de novo* synthesized ECM.

In the burn wound healing process, macrophages are considered to act as the primary regulators that activate fibroblasts, keratinocytes, and endothelial cells. They serve as grafts, regulators of dermal ECM synthesis, as well as se-

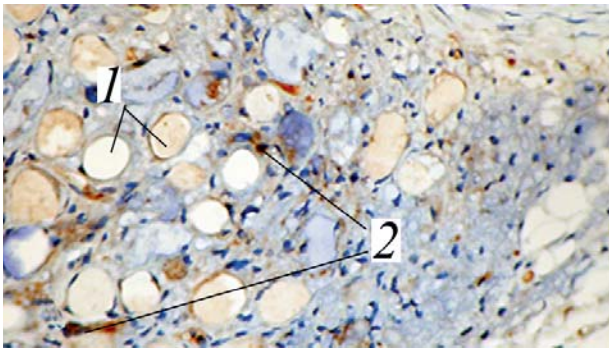

Рис. 6. Участок дермы в зоне ожога. α-SMA+ клетки через 2 месяца после введения филлера. 1 — микросферы 2 — α-SMA+ клетки. Иммуногистохимическая реакция. Увеличение ×400.
Fig. 6. Dermis in the burn area. α-SMA+ cells two months following the filler administration. 1 — microspheres 2 — α-SMA+ cells. Immunohistochemical reaction (x400 magnification).

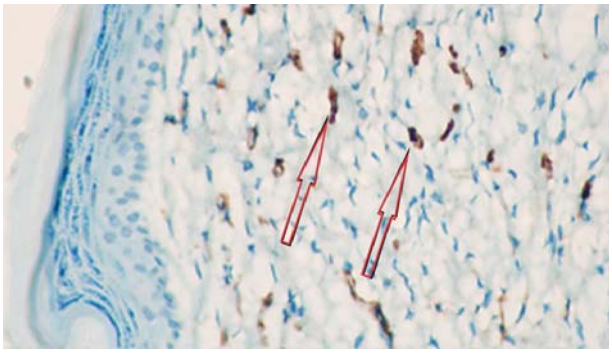

Рис. 7. Участок дермы в зоне ожога. α-SMA+ клетки (обозначено стрелками) через 4 месяца после введения филлера. Иммуногистохимическая реакция. Увеличение ×400.
Fig. 7. Dermis in the burn area. α-SMA+ cells (indicated by the arrows) four months following the filler administration. Immunohistochemical reaction (x400 magnification).

creting various growth factors that promote the proliferation and migration of fibroblasts, thus ensuring the re-epithelization and angiogenesis of the inflicted area.

The revealed dynamics of fibroblasts in the inflicted area can yield a dermis having collagen network composition and assembly characteristic of that *in vivo*. In this context, the normalization of intercellular communication in the dermis can also be expected.

The final stage of burn wound healing involves dermal remodeling, with the formation of a fibrous capsule. The granulation tissue fibroblasts are activated and acquire α-SMA expression, which indicates the transition of these cells into myofibroblasts. The contractile activity of cells leads to an increase in the stiffness and mechanical stress of the ECM. The relatively low density of this marker in dermal cells found in the studied areas suggests that the transition of fibroblasts into myofibroblasts occurs gradually here, which may indicate dermal remodeling without rough scar formation.

The current strategy of tissue engineering using the patient's endogenous cells to create an *in vivo* ECM similar to the original matrix may provide an alternative to skin repair in the post-burn healing process. At any rate, this approach would probably reduce the distinction between reparative and healthy tissue. The use of endogenous fibroblasts for ECM synthesis in the lesion area can yield dermis having collagen network composition and assembly characteristic of the *in vivo* dermis. In this case, it can be expected that communications between such cells as fibroblasts, macrophages, and keratinocytes will also be properly regulated.

In the process of dermal regeneration, the most promising approach involves activating the synthesis of collagen, which serves as a frame for dermal cells. By creating a stiff or porous frame effect, it is possible to control the assembly of the *de novo* synthesized EMC. This factor can bring the properties of the synthesized dermis into compliance with those of the patient's skin, minimizing, or at least decreasing, the structural differences between the restored dermis and the surrounding skin.

