УДК 616.5-089-74-089.819.843:577.1
https://doi.org/10.20538/1682-0363-2022-2-97-104

Morphological analysis of a local tissue response to subcutaneously implanted acellular dermal matrix fragments

Melkonyan K.I.[1], Verevkin A.A.[1], Sotnichenko A.S.[1], Rusinova T.V.[1], Kozmay Ya.A.[1], Asyakina A.S.[1], Kartashevskaya M.I.[1], Gurevich K.G [2], Bykov I.M.[1]

[1]Kuban State Medical University
4, Mitrofana Sedina Str., Krasnodar, 350063, Russian Federation

[2]A.I. Yevdokimov Moscow State University of Medicine and Dentistry
20/1, Delegatskaya Str., Moscow, 127473, Russian Federation

ABSTRACT

Acellular dermal matrices (ADMs) are gaining popularity as surgical materials for operations on the pelvic organs, as well as in burn therapy and plastic surgery. Evaluation of the biocompatibility of surgical materials is an important and necessary step in the development of new ADMs.

The aim of the study was to compare the results of subcutaneous implantation of ADM and native porcine skin in rats.

Materials and methods. To obtain ADMs, detergent – enzymatic decellularization was used. On days 7, 14, 21, and 60 after the implantation of ADMs (the experimental group) and native porcine skin (the control group), the animals were removed from the experiment. The histologic sections were stained with hematoxylin – eosin and Masson's trichrome stain, then an immunohistochemical reaction with antibodies to CD3 and CD68 was performed. Computer morphometry was carried out using the ImageJ software.

Results. On day 7 after the implantation, moderate sterile inflammation in the experimental group and pronounced sterile inflammation with eosinophil infiltration in the control group were observed. On day 14 of the experiment, the samples from the experimental group were characterized by a relatively low content of macrophages and T-lymphocytes with insignificant edema and no signs of ADM biodegradation. The control group showed pronounced inflammation, a large number of infiltrating macrophages and T lymphocytes, as well as fragmentation of collagen fibers. On day 21 of the experiment, a thin capsule was formed around ADM, there was a small number of infiltrating T lymphocytes and macrophages, the collagen fibers of the implant were intact. In the samples of the control group, there was pronounced inflammation with the presence of a significant number of lymphocytes and macrophages, as well as fragmentation and vascularization of the implant. On day 60 of the experiment, no inflammatory response was observed around ADM, biodegradation was minimal, and a dense fibrous capsule was formed around the fragment of the native porcine skin.

Conclusion. The experimental ADM has low immunogenicity and a low degree of biodegradation, which makes it possible to use it for further research to create efficient surgical material that is safe for use in clinical practice.

Keywords: regenerative medicine, skin, decellularization, morphological analysis, subcutaneous implantation, acellular dermal matrix

Conflict of interest. The authors declare the absence of obvious or potential conflict of interest related to the publication of this article.

Source of financing. The study was supported by Kuban Science Foundation within the research project No. MFI-P-20.1/11.

✉ *Melkonyan Karina I.,* kimelkonian@gmail.com

Conformity with the principles of ethics. The study was approved by independent Ethics Committee at Kuban State Medical University (Protocol No. 87 of 24.03.2020). The study was carried out in compliance with the principles of humanity set out in the European Council directives (86/609/EEC) and the ethical requirements of the Declaration of Helsinki revised in 2013.

For citation: Melkonyan K.I., Verevkin A.A., Sotnichenko A.S., Rusinova T.V., Kozmay Ya.A., Asyakina A.S., Kartashevskaya M.I., Gurevich K.G., Bykov I.M. Morphological analysis of a local tissue response to subcutaneously implanted acellular dermal matrix fragments. *Bulletin of Siberian Medicine.* 2022;21(2):97–104. https://doi.org/10.20538/1682-0363-2022-2-97-104.

Морфологический анализ местной тканевой реакции на подкожную имплантацию фрагментов ацеллюлярного дермального матрикса

Мелконян К.И.[1], Веревкин А.А.[1], Сотниченко А.С.[1], Русинова Т.В.[1], Козмай Я.А.[1], Асякина А.С.[1], Карташевская М.И.[1], Гуревич К.Г [2], Быков И.М.[1]

[1] Кубанский государственный медицинский университет (КубГМУ)
Россия, 350063, г. Краснодар, ул. им. Митрофана Седина, 4

[2] Московский государственный медико-стоматологический университет (МГМСУ) им. А.И. Евдокимова
Россия, 127473, г. Москва, ул. Делегатская, 20/1

РЕЗЮМЕ

Ацеллюлярные дермальные матриксы (АДМ) набирают большую популярность в качестве хирургических материалов при операциях на органах малого таза, в ожоговой терапии и пластической хирургии. Проверка биосовместимости материалов является важным и необходимым этапом при разработке новых АДМ.

