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Local biocompatibility and biochemical profile of hepatic cytolysis in subcutaneous implantation of polylactide matrices

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ABSTRACT

The aim of the study was to investigate local biocompatibility and systemic effects of nonwoven polylactide (PLA) matrices on blood and liver parameters after their subcutaneous implantation in Wistar rats.

Materials and methods. Bioabsorbable fibrous PLA matrices were produced by electrospinning and had dimensions (10 × 10 mm², thickness of no more than 0.5 mm; fiber diameter in the matrix ~1 μm) appropriate for subcutaneous implantation in white laboratory rats. Polymer implants were sterilized in ethylene oxide vapor. Thirty days after the implantation of PLA matrices, local biocompatibility according to GOST ISO 10993-6-2011, cellular parameters (total leukocyte count, hemogram, erythrocyte count, hemoglobin concentration), and biochemical blood parameters (lactate concentration, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels) were studied, and a standard histologic evaluation of the liver was performed.

Results. PLA matrix samples were mild local irritants on a scale of 1–1.9 points according to GOST ISO 10993-6-2011 criteria 30 days after the subcutaneous implantation. The median density of distribution of multinucleated giant cells (MNGCs) in the connective tissue around and in PLA matrices was 1,500 (1,350; 1,550) per 1 mm^2 of a slice. Pronounced leukocytic reaction due to lymphocytosis was noted (an increase by 1.7 times compared with a sham-operated (SO) control group, $p < 0.02$). The absence of a significant neutrophil count in the blood revealed sterile inflammation proceeding in the subcutaneous tissue around the PLA materials. Normalization of hepatic cytolysis markers (ALT and AST activity) in the blood without pronounced changes in the structure of the liver and the number of binuclear hepatocytes was noted. These markers were increased in SO controls (ALT up to 123% and AST up to 142%, $p < 0.001$ compared with values in the intact group).

Conclusion. Nonwoven PLA matrices are biocompatible with subcutaneous tissue, undergo bioresorption by MNGCs, and have a distant protective effect on the functional state of the liver in laboratory animals. Hypotheses on the detected systemic effect during subcutaneous implantation of PLA matrices were discussed; however, specific mechanisms require further study.

Keywords: nonwoven PLA matrix, rats, blood serum, alanine transaminase, aspartate transaminase, blood cells, binuclear hepatocytes

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Местная биосовместимость и биохимические маркеры цитолиза гепатоцитов при подкожной имплантации полилактидных матриц

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РЕЗЮМЕ

Цель. Исследование местной биосовместимости и системных эффектов нетканых полилактидных (PLA) матриксов на показатели крови и печени после подкожной имплантации крысам стока Wistar.

Материалы и методы. Биодеградируемые волокнистые PLA матриксы изготовлены методом электроспиннинга, имели размеры (10 × 10 мм^2, толщина не более 0,5 мм; диаметр волокон в матриксе ~1 мкм), пригодные для подкожного введения белым лабораторным крысам. Полимерные изделия стерилизовали в парах этиленоксида. Через 30 сут после имплантации PLA матриксов изучены местная биосовместимость согласно ГОСТ ISO 10993-6-2011, клеточные (общее количество лейкоцитов, гемограмма, число эритроцитов, концентрация гемоглобина) и биохимические показатели крови (концентрация лактата, активность аланинаминотрансферазы (АЛТ) и аспартатаминотрансферазы (АСТ)), определена стандартная гистологическая оценка состояния печени. Проведены компьютерная морфометрия цифровых изображений гистологических срезов и статистическая обработка результатов.

Результаты. Образцы PLA матрикса являлись легкими местными раздражителями в шкале 1–1,9 балла согласно критериям ГОСТ ISO 10993-6-2011 через 30 сут после подкожной имплантации. Медианная плотность распределения гигантских многоядерных клеток инородных тел (ГМКИТ) в соединительной ткани вокруг и в структуре PLA матриксов составила 1 500 (1 350; 1 550) на 1 мм^2 среза. Имела место выраженная лейкоцитарная реакция крови, обусловленная лимфоцитозом (в 1,7 раза по сравнению с ложнооперированным (ЛО) контролем, $p < 0,02$). Отсутствие значительного нейтрофилеза крови свидетельствовало об асептическом характере воспаления, протекающего в подкожной клетчатке вокруг PLA материалов. В крови отмечена нормализация маркеров цитолиза гепатоцитов (активности АЛТ и АСТ), повышенных у ЛО животных (АЛТ – до 123% и АСТ – до 142%, $p < 0,001$ в сравнении с интактными значениями), без выраженных изменений структуры печени и числа двуядерных гепатоцитов.