When using various skin substitutes in the treatment of thermal injuries, the regulatory role of endogenous cell-derived ECM is compromised since growth factors synthesized by fibroblasts are not provided to other cell types (such as keratinocytes and endothelial cells) as usual, with a disruption of intercellular signal transduction. This fact primarily affects the formation of the boundary between the dermis and the epidermis.

Tissue-engineered skin is known to form a flat dermal-epidermal border. When fibroblasts are used to synthesize endogenous ECM, a profile of the ridge is formed, having epithelial invaginations and follicular structures, which are typical for the physiological dermal-epidermal border.

CONCLUSION

Thus, the innovative idea consists in allowing old fibroblasts to partially restore their functions, while modifying molecular indicators of biological age in a group of cells newly emerged in the process of mitosis. This approach may well ensure ECM modulation yielding a dermis similar to that available.

Table 1. Number of animals providing primary outcomes in groups and their percentage depending on the group (observation period of two months)
Таблица 1. Количество животных с основными исходами в группах и их доли в зависимости от группы (срок наблюдения 2 месяца)

Groups of test animals	Animals providing the primary outcome		Significance level, *p*
	abs.	percentage	
Experimental (*n* = 15)	12	80	0.026
Control (*n* = 15)	6	40	

Note: The difference is significant at $p < 0.05$.
Примечание: различие значимо при $p < 0,05$.

Table 2. Number of animals providing primary outcomes in groups and their percentage depending on the group (observation period of four months)
Таблица 2. Количество животных с основными исходами в группах и их доли в зависимости от группы (срок наблюдения 4 месяца)

Groups of test animals	Animals providing the primary outcome		Significance level, *p*
	abs.	percentage	
Experimental (*n* = 15)	14	93	0.031
Control (*n* = 15)	9	60	

Note: The difference is significant at $p < 0.05$.
Примечание: различие значимо при $p < 0,05$.

FURTHER INFORMATION

Protocol registration

The study protocol (including research topic, key design features, and analysis plan) was prepared prior to the study and approved by the Independent Committee for Ethics of the Kuban State Medical University (Ministry of Healthcare of the Russian Federation).

Data access

Data on the conducted study can be freely accessed in machine-readable form to be used and republished without copyright and patent restrictions, as well as other control mechanisms.

COMPLIANCE WITH ETHICAL STANDARDS

The study content and design were approved by the Independent Committee for Ethics (Minutes No. 54 as of October 11, 2017) of the Kuban State Medical University (Mitrofana Sedina str., 4, Krasnodar, Russia). The maintenance and handling of animals were consistent with the principles of the Declaration of Helsinki on Humane Treatment of Animals and Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes.