Цель исследования – провести сравнительный анализ результатов подкожной имплантации крысам АДМ и нативной дермы свиньи.

Материалы и методы. Для получения АДМ использовали детергентно-энзиматический метод децеллюляризации. Через 7, 14, 21, 60 сут после имплантации АДМ (экспериментальная группа) и нативной дермы свиньи (контрольная группа) животных выводили из эксперимента. Гистологические срезы окрашивали гематоксилином и эозином, трихромом по Массону, выполняли иммуногистохимическую реакцию с антителами к CD3 и CD68. Компьютерную морфометрию проводили с помощью программы ImageJ.

Результаты. На 7-е сут в экспериментальной группе отмечалось умеренное асептическое воспаление, в контрольной группе – выраженное асептическое воспаление с эозинофилами в инфильтрате. На 14-е сут в экспериментальной группе показано относительно низкое содержание макрофагов и Т-лимфоцитов с незначительным отеком, без биодеградации АДМ. В контрольной группе выявлено выраженное воспаление, инфильтрация большим количеством макрофагов и Т-лимфоцитов, а также фрагментация коллагеновых волокон. На 21-е сут вокруг АДМ сформировалась тонкая капсула, в инфильтрате малое количество Т-лимфоцитов и макрофагов, коллагеновые волокна имплантата были интактны. В образцах контрольной группы – выраженное воспаление с присутствием значительного количества лимфоцитов и макрофагов, фрагментация и васкуляризация имплантата. На 60-е сут вокруг АДМ воспалительной реакции не наблюдалось, биодеградация была минимальной, вокруг фрагмента нативной дермы свиньи сформировалась плотная фиброзная капсула.

Заключение. Разработанный АДМ обладает низкой иммуногенностью и малой степенью биодеградации. Это позволяет использовать данную конструкцию для дальнейших исследований по созданию полноценного хирургического материала, безопасного для применения в клинической практике.

Ключевые слова: регенеративная медицина, дерма, децеллюляризация, морфологический анализ, подкожная имплантация, ацеллюлярный дермальный матрикс

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

Источник финансирования. Исследование выполнено при финансовой поддержке Кубанского научного фонда в рамках научного проекта № МФИ-П-20.1/11.

Соответствие принципам этики. Исследование одобрено независимым этическим комитетом КубГМУ (протокол № 87 от 24.03.2020).

Для цитирования: Мелконян К.И., Веревкин А.А., Сотниченко А.С., Русинова Т.В., Козмай Я.А., Асякина А.С., Карташевская М.И., Гуревич К.Г., Быков И.М. Морфологический анализ местной тканевой реакции на подкожную имплантацию фрагментов ацеллюлярного дермального матрикса. *Бюллетень сибирской медицины.* 2022;21(2):97–104. https://doi.org/10.20538/1682-0363-2022-2-97-104.

INTRODUCTION

In recent years, researchers have widely used decellularized (acellular) materials to create tissue-engineered scaffolds and culture cells on them [1]. There are various methods of decellularization in regenerative medicine [2]. It is possible to create tissue-specific scaffolds that most accurately mimic the physical and chemical signals that are essential for cell adhesion, proliferation, migration, differentiation, and restoration of function [3].

Some of the most promising advances in surgical practice are acellular dermal matrices (ADMs) [4]. ADMs are gaining increasing popularity as surgical materials for operations on the pelvic organs, in burn therapy, and plastic surgery, including reconstructive mammoplasty [5]. In plastic surgery, they are used both in radical skin-sparing mastectomy and in secondary operations for breast deformities as materials supporting the endoprosthesis [6]. ADMs can be incorporated into the patient's connective tissues with further ingrowth of vessels and nerves and, therefore, function as these tissues [7]. The advantages of ADMs include the possibility to avoid the use of tissue expanders, reduction of postoperative pain, decrease in operation time, optimization of regeneration processes, and a better aesthetic result [7, 8]. A particular advantage of ADM is the presence of collagen as one of the main components of the dermis. Compared with scaffolds based on other biological or synthetic polymers, collagen scaffolds are optimal for cell growth and adhesion both *in vitro* and *in vivo*, have good biocompatibility and low immunogenicity, and make it possible to regulate biodegradation due to their ability to form complexes with biologically active substances. The latter stimulates proliferation of fibroblasts and formation of patient's own tissues [6].