Заключение. Нетканые PLA матриксы биосовместимы с подкожной клетчаткой, подвергаются биорезорбции ГМКИТ, обладают дистантным протекторным действием на функциональное состояние печени у лабораторных животных. Обсуждены гипотезы обнаруженного системного эффекта при подкожной имплантации PLA матриц. Однако конкретные механизмы требуют дальнейшего изучения.

Ключевые слова: нетканый матрикс из полимолочной кислоты, крысы, сыворотка крови, аланинаминотрансфераза, аспартатаминотрансфераза, клетки крови, двуядерные гепатоциты

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

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INTRODUCTION

Polylactic acid (polylactide, PLA) is one of the synthetic biodegradable polymers consisting of analogs of natural monomers. It is actively used in various applications of tissue engineering [1] as implants [2], as well as in the form of matrices for drug and cell delivery [3].

PLA materials are approved for clinical use. The surface of nonwoven fibrous matrices makes it possible to use various methods of loading and release of significant concentrations of drugs and biological

molecules [4]. However, the accumulation of lactate, which depends on the rate of PLA degradation, can provoke local inflammation and/or systemic toxicity [5].

Electrospinning is a fast growing technology for obtaining nonwoven fibrous scaffolds made of nanosized interconnected fibers (5 nm – 1 μm in diameter) that form microsized (~100 μm) interconnected pores [6, 7]. Thereby their architecture allows to reproduce to a certain extent the structure of the natural extracellular matrix in various biological tissues. On the other hand, a large surface area at low density [6] promotes increased biodegradation of fibrous PLA materials [8] with the release of high doses of lactic acid, which can result in adverse events masking or eliminating the therapeutic effect of cells and drug and biological molecules immobilized on the implant.

Therefore, the aim of the study was to research local biocompatibility and systemic effects of nonwoven PLA matrices on blood and liver parameters after subcutaneous implantation in Wistar rats.

MATERIALS AND METHODS

The experimental study was conducted *in vivo* on 20 mature white male Wistar rats weighing 280–300 g. The animals were kept in standard vivarium conditions in the Laboratory of Biological Models at Siberian State Medical University (Tomsk) and received a standard diet. The study was carried out in compliance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Nonwoven PLA matrices (10 × 10 mm^2, thickness of no more than 0.5 mm; average fiber diameter in the matrix ~1 μm) obtained in Tomsk Polytechnic University using electrospinning as described earlier [8] were used as test products. The raw material was poly(DL-lactide) PURASORB (Corbion, Netherlands). Nonwoven PLA matrices were sterilized in 100% ethylene oxide vapor at 37 ˚C for 9 hours in the 3M Steri-Vac Sterilizer/Aerator gas sterilizer (3M, USA) according to ISO 11135-2017 (Sterilization of healthcare products. Ethylene oxide. Requirements for the development, validation, and routine control of a sterilization process for medical devices).

PLA matrices were injected subcutaneously through a median abdominal incision and a formed lateral subcutaneous pocket in 10 rats under CO_2 anesthesia (1 matrix per animal) as described earlier [9]. After placing the samples in the axillary pocket, interrupted atraumatic sutures (thread 4.0) were applied. The skin around the sutures was treated with an aseptic agent. The control group consisted of 10 sham-operated rats. Sham surgery involved a median skin incision, via which forceps were inserted subcutaneously; a lateral pocket was formed, and then the wound was closed without implantation of a matrix. Blood biochemistry tests were also conducted in 10 intact animals.

Thirty days after the implantation, the animals were euthanized by carbon dioxide inhalation in compliance with the rules and norms of the European Community (86/609EEC), the Declaration of Helsinki, and orders of the USSR Ministry of Healthcare (No. 742 of 13.11.1984 and No. 48 of 23.01.1985).

Blood was collected from decapitated animals in Vacuette blood collection tubes (BD Diagnostics, USA) to obtain blood serum. We determined lactate concentrations and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels using kits for the Random Access A25 Biochemistry Analyzer (BioSystems S.A., Spain) according to the manufacturer's instructions. Blood parameters (total leukocyte count (TLC), hemogram, erythrocyte count, hemoglobin) were examined using standard hematologic methods [10].

