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Application of a hydrogel derived from porcine dermis for experimental treatment of superficial wounds

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ABSTRACT

Aim. To study the efficacy of dermal hydrogel application in the experimental treatment of superficial scarified wounds in rats.

Materials and methods. The hydrogel was obtained from porcine dermis by alkaline hydrolysis. The DNA concentration was determined using the Nano Drop ND-1000 spectrophotometer. The study included 30 male Sphinx rats. Scarified wounds were created on the rat skin, then the rats were divided into two groups: group 1 – rats without treatment, or control group (n = 15), group 2 – rats with wound treatment with the dermal hydrogel for 5 days, or experimental group (n = 15). On day 3, 7, and 14 of the experiment, we explanted skin samples from the wound area and performed routine H&E staining.

Results. On day 3 of the experiment, moderate inflammation, edema, and collagen fiber disorganization were revealed in the experimental group, and pronounced inflammation with purulent exudate was found in the control group. On day 7 of the experiment, inflammation and foci of stratified epithelium were detected in the control group. The histologic analysis of the skin samples from the experimental group showed pronounced plethora of the vessels, necrotic changes of the dermis, and edema. The total thickness of the epidermis and the thickness of its stratum corneum were greater than in the control group samples. On day 14, the differences between the groups were minimal and the epidermis was thickened in the experimental group animals.

Conclusion. The study examined the effects of the dermal hydrogel on scarified wounds in rats. We found faster skin regeneration (by 1.5–2 days) in the experimental group compared to the controls. Besides, the rats of the experimental group were characterized by an increase in the number of fibroblasts in the dermis and thickened epidermis in the affected area.

Keywords: dermal hydrogel, scarified wound, morphological analysis, extracellular matrix

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Kuban State Medical University (Protocol No. 102 of 01.10.2021).

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Применение гидрогеля на основе дермы свиньи для экспериментального лечения поверхностных ран

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РЕЗЮМЕ

Цель – изучение эффективности использования дермального гидрогеля при экспериментальном лечении поверхностных скарифицированных ран у крыс.

Материалы и методы. Гидрогель получали из свиной дермы химическим методом с применением щелочного гидролиза. В полученных образцах гидрогеля определяли содержание ДНК с помощью спектрофотометра Nano Drop ND-1000. Исследование проведено на 30 самцах крыс породы сфинкс. Крысам наносили скарифицированные раны, затем животные были разделены на две группы: группа 1 – без лечения, или контрольная группа (n = 15), группа 2 – лечение раны дермальным гидрогелем в течение 5 сут (n = 15). На 3-и, 7-е и 14-е сут эксплантировались образцы кожи из области раны, которые подвергались гистологическому исследованию.

Результаты. На 3-и сут эксперимента в образцах кожи животных группы 2 отмечалось умеренное воспаление с поверхностным отеком и дискомплексацией коллагеновых волокон, а контрольной группы – выраженное воспаление с гнойным экссудатом. На 7-е сут эксперимента у крыс контрольной группы наблюдали воспаление, однако отмечали очаги пролиферации многослойного эпителия. Гистологический анализ кожи животных группы 2 продемонстрировал более выраженное полнокровие сосудов, некротические изменения дермы и ее отек. Общая толщина эпидермиса и толщина его рогового слоя была больше, чем в образцах контрольной группы. На 14-е сут эксперимента различия между изучаемыми группами были минимальны, отмечали утолщение эпидермиса у животных группы 2 по сравнению с контрольной группой.

Заключение. В проведенном исследовании продемонстрировано, что при использовании гидрогеля на основе дермы свиньи для лечения скарифицированных ран крыс полное восстановление кожи в пораженной области наступало на 1,5–2 сут быстрее, чем в контрольной группе. Помимо этого было зарегистрировано увеличение количества фибробластов в дерме и утолщение эпидермиса относительно аналогичного показателя у крыс контрольной группы.