There are several ADM-based surgical materials used in reconstructive surgery for soft tissue restoration – AlloDerm, Strattice, DermaMatrix, SurgiMend, Permacol, Veritas, and FlexHD. Some analogs cannot be used in Russian surgical practice due to legislation (AlloDerm, DermaMatrix, Dermalogen, AlloDerm, Cymetra), while materials from bovine collagen can cause a severe allergic reaction.

The most promising are ADMs based on porcine skin (Evolence, Strattice, Fibroquel, Permacol) that significantly reduce the risk of allergic complications in surgical practice [9]. The possibility of obtaining porcine skin from secondary raw materials increases the efficiency of the method for obtaining the material. Nevertheless, these ADMs are extremely expensive, which poses a challenge for modern regenerative medicine to create a more economical analog. The first stage in the creation of any ADM is assessment of its biocompatibility [10]. It should not damage cells or cause rejection, while its biomechanical properties should be comparable to those of native tissues. Therefore, to assess the characteristics of the matrix, it is necessary to compare early and long-term results of heterotopic xenotransplantation of the developed ADM and native porcine skin.

The aim of the study was to conduct a comparative morphological analysis of the results of subcutaneous implantation of the developed ADM and native porcine skin in rats.

MATERIALS AND METHODS

Samples of native skin of the Landrace pig with a thickness of 0.7 mm were taken after preliminary removal of the epithelial layer with a dermatome under sterile conditions and frozen at –80 °C. To obtain ADM, the skin was incubated for 6 hours (3 cycles, 2 hours each) at 37° C in a trypsin – Versene solution (Biolot, Russian Federation). Then, 2 treatment cycles were carried out with 1% Triton X-100 (SigmaAldrich, USA) and 4% sodium deoxycholate solution (SigmaAldrich, USA) combined with 0.002 M Na_2-EDTA for a total duration of 12 hours at room temperature in the incubator shaker (170 rpm). Then the samples were incubated in a solution of porcine pancreatic DNase (SigmaAldrich, USA; 2,000 U in 200 ml of phosphate-buffered saline (PBS)) at 37°

C for 4 hours. To ensure sterility of the samples, 1% solution of gentamicin and amphotericin B was added. The samples were washed with deionized water for 10 minutes between the cycles and at each change of solution. For the quantitative determination of residual DNA in the matrix, a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA) and a DNeasy Blood and Tissue Kit (Qiagen, Sweden) were used. To assess the quality of decellularization, the samples of decellularized skin were stained with hematoxylin – eosin (Histolab, Sweden) and DAPI fluorophore (4', 6-diamidino-2-phenylindole; Sigma Aldrich, USA).

Subcutaneous implantation of the samples was performed on male Wistar rats weighing 200–230 g and aged 6 months ($n = 32$). The samples (3 x 3 mm) were placed in a subcutaneous pocket on the withers. The animals were divided into the control and the experimental groups, each of which included 4 subgroups of 4 animals. The rats of the control group were implanted with samples of native porcine skin, and those of the experimental group – with ADM samples.

Biopsy material was taken for histologic and immunohistochemical analysis on day 7, 14, 21, and 60 after the subcutaneous implantation. The morphological analysis was performed on five independent biopsy fragments of the samples, for each of them nine sections were performed and analyzed. Tissue staining with hematoxylin – eosin and Masson's stain (BioVitrum, Russian Federation) was performed according to the standard protocol. Immunohistochemical study used heat-induced antigen retrieval, as well as polyclonal antibodies to T-lymphocyte receptor – CD3 (cat. Number ab11089, Abcam, UK) and to the macrosialin of monocytes and macrophages – CD68, (cat. Number ab955, Abcam, UK). All the samples before and after the implantation were examined using the Olympus CX 41 microscope (Olympus, Japan) at different magnifications. Computerized morphometry was performed using the ImageJ software (National Institution of Health, USA) and the IHC profiler. The diameter of the blood vessels and collagen fibers was assessed using the freehand selection tool. For quantitative analysis of the immunohistochemistry results, we used color segmentation with highlighting of the green channel, color binarization, and the particle analyzer tool.