СООТВЕТСТВИЕ ПРИНЦИПАМ ЭТИКИ

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REFERENCES

1. Zhang X., Kang X., Jin L., Bai J., Liu W., Wang Z. Stimulation of wound healing using bioinspired hydrogels with basic fibroblast growth factor (bFGF). *Int. J.Nanomedicine*. 2018;13:3897-3906. DOI: 10.2147/IJN.S168998
2. Velnar T., Bunc G., Klobucar R., Gradisnik L. Biomaterials and host versus graft response: a short review. *Bosn. J. Basic. Med. Sci*. 2016;16(2):82-90. DOI: 10.17305/bjbms.2016.525
3. Urciuolo F., Casale C., Imparato G., Netti P.A. Bioengineered Skin Substitutes: the Role of Extracellular Matrix and Vascularization in the Healing of Deep Wounds. *J.Clin. Med*. 2019;8(12):2083. DOI: 10.3390/jcm8122083
4. Hesketh M., Sahin K.B., West Z.E., Murray R.Z. Macrophage Phenotypes Regulate Scar Formation and Chronic Wound Healing. *Int.J.Mol.Sci*. 2017;18(7):1545. DOI: 10.3390/ijms18071545
5. Zavgorodniaia M.I., Makeieva L.V., Slavcheva O.S., Sulaieva O.N Cellular and molecular basics of the wound healing. *Morphologia*.2016; 10(3): 19–23 (In Russ., English abstract). DOI:10.26641/1997-9665.2016.3.19-23
6. Gorokhova V.S., Chernovol P.A., Chernovol V.P., Sulaieva O.N. The variability of response of thrombocytes to ADP: from theory of thrombogenesis to practical application of plasma rich in thrombocytes. *Klin. Lab. Diagn*. 2016;61(6):363-367 (In Russ., English abstract). DOI: 10.18821/0869-2084-2016-6-363-367
7. Gilroy D., De Maeyer R. New insights into the resolution of inflammation.*Semin.Immunol*. 2015;27(3):161-168. DOI: 10.1016/j.smim.2015.05.003
8. Donati G., Watt F.M. Stem cell heterogeneity and plasticity in epithelia. *Cell Stem.Cell*. 2015;16(5):465-476. DOI: 10.1016/j.stem.2015.04.014
9. Rittié L. Cellular mechanisms of skin repair in humans and other mammals. *J. Cell Commun. Signal*. 2016;10(2):103-120. DOI: 10.1007/s12079-016-0330-1
10. Motwani M.P., Flint J.D., De Maeyer R.P., Fullerton J.N., Smith A.M., Marks D.J., Gilroy D.W. Novel translational model of resolving inflammation triggered by UV-killed E. coli. *J. Pathol. Clin. Res*. 2016;2(3):154-165. DOI: 10.1002/cjp2.43
11. Schlader Z.J., Vargas N.T. Regulation of Body Temperature by Autonomic and Behavioral Thermoeffectors. *Exerc.Sport. Sci. Rev*. 2019;47(2):116-126. DOI: 10.1249/JES.0000000000000180
12. Lombardi B., Casale C., Imparato G., Urciuolo F., Netti P.A. Spatiotemporal Evolution of the Wound Repairing Process in a 3D Human Dermis Equivalent. *Adv. Healthc. Mater.*2017;6(13). DOI: 10.1002/adhm.201601422
13. Martorina F., Casale C., Urciuolo F., Netti P.A., Imparato G. In vitro activation of the neuro-transduction mechanism in sensitive organotypic human skin model.*Biomaterials*.2017;113:217-229. DOI: 10.1016/j.biomaterials.2016.10.051
14. Boyce S.T., Lalley A.L. Tissue engineering of skin and regenerative medicine for wound care. *Burns.Trauma*.2018;6:4. DOI: 10.1186/s41038-017-0103-y
15. Fetah K., Tebon P., Goudie M.J., Eichenbaum J., Ren L., Barros N.,Nasiri R., Ahadian S., Ashammakhi N., Dokmeci M.R., Khademhossein A. The emergence of 3D bioprinting in organ-on-chip systems.*Progress in Biomedical Engineering*. 2019; 1(1): 012001. DOI:10.1088/2516-1091/ab23df
16. Tarassoli S.P., Jessop Z.M., Al-Sabah A., Gao N., Whitaker S., Doak S., Whitaker I.S. Skin tissue engineering using 3D bioprinting: An evolving research field. *J. Plast. Reconstr. Aesthet. Surg*. 2018;71(5):615-623. DOI: 10.1016/j.bjps.2017.12.006
17. Boulet L.P. Airway remodeling in asthma: update on mechanisms and therapeutic approaches. *Curr. Opin.Pulm. Med*. 2018;24(1):56-62. DOI: 10.1097/MCP.0000000000000441
18. Roy B., Yuan L., Lee Y., Bharti A., Mitra A., Shivashankar G.V. Fibroblast rejuvenation by mechanical reprogramming and redifferentiation. *Proc. Natl. Acad. Sci. USA*. 2020;117(19):10131-10141. DOI: 10.1073/pnas.1911497117
19. PutlyaevV.I., Yevdokimov P.V., Klimashina E.S., Rodin I.A., Safronova T.V., Garshev A.V., Mamonov S.A., ZorinV.N. Stereolithographic 3D printing of bioceramic scaffolds of a given shape and architecture for bone tissue regeneration. *Inorganic Materials: Applied Research*. 2019; 10(5): 1101-1108(In Russ., English abstract). DOI: 10.1134/S2075113319050277
20. Shinde A.V., Frangogiannis N.G. Mechanisms of Fibroblast Activation in the Remodeling Myocardium. *Curr.Pathobiol. Rep*. 2017;5(2):145-152. DOI: 10.1007/s40139-017-0132-z
21. Wahlsten A., Rütsche D., Nanni M., Giampietro C., Biedermann T., Reichmann E., Mazza E. Mechanical stimulation induces rapid fibroblast proliferation and accelerates the early maturation of human skin substitutes. *Biomaterials*. 2021;273:120779. DOI: 10.1016/j.biomaterials.2021.120779
22. Meleshina A.V., Bystrova A.S., Rogovaya O.S., Vorotelyak E.A., Vasiliev A.V., Zagaynova E.V. Skin tissue-engineering constructs and stem cells application for the skin equivalents creation (review). *Modern Technologies in Medicine*. 2017; 9(1): 198-220. DOI: 10.17691/stm2017.9.1.24