Ключевые слова: дермальный гидрогель, скарифицированная рана, морфологический анализ, внеклеточный матрикс

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Исследование было одобрено локальным независимым этическим комитетом ФГБОУ ВО КубГМУ Минздрава России (протокол № 102 от 01.10.2021).

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INTRODUCTION

Hydrogel dressings are the most promising material for the treatment of superficial wounds, as they prevent the formation of adhesions in the adjacent tissues, exert an analgesic effect, and have a positive effect on wound healing due to the contained biologically active components. Hydrogel dressings are semi-permeable to promote wound hydration, eschar rehydration, and autolytic wound debridement [1].

Hydrogels based on natural biologically active polymers (collagen, hyaluronic acid, chitosan, alginate, etc.) are biocompatible and biodegradable, have low cytotoxicity, and regulate the proliferation and functioning of fibroblasts, keratinocytes, macrophages, and endothelial cells [2]. These biomaterials can acquire anti-inflammatory and antibacterial properties when growth factors and bioactive peptides are added.

A promising material for biopolymer-based hydrogels is the extracellular matrix (ECM), which consists of collagen, elastin, glycosaminoglycan, and other biologically active molecules [3]. The source of ECM in most cases are animal tissues, in particular, the dermis of pigs and cattle, however, the technologies for their processing are quite expensive and time-consuming [4]. Recently, there has been an increased interest in the development of an optimal method for obtaining hydrogels based on ECM of animal tissues. The aim of this study was to evaluate the developed hydrogel based on porcine dermal ECM as a wound dressing for the experimental treatment of superficial wounds in laboratory animals.

MATERIALS AND METHODS

The research was carried out in compliance with the principles of humanity set out in the directives of the European Community (86/609/EEC) and the requirements of the Declaration of Helsinki, revision 2013. All manipulations met the requirements of the Order No. 708n of the Ministry of Health of Russia of 23.08.2010 “On Approval of the Rules of Laboratory Practice”, the Bioethics Committee, and the Federal Law of the Russian Federation on the Protection of Animals (Article 4 of the Law of the Russian Federation “On the Protection of Animals from Cruelty” of 01.12. 1999).

The material for the creation of the dermal hydrogel was the skin of the Landrace pig (male, aged 2 months) weighing 13.4 kg. The animal was anesthetized with solutions of Zoletil (1 mg / kg; Zoletil 100, Virbac, France) and Xylazine (4 ml / kg; Rometar, Spofa, Czech Republic). Dermal samples with a thickness of 0.5 ± 0.05 mm were obtained from the lateral surface of the body after preliminary mechanical removal of the epithelial layer with an electrodermatome (disk knife diameter 100 mm) under sterile conditions. The collected dermis was stored for 1–6 months at –80 °C for preliminary cryodestruction of the cellular elements in the dermis.The porcine skin samples were chemically decellularized, in particular treated with 5% aqueous NaOH solution, at a sample weight per solution volume ratio of 1:5 for 22.5 hours. After that, the samples were washed with deionized water until a stable neutral pH was reached. DNA content was determined in the dermal hydrogel samples with the Nano Drop-1000 spectrophotometer (Thermo Fisher Scientific Inc., USA) using the reagent kit (General DNA Quantification Kit, Abcam, UK) according to the manufacturer’s protocol.The efficiency of the obtained dermal hydrogel was studied on 30 male Sphinx rats (weight 160–200 g, age 3–4 months), kept in a vivarium with a balanced diet and natural light. The rats were divided into two groups: group 1 – rats without dermal hydrogel treatment or control group (n = 15), group 2 – rats with dermal hydrogel treatment (n = 15). Under general gaseous anesthesia with Isoflurane (induction 2–5%, flow 0.25–4%; Laboratorios Karizoo, Spain), scarified wounds 30 x 20 x 2 mm in size were created in the rats in the area of the withers along the marked surface. The wounds in the rats of group 2 were treated daily for 5 days with 0.5 g dermal hydrogel. After the surgery, all animals were injected with the analgesic drug Ketoprofen 10% (5 mg / kg; Nita-Pharm, Russia) and the antibiotic Convenia (4 mg / kg; Convention, Zoetis, USA). On day 3, 7, and 14 of the experiment, the skin samples (8 mm in diameter) were explanted in adjacent native tissues using a skin biopsy device (Medax, Italy); then these samples were histologically stained with hematoxylin and eosin.