Statistical processing of the data was performed using the MedCalc Statistical Software (Belgium). The Shapiro – Wilk test was used to check the nature of the distribution in the variational series. Since the distribution was different from normal, the results were presented as the median, as well as the first and the third quartiles Me [Q_1; Q_3]. The significance of differences was assessed using the Mann – Whitney U-test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The spectrophotometry data showed that the quantitative DNA content in the native porcine skin was 314.4 [300.7; 333.7] ng per 1 mg of tissue, and in ADM – 60.14 [55.34; 63.58] ng per 1 mg of tissue, which complied with the criteria for tissue decellularization quality [11]. The relatively low content of residual nucleic acids in ADM indicated low immunogenic potential of the matrix.

ADM had specific milky-white color; the histologic structure examination revealed no intact cells or nuclear fluorescence after DAPI staining (Fig. 1).

On day 7 after the implantation, pronounced sterile inflammation was registered in the animals of the experimental group. The immunohistochemical analysis and computerized morphometry detected 12.70 [12.19; 13.09] macrophages and 1.87 [1.56; 2.04] lymphocytes per 1 mm^2 of the section and an insignificant number of neutrophils in the cell infiltrate. Intact

Fig. 1. Stages of skin decellularization and histologic analysis of the resulting ADM: *a* – appearance of the native porcine skin, *b* – appearance of ADM, c – hematoxylin – eosin staining, no cell nuclei after decellularization; x20

collagen fibers of the implanted structure with thickness of 12.88 [11.87; 13.60] μm were clearly visible when stained with the Masson's trichrome stain. In the meantime, in the samples of the control group, pronounced sterile inflammation with an admixture of eosinophils in the inflammatory infiltrate and congestion of skin vessels and muscular arteries were identified. Infiltration of the implant with blood and inflammatory cells was noted – 9.03 [8.94; 9.16] macrophages and 1.91 [1.84; 1.99] lymphocytes per 1 mm^2 of the section (Fig. 2) were revealed after staining with the Masson's trichome. It was noted that the number of lymphocytes in the experimental group did not differ from that of the control group (p = 0.062), and the number of macrophages was significantly greater (p = 0.043) (Fig. 3).

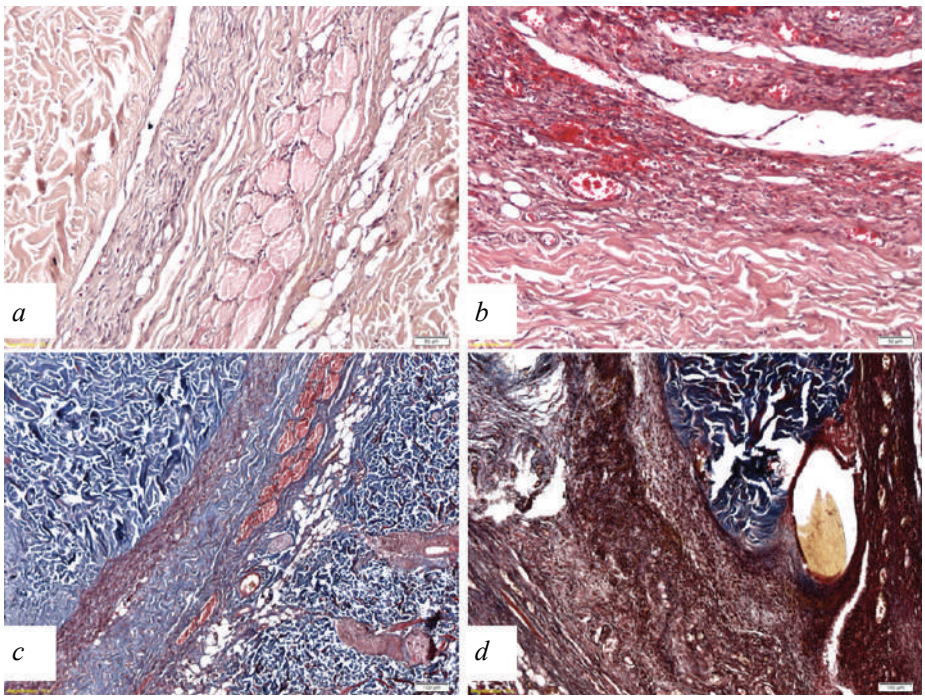

Fig. 2. Histologic evaluation of ADM and native porcine skin specimens after subcutaneous implantation in the experimental animals on day 7: *a* – ADM, hematoxylin – eosin, *b* – native porcine skin, hematoxylin – eosin, *c* – ADM, Masson's trichrome, *d* – native porcine skin, Masson's trichrome; x20