23. Lorenc Z.P., Bass L.M., Fitzgerald R., Goldberg D.J., Graivier M.H. Physiochemical Characteristics of Calcium Hydroxylapatite (CaHA). *Aesthet. Surg. J*. 2018; 38(suppl_1): S8-S12. DOI: 10.1093/asj/sjy011

24. Michalek I.M., Lelen-Kaminska K., Caetano Dos Santos F.L. Peptides stimulating synthesis of extracellular matrix used in anti-ageing cosmetics: Are they clinically tested? A systematic review of the literature. *Australas J. Dermatol*. 2019; 60(4): e267-e271. DOI: 10.1111/ajd.13036

25. Shpichka A., Butnaru D., Bezrukov E.A., Sukhanov R.B., Atala A., Burdukovskii V., Zhang Y., Timashev P. Skin tissue regeneration for burn injury. *Stem Cell Res. Ther*. 2019;10(1):94. DOI: 10.1186/s13287-019-1203-3

26. Urciuolo F., Casale C., Imparato G., Netti P.A. Bioengineered Skin Substitutes: the Role of Extracellular Matrix and Vascularization in the Healing of Deep Wounds. *J. Clin. Med*. 2019; 8(12): 2083. DOI: 10.3390/jcm8122083

27. Zhang Y., Liang H., Luo Q., Chen J., Zhao N., Gao W., Pu Y., He B., Xie J. In vivo inducing collagen regeneration of biodegradable polymer microspheres. *Regen. Biomater.* 2021;8(5):rbab042. DOI: 10.1093/rb/rbab042

28. Akinbiyi T., Othman S., Familusi O., Calvert C., Card E.B., Percec I. Better Results in Facial Rejuvenation with *Fillers. Plast.Reconstr. Surg. Glob. Open*. 2020;8(10):e2763. DOI: 10.1097/GOX.0000000000002763

29. Mogilnaya G.M., Fomicheva E.V. Dermal response to combined double filler administration. *Kuban Scientific Medical Bulletin*. 2020; 27(4): 72-81(In Russ., English abstract). DOI:10.25207/1608-6228-2020-27-4-72-81

СПИСОК ЛИТЕРАТУРЫ

1. Zhang X., Kang X., Jin L., Bai J., Liu W., Wang Z. Stimulation of wound healing using bioinspired hydrogels with basic fibroblast growth factor (bFGF). *Int. J. Nanomedicine*. 2018; 13: 3897–3906. DOI: 10.2147/IJN.S168998

2. Velnar T., Bunc G., Klobucar R., Gradisnik L. Biomaterials and host versus graft response: a short review. *Bosn. J. Basic. Med. Sci*. 2016; 16(2): 82–90. DOI: 10.17305/bjbms.2016.525

3. Urciuolo F., Casale C., Imparato G., Netti P.A. Bioengineered Skin Substitutes: the Role of Extracellular Matrix and Vascularization in the Healing of Deep Wounds. *J. Clin. Med*. 2019; 8(12): 2083. DOI: 10.3390/jcm8122083