Statistical processing of the obtained results on the content of DNA and morphometric data was carried out using the Graph Pad Prism version 6.04 and Microsoft Excel 2016 software. The results were presented as $M \pm S$, where M is the arithmetic mean, and S is the standard deviation. The differences were considered significant at $p < 0.05$, the significance of differences was calculated according to the Mann – Whitney test. To quantify histologic changes in the porcine dermis, computer morphometry was used by the ImageJ program (National Institution of Health, USA) and the IHC metrics plugin. Epidermal changes in the samples were evaluated using the Freehand Selection Tool.

RESULTS

The porcine skin samples after chemical decellularization acquired a gel-like structure after 22.5 hours (Fig. 1). The dermal hydrogel was clear, dense, and homogeneous. The finished dermal hydrogel contained a 1% solution of an antimycotic antibiotic (Gibco, Thermo Fisher Scientific, USA) and was stored under sterile conditions at a temperature of +4 °C.

The hydrogel based on the porcine dermis was an oxyphilic structure, which was predominantly homogeneous due to pronounced swelling of the polymers (Fig. 2, *b*).

The comparative quantitative analysis of the DNA content in the dermal hydrogel and native dermal samples showed that the amount of DNA in the dermal hydrogel decreased to 33.19 ng / mg of dry matter, $p < 0.05$ (17.43%) relative to the DNA content in the native dermis (190 .45 (100%) ng / mg of dry matter). The data obtained corresponded to the quality criterion for decellularized tissues – no more than 50 ng of DNA per 1 mg of dry tissue mass [5].The results of the study of dermal hydrogel showed that it has a fairly pronounced reparative effect and accelerates the process of wound healing in comparison with the animals in the control group. Thus, on day 9 of the study, the rats of group 2 showed no visual signs of inflammation, tissue necrosis, or scarring (Fig. 3).

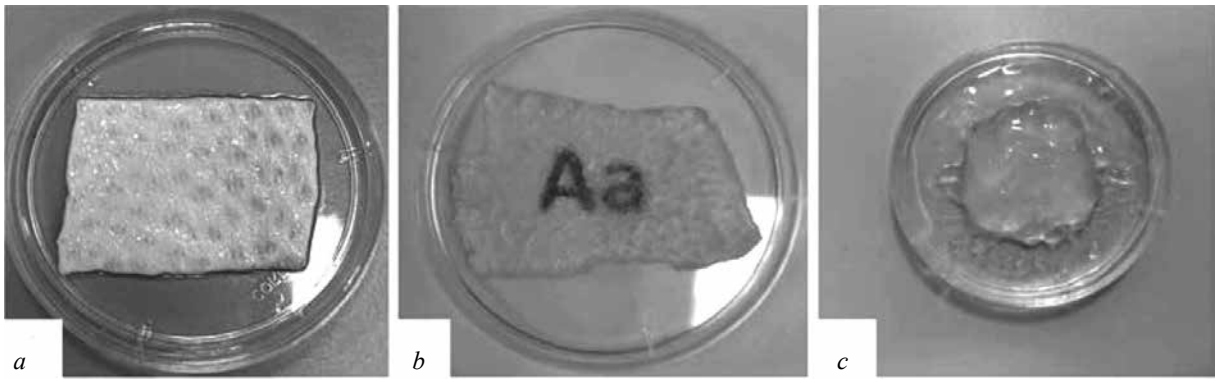

Fig. 1. The samples: *a* – native porcine dermis, *b* – dermis after 5 hours of treatment, *c* – sample of dermal hydrogel