In accordance with ISO 10993-6-2011 (Medical devices. Biological evaluation of medical devices. Part 6. Tests for local effects after implantation), we conducted a macroscopic assessment of subcutaneous soft tissue surrounding the implantation site for the local tissue reaction (local biocompatibility *in vivo*): the presence or absence of signs of inflammation (alteration, exudation, proliferation), hyperemia of vessels in the recipient bed, and encapsulation of samples. The results of the semi-quantitative macroscopic assessment of the local reaction to PLA matrices were recorded on the following scale (in points): no irritating effect (0–0.9 points); mild irritant (1–1.9 points); moderate irritant (2–2.9 points); strong irritant (3–4 points).

For the microscopic analysis, the implants with surrounding tissues were carefully removed from the subcutaneous pocket; after opening the abdominal cavity, a part of the liver was taken for the histologic assessment after subcutaneous implantation of PLA matrices. The analyzed fragments were fixed in 10% neutral buffered formalin. The fragments were dehydrated in eight changes of isopropanol-based dehydrating solution (IsoPrep, BioVitrum, Russia) according to the manufacturer's instruction. The

studied objects were placed in the Histomix paraffin medium (BioVitrum, Russia), thin (5–7 μm) sections were prepared on a microtome perpendicular to the surface of the tissue plates and the studied samples.

Slices mounted on slides were stained with Gill's hematoxylin (BioVitrum, Russia) and eosin for histologic examinations under standard conditions. A total of 10 serial sections were obtained from each tissue sample. Then they were stained and examined. Microslides were studied in transmitted light of the ZEISS Axio Observer A1 microscope (Germany) at various magnifications (40–400). Digital images of the stained histologic sections were obtained using the AxioVision 4.8 software (ZEISS, Germany). We determined the intensity of cellular resorption of PLA matrices (based on the number of multinucleated foreign body giant cells, FBGCs) and the activity of regenerative processes in the liver (measured by the number of binuclear hepatocytes) in 10 randomly selected fields of vision in each histologic sample using computer morphometry of sections stained with hematoxylin and eosin, as described in the literature [11].

The standard Statistica software package v.13.3 was used for statistical description and testing of statistical hypotheses in order to evaluate the data obtained. We used the Shapiro – Wilk test to analyze normally distributed data. The data were presented as the median and the interquartile range *Me* (Q_1; Q_3). The nonparametric Mann – Whitney test was used to evaluate statistical differences in the samples. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In addition to their principal function, implants can activate local regenerative processes, cause a systemic reaction of the body mediated through the circulatory system. At the same time, biodegradable materials (for example, polylactides) can affect the body with the properties of their surface and biodegradation and bioresorption products [5]. The study of the local biocompatibility of PLA matrices established that there were no macroscopic signs of inflammation (alteration, exudation) in the tissues surrounding the implants 30 days after the subcutaneous implantation of the tested samples (Fig. 1). The severity of hyperemia in the recipient vascular bed and encapsulation of the samples (cell proliferation reaction) in the study groups corresponded mainly to the absence of irritation (0 points), mild irritation (1 point), and in a few cases (with encapsulation) – to moderate irritation (2 points) (Table 1). Thus, the studied PLA matrix samples were mild irritants on a scale of 1–1.9 points according to the criteria of ISO 10993-6-2011.

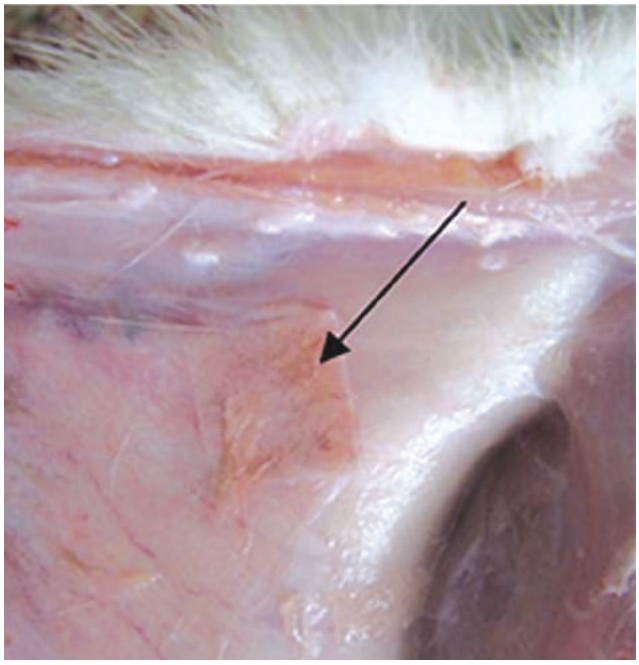


Fig. 1. The condition of the tissues surrounding the PLA matrix in the axillary subcutaneous pocket in rats 30 days after the implantation