4. Hesketh M., Sahin K.B., West Z.E., Murray R.Z. Macrophage Phenotypes Regulate Scar Formation and Chronic Wound Healing. *Int. J. Mol. Sci*. 2017; 18(7): 1545. DOI: 10.3390/ijms18071545

5. Завгородняя М.И., Макеева Л.В., Славчева О.С., Сулаева О.Н. Клеточные и молекулярные основы заживления ран. *Morphologia*. 2016; 10(3): 19–23. DOI: 10.26641/1997-9665.2016.3.19-23

6. Горохова В.С., Черновол П.А., Черновол В.П., Сулаева О.Н. Вариабельность ответа тромбоцитов на АДФ: от теории тромбогенеза к практическому применению богатой тромбоцитами плазмы. *Клиническая лабораторная диагностика*. 2016; 61(6): 363–367. DOI: 10.18821/0869-2084-2016-6-363-367

7. Gilroy D., De Maeyer R. New insights into the resolution of inflammation. *Semin. Immunol*. 2015; 27(3): 161–168. DOI: 10.1016/j.smim.2015.05.003

8. Donati G., Watt F.M. Stem cell heterogeneity and plasticity in epithelia. *Cell Stem.Cell*. 2015; 16(5): 465–476. DOI: 10.1016/j.stem.2015.04.014

9. Rittié L. Cellular mechanisms of skin repair in humans and other mammals. *J. Cell Commun. Signal*. 2016; 10(2): 103–120. DOI: 10.1007/s12079-016-0330-1

10. Motwani M.P., Flint J.D., De Maeyer R.P., Fullerton J.N., Smith A.M., Marks D.J., Gilroy D.W. Novel translational model of resolving inflammation triggered by UV-killed E. coli. *J. Pathol. Clin. Res*. 2016; 2(3): 154–165. DOI: 10.1002/cjp2.43

11. Schlader Z.J., Vargas N.T. Regulation of Body Temperature by Autonomic and Behavioral Thermo effectors. *Exerc. Sport. Sci. Rev*. 2019; 47(2): 116–126. DOI: 10.1249/JES.0000000000000180

12. Lombardi B., Casale C., Imparato G., Urciuolo F., Netti P.A. Spatiotemporal Evolution of the Wound Repairing Process in a 3D Human Dermis Equivalent. *Adv. Healthc. Mater*. 2017; 6(13). DOI: 10.1002/adhm.201601422

13. Martorina F., Casale C., Urciuolo F., Netti P.A., Imparato G. In vitro activation of the neuro-transduction mechanism in sensitive organotypic human skin model. *Biomaterials*. 2017; 113: 217–229. DOI: 10.1016/j.biomaterials.2016.10.051

14. Boyce S.T., Lalley A.L. Tissue engineering of skin and regenerative medicine for wound care. *Burns. Trauma*. 2018; 6: 4. DOI: 10.1186/s41038-017-0103-y

15. Fetah K., Tebon P., Goudie M.J., Eichenbaum J., Ren L., Barros N., Nasiri R., Ahadian S., Ashammakhi N., Dokmeci M.R., Khademhossein A. The emergence of 3D bioprinting in organ-on-chip systems. *Progress in Biomedical Engineering*. 2019; 1(1): 012001. DOI: 10.1088/2516-1091/ab23df

16. Tarassoli S.P., Jessop Z.M., Al-Sabah A., Gao N., Whitaker S., Doak S., Whitaker I.S. Skin tissue en-

gineering using 3D bioprinting: An evolving research field. *J. Plast. Reconstr.Aesthet. Surg*. 2018; 71(5): 615–623. DOI: 10.1016/j.bjps.2017.12.006

17. Boulet L.P. Airway remodeling in asthma: update on mechanisms and therapeutic approaches. *Curr. Opin. Pulm. Med*. 2018; 24(1): 56–62. DOI: 10.1097/MCP.0000000000000441

18. Roy B., Yuan L., Lee Y., Bharti A., Mitra A., Shivashankar G.V. Fibroblast rejuvenation by mechanical reprogramming and redifferentiation. *Proc. Natl. Acad. Sci. USA*. 2020; 117(19): 10131–10141. DOI: 10.1073/pnas.1911497117