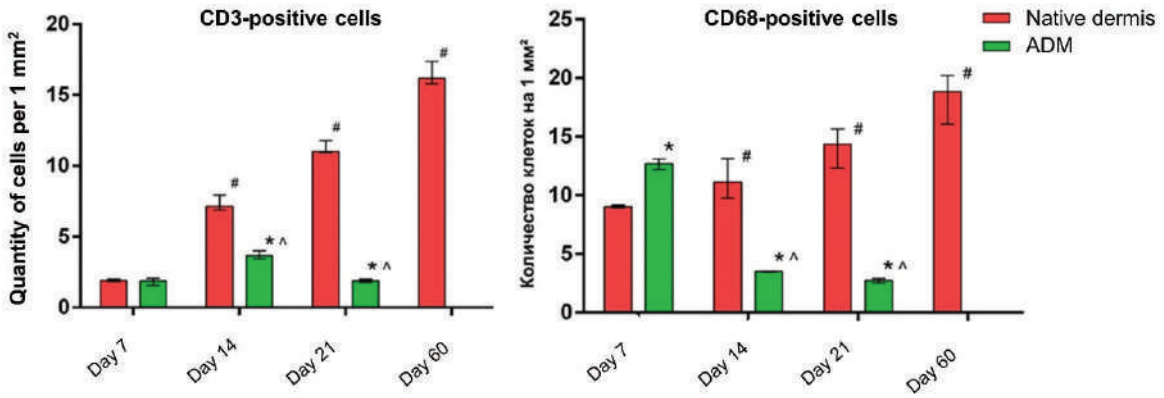

Fig. 3. Immunophenotyping of the inflammatory infiltrate at different periods of the experiment: *a* – changes in the content of T-lymphocytes (on the left), *b* – changes in the content of macrophages (on the right), *Me* [Q_1; Q_3]. * – the significance of the differences between the control and experimental groups, $p < 0.05$; # – the significance of the differences for the values in the control group for different periods, $p < 0.05$; ^ – the significance of the differences for the values in the experimental group for different periods, $p < 0.05$

On day 14, the formation of a thin connective tissue capsule around the implanted structure was noted in the experimental group. The thickness of the capsule was 18.17 [14.73; 20.32] μm, single macrophages (3.50 [3.44; 3.54] $CD68^+$ cells per 1 mm^2 of the section according to the computerized morphometry findings) were observed in the capsule walls. There was a thin layer of granulation tissue outside the capsule. Weakly pronounced vascular congestion was noted at the site of the implantation; the average diameter of the vessels was 30.97 [29.71; 31.82] μm. No evidence of degradation of ADM collagen fibers and no evidence of ADM infiltration by inflammatory cells were identified. We observed inflammation around the foreign body with predominance of a relatively large number of macrophages (11.13 [9.79; 13.11] $CD68^+$ cells according to the computerized morphometry data) in the samples of native porcine skin compared with the same parameter in the experimental group on day 14 ($p = 0.007$) and in the control group on day 7 ($p = 0.033$). Around the implant, a denser, thick-walled capsule with the average thickness of 165.2 [152.90; 188.80] μm, compared with the experimental group ($p = 0.003$), was formed.

The collagen fibers of the implant were fragmented, edematous, infiltrated with blood with an admixture of neutrophils and macrophages, and characterized by a pronounced oxyphilic reaction of the medium. A large number of thin congested vessels were found in immediate proximity to the capsule (the average diameter was 20.81 [19.41; 21.61] μm) (Fig. 4). There was a greater number of CD3-positive cells in both groups relative to the previous period of sample explantation – 3.66 [3.42; 4.01] cells per 1 mm^2 of the section for the experimental group ($p = 0.039$) and 7.14 [6.87; 7.93] cells per 1 mm^2 of the section for the control group ($p = 0.041$).