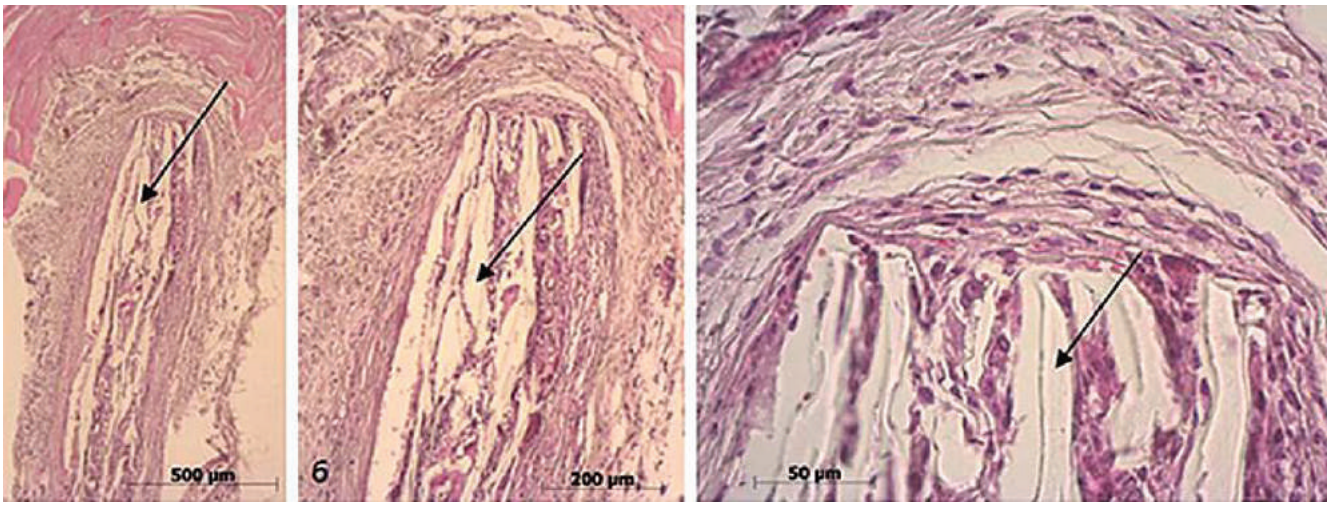
Table 1

Macroscopic signs of changes in the tissues surrounding the PLA matrix 30 days after the subcutaneous implantation in Wistar rats, *Me* (Q_1; Q_3)

Study groups	Inflammation	Hyperemia around the sample (postoperative scar)	Matrix encapsulation
Sham surgery, $n = 10$	0 (0; 0)	0 (0; 1)	–
Implantation of PLA matrix, $n = 10$	0 (0; 0)	1 (1; 1.5)	1.5 (1; 1.5)

The presence of a well-formed connective tissue capsule around the implants was revealed during the examination of microscopic changes (Fig. 2). In the capsule, two layers could be well identified: a thin (thickness of no more than 50 μm) inner layer in contact with the PLA matrix formed by dense fibrous connective tissue. Encapsulation with a thin layer of connective tissue is characteristic of relatively bioinert artificial materials [5]. Thin collagen fiber bundles were parallel to the implant surface and grew into the implant structure, mainly from the edge of the tested samples. The second layer of the capsule was located outside the previous one. It directly contacted

the surrounding tissues and was formed by loose connective tissue with microvessels. Collagen fiber bundles diverged in different directions, regardless of the implant surface. Both fibroblasts and inflammatory cells (polymorphonuclear leukocytes, lymphocytes, and macrophages) were found between them.