19. Путляев В.И., Евдокимов П.В., Мамонов С.А., Зорин В.Н., Климашина Е.С., Родин И.А., Сафронова Т.В., Гаршев А.В. Стереолитографическая 3D-печать биокерамических матриксов заданной формы и архитектуры для регенерации костной ткани. *Перспективные материалы*. 2019; 5: 28–40. DOI: 10.30791/1028-978X-2019-5-28-40

20. Shinde A.V., Frangogiannis N.G. Mechanisms of Fibroblast Activation in the Remodeling Myocardium. *Curr. Pathobiol. Rep*. 2017; 5(2): 145–152. DOI: 10.1007/s40139-017-0132-z

21. Wahlsten A., Rütsche D., Nanni M., Giampietro C., Biedermann T., Reichmann E., Mazza E. Mechanical stimulation induces rapid fibroblast proliferation and accelerates the early maturation of human skin substitutes. *Biomaterials*. 2021; 273: 120779. DOI: 10.1016/j.biomaterials.2021.120779

22. Meleshina A.V., Bystrova A.S., Rogovaya O.S., Vorotelyak E.A., Vasiliev A.V., Zagaynova E.V. Skin tissue-engineering constructs and stem cells application for the skin equivalents creation (review). *Modern Technologies in Medicine*. 2017; 9(1): 198–220. DOI: 10.17691/stm2017.9.1.24

23. Lorenc Z.P., Bass L.M., Fitzgerald R., Goldberg D.J., Graivier M.H. Physiochemical Characteristics of Calcium Hydroxylapatite (CaHA). *Aesthet. Surg. J*. 2018; 38(suppl_1): S8–S12. DOI: 10.1093/asj/sjy011

24. Michalek I.M., Lelen-Kaminska K., Caetano Dos Santos F.L. Peptides stimulating synthesis of extracellular matrix used in anti-ageing cosmetics: Are they clinically tested? A systematic review of the literature. *Australas J. Dermatol*. 2019; 60(4): e267–e271. DOI: 10.1111/ajd.13036

25. Shpichka A., Butnaru D., Bezrukov E.A., Sukhanov R.B., Atala A., Burdukovskii V., Zhang Y., Timashev P. Skin tissue regeneration for burn injury. *Stem Cell Res. Ther*. 2019; 10(1): 94. DOI: 10.1186/s13287-019-1203-3

26. Urciuolo F., Casale C., Imparato G., Netti P.A. Bioengineered Skin Substitutes: the Role of Extracellular Matrix and Vascularization in the Healing of Deep Wounds. *J. Clin. Med*. 2019; 8(12): 2083. DOI: 10.3390/jcm8122083

27. Zhang Y., Liang H., Luo Q., Chen J., Zhao N., Gao W., Pu Y., He B., Xie J. In vivo inducing collagen regeneration of biodegradable polymer microspheres. *Regen. Biomater*. 2021; 8(5): rbab042. DOI: 10.1093/rb/rbab042

28. Akinbiyi T., Othman S., Familusi O., Calvert C., Card E.B., Percec I. Better Results in Facial Rejuvenation with *Fillers. Plast.Reconstr. Surg. Glob. Open*. 2020; 8(10): e2763. DOI: 10.1097/GOX.0000000000002763

29. Могильная Г.М., Фомичева Е.В. Характер ответной реакции дермы на сочетанное введение двух филлеров. *Кубанский научный медицинский вестник*. 2020; 27(4): 72–81. DOI: 10.25207/1608-6228-2020-27-4-72-81

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Approval of the final version of the paper — agreement to be accountable for all aspects of the work, the integrity of all parts of the paper, and its final version.

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Подготовка и редактирование текста — составление черновика рукописи, его критический пересмотр с внесением ценного замечания интеллектуального содержания.

Утверждение окончательного варианта статьи — принятие ответственности за все аспекты работы, целостность всех частей статьи и ее окончательный вариант.

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Ресурсное обеспечение исследования — предоставление инструментария.

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