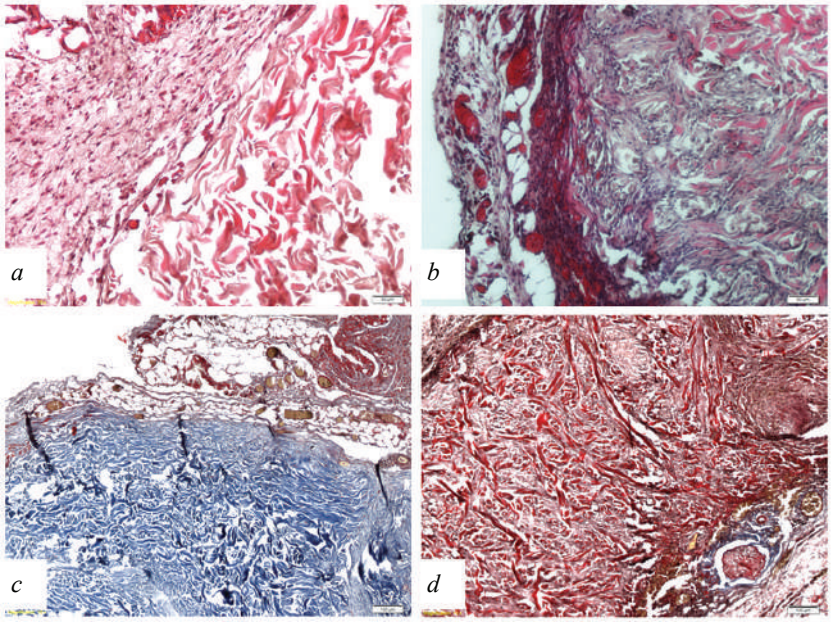

Fig. 4. Histologic evaluation of ADM and native porcine skin specimens after subcutaneous implantation in the experimental animals on day 14: *a* – ADM, hematoxylin – eosin, *b* – native porcine skin, hematoxylin – eosin, *c* – ADM, Masson's trichrome, *d* – native porcine skin, Masson's trichrome; x20

On day 21 after the ADM implantation, the thickness of the capsule around the implant was 10.02 [9.95; 10.10] μm, and a small number of inflammatory cells was observed – 2.73 [2.54; 2.89] $CD68^+$ macrophages and 1.90 [1.76; 1.99] $CD3^+$ lymphocytes per 1 mm^2 of the section, which was significantly lower than in the control group ($p = 0.012$ and $p = 0.009$, respectively). The average capillary diameter was 26.61 [19.48; 31.52] μm; no congested vessels were observed. The collagen fibers of the encapsulated ADM were completely preserved and had the diameter of 11.67 [10.02; 14.50] μm. There was no accumulation of leukocytes in the ADM or its infiltration with blood. At the same time, the formation of a dense connective tissue capsule with the thickness of 66.53 [61.24; 7.59] μm was observed in the animals of the control group. Numerous small clusters of T-lymphocytes, CD68-positive macrophages (the content was 14.37 [12.33; 15.65] cells per 1 mm^2 of the section), and foreign body cells were detected. Moreover, along with pronounced encapsulation, the presence of congested vessels with the diameter of 23.80 [19.24;

27.76] μm was noted inside the sample, which indicated active sample biodegradation. The collagen fibers were swollen and partially destroyed – their thickness ranged from 5 to 37 μm. Macrophages and T-lymphocytes were present in the thickness of the implant, which also confirmed immune rejection.

On day 60 after the subcutaneous implantation, the ADM was surrounded by the connective tissue capsule (its thickness was 13.83 [12.03; 15.54] μm), and inflammation was completely absent. Immunohistochemistry did not show the presence of macrophages and T-lymphocytes at the implantation site. Atrophy of surrounding tissues was absent; focal reactive lipomatosis was noted. The collagen fibers of the implant with the diameter of 15.06 [12.45; 15.99] μm were completely preserved, biodegradation was minimal. In the control group, significant implant degradation due to its lysis by macrophages and persistent perifocal inflammation were observed. A quantitative assessment showed the presence of 15.79 [14.50; 17.67] T-lymphocytes and 18.86 [16.09; 20.22] macrophages per 1 mm^2 of the section, which was significantly higher than the previous control value ($p = 0.008$ and $p = 0.048$, respectively). A multilayer capsule with the thickness of 107.20 [91.32; 117.50] μm formed around the fragment of the native porcine skin, the vessels surrounding it were congested and had the diameter of 28.50 [26.32; 31.45] μm (Fig. 5).