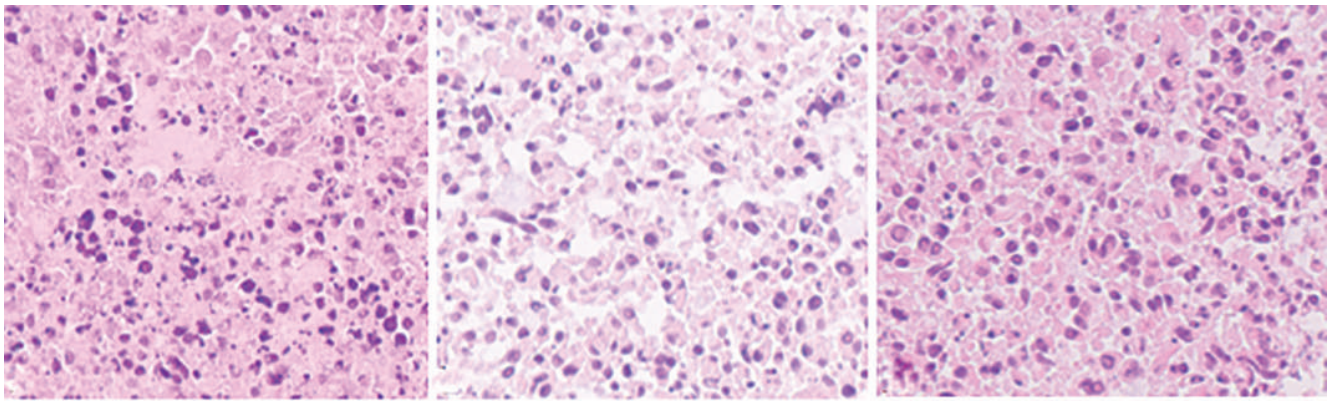


a *b* *c*

Fig. 2. The condition of the connective tissue capsule around and in the structure of the PLA scaffold at different magnifications (*a* – 50; *b* – 100; *c* – 400). Staining with hematoxylin and eosin. The arrows indicate the transparent substance of the polymer scaffold

The cellular composition of the connective tissue was represented, in particular, by FBGCs (Fig. 3). FBGCs were often found inside the implant cavity and were located separately (Fig. 3*a*) or in small clusters of up to three cells (Fig. 3*b*). According to computer morphometry data, the median distribution density of FBGCs in the connective tissue around and in the structure of PLA matrices was 1,500 (1,350; 1,550) per 1 mm^2 of the slice. The formation and accumulation of FBGCs at the site of implantation with a high surface-area-to-volume ratio is a typical reaction of the host organism that indicates active cellular resorption of the matrix substance [5].

Fibrocytes and fibroblasts were located between collagen fiber bundles (Fig. 3*c*). Mononuclear (lymphocytes, macrophages) and polymorphonuclear leukocytes (PMNs) diffused, but formed clusters near FBGCs (Fig. 3*a, b*).



a *b* *c*

Fig. 3. Tissue response to PLA matrix 30 days after the subcutaneous implantation in Wistar rats: *a, b* – mononuclear and polymorphonuclear leukocytes, multinucleated FBGCs (marked with arrows); *c* – fibroblasts between the polymer fibers of the implant. Stained with hematoxylin and eosin

Thus, the local reaction to PLA matrices 30 days after the subcutaneous implantation in rats apparently reflected the transition from acute to chronic productive inflammation with the change of leukocytes infiltrating the damaged area from PMNs to mononuclear forms. After the catabolic phase, which promotes rehabilitation of the inflammatory focus, anabolic processes are activated [12], as secretion of proinflammatory cytokines stops and secretion of anti-inflammatory / regenerative factors begins [13–15].

On the one hand, the formation and accumulation of FBGCs at the site of implantation demonstrated pronounced cellular resorption of PLA matrices. At the same time, they are crucial for the development of a local granulomatous inflammatory reaction to the implant, mediated primarily through the tumor necrosis factor (TNF)α secreted by them [5]. TNFα is a systemic cytokine that induces the expression of epidermal growth factor receptors (EGFR) [16] and activates the production of growth hormone (GH) by cells of the adenohypophysis [17]. In turn, EGFR and GH have an anabolic effect and enhance regenerative processes in organs and tissues [18, 19].