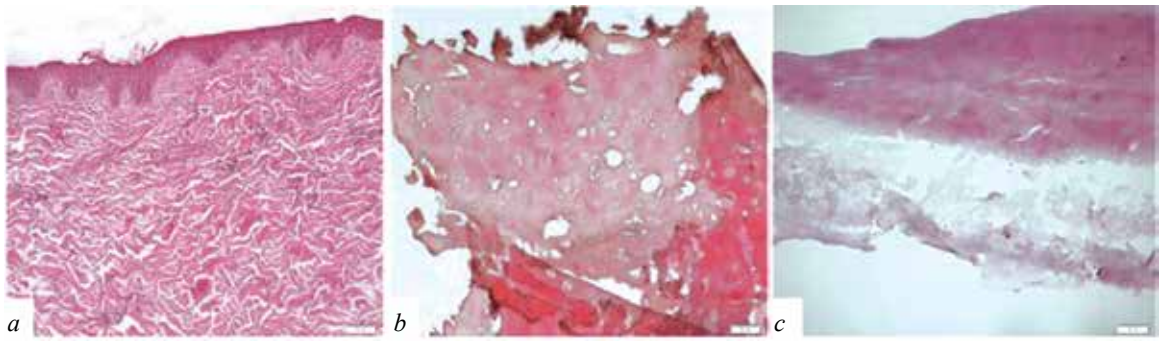

Fig. 2. Morphological analysis of the hydrogel: *a* – native porcine dermis, *b* – dermis after 5 hours of treatment, *c* – dermis after 22.5 hours of the treatment

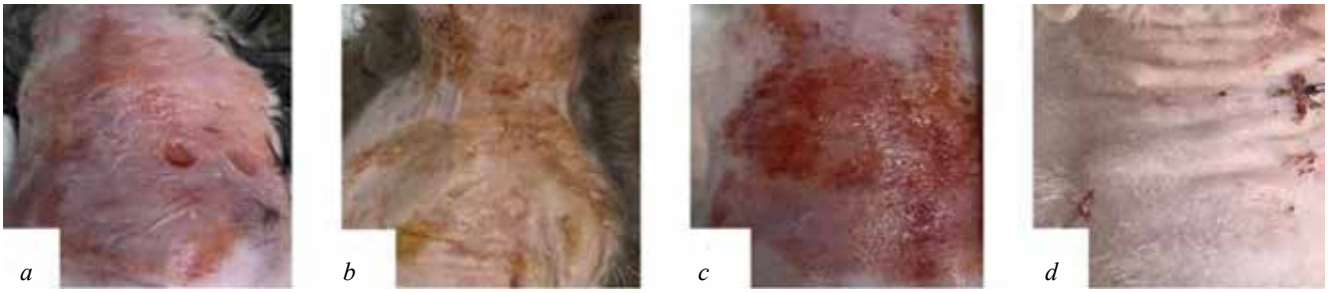

Fig. 3. The animals after the creation of a superficial scarified wound: *a, b* – the control group, no treatment; *c, d* – the experimental group, treatment with dermal hydrogel; *a, c* – right after the surgery; *b, d* – day 9 of the experiment

In the skin samples of untreated rats obtained on day 3 of the experiment, pronounced necrotic changes were observed, as well as obvious signs of inflammation and fibrinous purulent exudate (Fig. 4, *a*). On day 3 of the experimental treatment, the wounds treated with dermal hydrogel had moderately pronounced signs of inflammation. However, structural changes in this case were manifested through a pronounced edema of the wound surface with collagen fiber disorganization (Fig. 4, *d*).