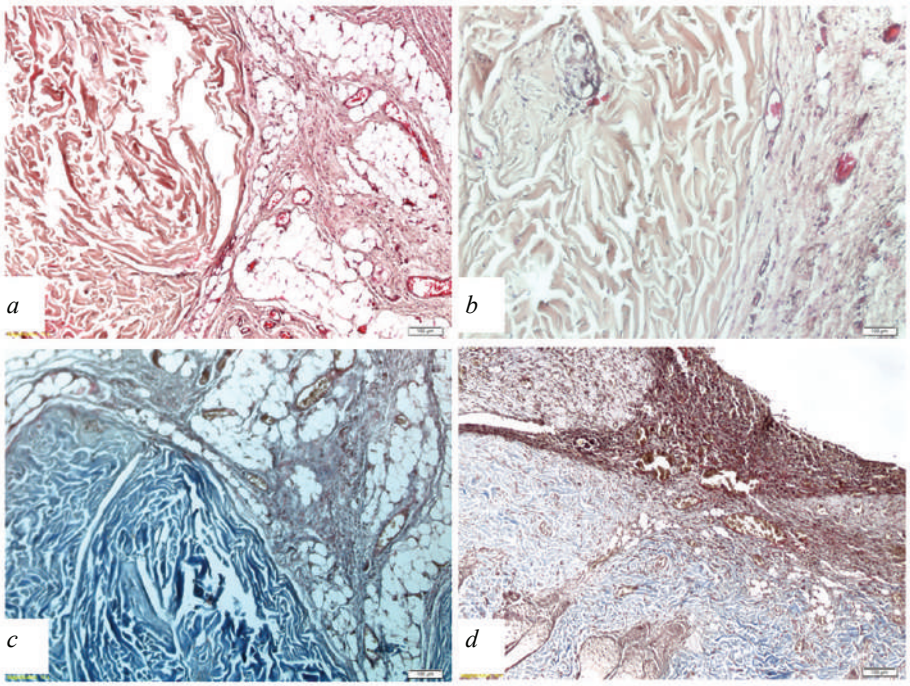

Fig. 5. Histologic evaluation of ADM and native porcine dermis specimens after subcutaneous implantation in the experimental animals on day 60: *a* – ADM, hematoxylin – eosin, *b* – native porcine skin, hematoxylin – eosin, *c* – ADM, Masson's trichrome, *d* – native porcine skin, Masson's trichrome; x20

Therefore, the results of immunophenotyping of the inflammatory infiltrate around and directly inside the implant at different periods of the experiment suggested that the ADM had minimal immunogenicity compared with the native porcine skin. A relatively weak inflammatory response and a thin connective tissue capsule around the implanted fragment, noted at later stages of the experiment, confirmed low antigenic properties of the ADM. Collagen fibers, which are the main component of the extracellular matrix that determine its mechanical properties, remained practically intact throughout the entire experiment. In the meantime, the implanted native porcine skin underwent significant biodegradation due to a pronounced inflammatory response, which was confirmed by the fragmentation of collagen fibers, the presence of congested capillaries, and a dense connective tissue capsule surrounding the implanted fragment.

CONCLUSION

The study showed that the developed ADM was characterized by low immunogenicity and a low degree of biodegradation. It allows to test the protocol of its development and use this construct as a starting point for further research on the biological and biomechanical properties of ADM to create valuable surgical material that is safe for application in clinical practice.

REFERENCES

1. Brouki Milan P., Pazouki A., Joghataei M.T., Mozafari M., Amini N., Kargozar S. et al. Decellularization and preservation of human skin: A platform for tissue engineering and reconstructive surgery. *Methods.* 2020;171:62–67. DOI: 10.1016/j.ymeth.2019.07.005.
2. Gholipourmalekabadi M., Bandehpour M., Mozafari M., Hashemi A., Ghanbarian H., Sameni M. et al. Decellularized human amniotic membrane: More is needed for an efficient dressing for protection of burns against antibiotic-resistant bacteria isolated from burn patients. *Burns*. 2015;41(7):1488–1497. DOI: 10.1016/j.burns.2015.04.015.
3. Nowacki M., Nowacka K., Kloskowski T., Pokrywczyńska M., Tyloch D., Rasmus M. et al. Are agricultural and natural sources of bio-products important for modern regenerative medicine? A review. *Ann. Agric. Environ. Med*. 2017;11;24(2):207–212. DOI: 10.5604/12321966.1235171.
4. Hsu P.W., Salgado C.J., Kent K., Finnegan M., Pello M., Simons R. et al. Evaluation of porcine dermal collagen (Permacol) used in abdominal wall reconstruction. *J. Plast. Reconstr. Aesthet. Surg.* 2009;62(11):1484–1489. DOI: 10.1016/j.bjps.2008.04.060.
5. JoAnna Nguyen T., Carey J.N., Wong A.K. Use of human acellular dermal matrix in implant- based breast reconstruction: evaluating the evidence. *J. Plast. Reconstr.Aesthet. Surg*. 2011;64(12):15531361. DOI: 10.1016/j.bjps.2011.02.001.
6. Shekhter A.B., Fayzullin A.L., Vukolova M.N., Rudenko T.G., Osipycheva V.D., Litvitsky P.F. Medical applications of collagen and collagen-based materials. *Cur. Med. Chem*. 2019;26(3):506–516. DOI: 10.2174/0929867325666171205170339.
7. Hartzell T.L., Taghinia A.H., Chang J., Lin S.J., Slavin S.A. The use of human acellular dermal matrix for the correction of secondary deformities after breast augmentation: results and costs. *Plast. Reconstr. Surg*. 2010;126(5):1711–1720. DOI: 10.1097/PRS.0b013e3181ef900c.
8. Gumenyuk A.S., Ushmarov D.I., Gumenyuk S.E., Gaivoronskaya T.V., Sotnichenko A.S., Melkonyan K.I., et.al. Prospects for the use of multilayer wound dressings based on chitosan in dental practice. *Kuban Scientific Medical Bulletin*. 2020;27(1):27–39 (in Russ.). DOI: 10.25207/1608-6228-2020-27-1-27-39.
9. Lucey P., Goldberg D.J. Complications of collagen fillers. *Facial. Plast. Surg*. 2014;30(6):615–622. DOI: 10.1055/s-0034-1396904.
10. Liu Y., Bharadwaj S., Lee S., Atala A., Zhang Y. Optimization of a natural collagen scaffold to aid cell-matrix penetration for urologic tissue engineering. *Biomaterials*. 2009;30(23–24):3865–3873. DOI: 10.1016/j.biomaterials.2009.04.008.
11. Crapo P.M., Gilbert T.W., Badylak S.F. An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2011;32(12):3233–3243. DOI: 10.1016/j.biomaterials.2011.01.057.

Authors contribution

Melkonyan K.I., Rusinova T.V. – conception and design, substantiation of the manuscript and critical revision of the manuscript for important intellectual content. Verevkin A.A., Sotnichenko A.S. – carrying out of the experiment, analysis and interpretation of the data, drafting of the manuscript. Kozmay Ya.A., Asyakina A.A., Kartashevskaya M.I. – review of literature on the topic of the article, analysis and interpretation of the data. Gurevich K.G., Bykov I.M. – final approval of the manuscript for publication.

Authors information

Melkonyan Karina I. – Cand. Sci. (Med.), Associate Professor, Head of the Central Research Laboratory, Kuban State Medical University, Krasnodar, kimelkonian@gmail.com, http://orcid.org/0000-0003-2451-6813

Verevkin Alexander A. – Researcher, Laboratory for Basic Research in Regenerative Medicine, Kuban State Medical University, Krasnodar, vilehand@bk.ru, http://orcid.org/0000-0002-4159-2618

Sotnichenko Alexander S. – Cand. Sci. (Med.), Head of the Laboratory for Basic Research in Regenerative Medicine, Kuban State Medical University, Krasnodar, alex24.88@mail.ru, http://orcid.org/0000-0001-7322-0459

Rusinova Tatyana V. – Cand. Sci. (Biology), Researcher, Central Research Laboratory, Kuban State Medical University, Krasnodar, rusinova.tv@mail.ru, http://orcid.org/0000-0003-2962-3212

Kozmai Yana A. – Junior Researcher, Laboratory for Basic Research in Regenerative Medicine, Kuban State Medical University, Krasnodar, yana.kozmay@gmail.com, http://orcid.org/0000-0001-5043-4315

Asyakina Alevtina S. – Laboratory Assistant, Laboratory for Basic Research in Regenerative Medicine, Kuban State Medical University, Krasnodar, alevtina.asyakina@mail.ru, http://orcid.org/0000-0002-5596-7783

Kartashevskaya Marina I. – Cand. Sci. (Med.), Assistant, Department of Dermatovenereology, Kuban State Medical University, Krasnodar, marinaikar@mail.ru, http://orcid.org/0000-0001-9060-2969

Gurevich Konstantin G. – Dr. Sci. (Med.), Professor, Head of the UNESCO Division "Healthy lifestyle – the key to successful development", A.I.Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, kgurevich@mail.ru, https://orcid.org/0000-0002-7603-6064

Bykov Ilya M. – Dr. Sci. (Med.), Professor, Head of the Department of Fundamental and Clinical Biochemistry, Kuban State Medical University, Krasnodar, ilya.bh@mail.ru, https://orcid.org/0000-0002-1787-0040

(✉) **Melkonyan Karina I.,** kimelkonian@gmail.com

Received 19.04.2021;
approved after peer review 20.09.2021;
accepted 05.10.2021