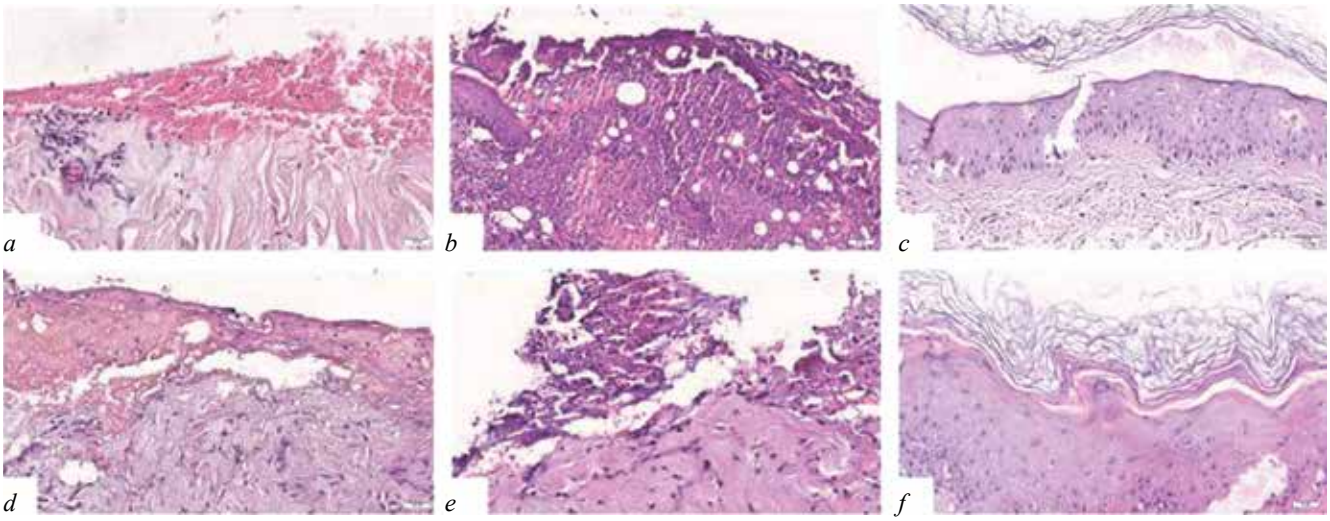

Fig. 4. Porcine skin from the wound area with adjacent tissues: *a, b, c* – the control group, no treatment; *d, e, f* – group 2, treatment with dermal hydrogel. *a, d* – day 3; *b, e* – day 7; *c, f* – day 14 of the experiment. Hematoxylin – eosin staining, x 100 magnification

On day 7, signs of inflammation were observed in the skin samples of the control rats, in particular, moderately pronounced infiltration. Against the background of resolving inflammation, signs of epidermal regeneration were noted in the form of foci of stratified epithelium proliferation (Fig. 4, *b*). The analysis of the skin samples of the animals of group 2 on day 7 showed more pronounced plethora of the microvasculature, necrotic changes in the dermis, and its edema (Fig. 4, *e*). Thickness of the epidermis in the rats of group 1 was 64.09 [52.82; 71.40] μm, which is less than that in the control animals (23.10 [16.56; 33.17] μm. The thickness of the stratum corneum in the epidermis of the control rats was also less ($p < 0.05$).

In the study of the porcine skin samples obtained 14 days after the gel was applied, the differences in the groups of animals were minimal: there was a slight difference in the thickness of the epidermis between the animals of group 2 (183.25 [155.07; 202.20] μm) and control rats (168.11 [144.26; 190.01] μm; $p < 0.05$; Fig. 4 *c, f*).

DISCUSSION

The most promising material for treatment of wounds are hydrogel dressings, whose high therapeutic efficacy as wound healing agents is proved by positive results of many studies [6]. It is known that hydrogels play a key role in the delivery of bioactive molecules and cellular products to the damaged area, unlike other types of modern wound dressings. Biological hydrogels promote autolysis of necrotic tissues, and their main property is a high degree of hydration to ensure a bactericidal effect and create optimal conditions for healing [7, 8].

Currently, an active search is underway for the most "perfect" collagen-containing hydrogel. There are numerous studies and developments for obtaining hydrogels from various tissues. The closest to our proposed method of processing the dermis to obtain a hydrogel is the method proposed by N.V. Kalmykova et al. [9]. The author obtained an ECM from the dermis of cattle in several ways – by treating with a 1M NaOH solution and a NaOH solution at a lower concentration with the addition of Na_2SO_4 and H_3BO_3 solutions and subsequent lyophilization of the obtained material. As a result of the processing of the dermis, they received a lyophilized ECM with a high content of collagen, but an additional processing step was necessary to obtain its hydrogel form.

In another study, F.T. Rodrigez et al. [10] developed a wound healing material based on the

porcine dermis treated with solutions of concentrated alkali and alkaline earth metal salts. The resulting material was further treated with a cross-linking agent, glutaraldehyde. However, the addition of crosslinkers can affect the toxicity and immunogenicity of the resulting material. Q.W. Tan et al. [11] obtained a hydrogel from porcine adipose tissue, which was decellularized using solutions of sodium dodecyl sulfate, pepsin, and hydrochloric acid, which are quite expensive. The dermal hydrogel proposed by us was obtained on the basis of porcine dermis, which is a less immunogenic biological material than synthetic materials or materials obtained using synthetic detergents. In addition, in our proposed method, there is no additional lyophilization step.

In the study, when using dermal hydrogel for the treatment of scarified wounds, on day 14, complete restoration of the skin in the affected area, a large number of fibroblasts, and thickening of the epidermis were noted compared to the animals in the control group. This is also confirmed by the data of other researchers, for example, H. Fujisaki et al. [12] noted that collagen hydrogels containing mainly type IV and I collagens support adhesion, proliferation, and growth of fibroblasts. It is known that collagen has a positive effect on early stages of wound healing, as it promotes platelet aggregation, stimulates the formation of granulation tissue, etc. Collagen lysis contributes to the enrichment of the wound with amino acids, which leads to the activation of protein biosynthesis in skin cells. Thus, in a study by T. M. Cherdantseva et al. [13], there was a slower increase in the area of granulation tissue, a slower decrease in the number of mast cells, and a decrease in their area and degranulation coefficient compared to animals in the control group. The author noted that in other studies on the effect of collagen-containing wound dressings, the ability of collagen to reversibly bind growth factors, protecting them from proteolysis, was revealed, which explains slower formation of granulation tissue in the group of rats whose burn wound was treated with a collagen matrix. Thus, due to its bioactive properties, the dermal hydrogel obtained by us promotes accelerated healing of scarified wounds, which correlates with the data of other researchers on the study of collagen-containing wound dressings. The simplicity and low cost of the technology for obtaining hydrogel from porcine dermis make it a potentially promising and competitive domestic biological material for wound healing.

CONCLUSION

The study demonstrated the effectiveness of using a dermal hydrogel based on the porcine dermal ECM in the experimental treatment of superficial scarified wounds. The use of the dermal hydrogel on scarified animal wounds led to earlier full recovery of the skin in the affected area, a greater number of fibroblasts, and more significant thickening of the epidermis compared to the control animals.The developed dermal hydrogel makes it possible to effectively protect the wound from bacterial microflora, accelerate wound healing, and create optimal conditions for active regeneration in the affected area. Further research on the use of dermal hydrogel as a therapeutic drug for wounds of various types will allow for the creation of a highly effective wound healing agent that has significant advantages among existing wound dressings.

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Authors' contribution

Melkonyan K.I., Rusinova T.V. – conception and design, justification of the manuscript, critical revision of the manuscript for important intellectual content. Kozmay Ya.A., Chuprynin G.P., Kartashevsky I.I. – carrying out of the experiment, analysis and interpretation of the data, drafting of the manuscript. Storozhuk S.V., Kartashevskaya M.I., Selezneva I.I. – review of literature on the research topic, analysis and interpretation of the data. Gurevich K.G. – final approval of the manuscript for publication.

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