On the other hand, the utilization of the PLA matrix is accompanied by the release of lactic acid, which can have a systemic effect on the body. The liver and the kidneys are target organs for lactate circulating in the bloodstream [20]. Therefore, biochemical blood parameters are assessed to determine the biological safety of polymer scaffold biodegradation products and the capacity of the liver.

The polylactic base of the tested materials could lead to an increase in lactate monomers in the blood of animals in case of massive biodegradation. However, statistically significant changes in the concentration of lactic acid in the blood were not observed 30 days after the implantation (Table 1). A fivefold increase in *in vitro* dissolution of the tested PLA matrices was noted by day 35 of immersion into the nutrient medium [8]. Cellular resorption of the implant can accelerate its destruction *in vivo*, however, the distribution density of FBGCs in the fibrous matrix was insignificant (Fig. 3). We cannot exclude the influence of implants and their biodegradation products on liver function, mediated through distant (stress-regulating) regulatory systems of the body.

It is known that subcutaneous implantation induces a stress reaction in animals [21], which is realized through local (cellular microenvironment) and distant life support systems of the body [19]. In our study, the activity of AST and ALT in the blood of intact Wistar rats (Table 2) was close to that in other vivaria [22]. The sham operation was accompanied by an increase in the activity of ALT (up to 123%) and AST (up to 142%, $p < 0.001$) in the blood serum compared with the values in intact animals 30 days after the surgery. These functional tests suggest damage to the liver parenchyma [23] caused by postoperative stress. At the same time, subcutaneous implantation of the PLA matrix led to normalization of hepatocyte cytolysis markers (almost to the baseline level), which indicated the distant hepatoprotective effect of the PLA material during surgery (Table 2).

Table 2

Biochemical blood parameters in Wistar rats 30 days after the subcutaneous implantation of PLA matrices, *Me* (Q_1; Q_3)

Group	Lactate, mM	ALT, UI / l	AST, UI / l
Intact animals, $n = 10$	–	67.14 (60.47; 69.59)	185.14 (174.16; 216.07)
Sham-operated animals, $n = 10$	4.93 (4.5; 5.71)	82.58 (75.61; 84.36)* $p < 0.001$	262.90 (246.60; 304.60)* $p < 0.001$
Implantation of PLA matrix, $n = 10$	4.28 (3.50; 5.40)	67.34 (65.61; 72.95)# $p < 0.003$	225.10 (209.10; 250.0)# $p < 0.02$

* with the baseline level (intact animals); # with sham-operated rats.

Having obtained data on the functional changes, we were to study the effect of PLA matrices on the liver structure, since subcutaneous implantation of scaffolds can stimulate hepatocyte regeneration [24]. The number of binuclear hepatocytes (dividing and non-dividing) is one of the principal indicators of liver regeneration. Slow subthreshold loss of hepatocytes is not compensated by the body when the loss becomes critical (10% of working hepatocytes for the liver), which leads to irreversible processes and death of the body due to liver failure [25].

During the examination of the histologic preparations of the liver in sham-operated (SO) rats, we observed moderate hyperemia of the central veins and sinusoidal capillaries, while the structure and shape of the classic hepatic lobules were preserved. Subcutaneous implantation of PLA matrices did not cause visible destructive changes in the liver by day 30 of the follow-up (Fig. 4). However, the increase in the number of binuclear hepatocytes (Fig. 4) did not reach statistical differences in comparison with the controls (Table 2).

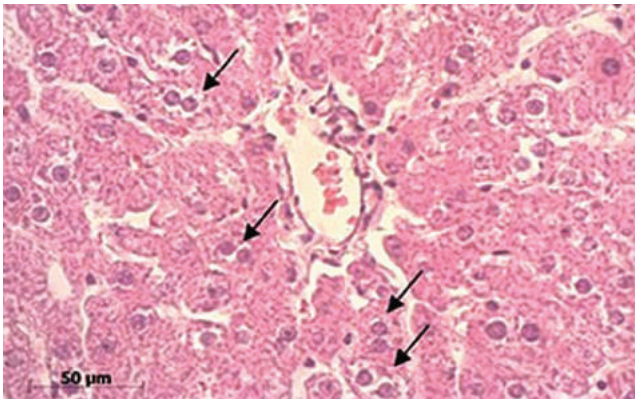


Fig. 4. Histologic image of the liver of Wistar rats 30 days after the subcutaneous implantation of PLA matrices: staining with hematoxylin and eosin; arrows indicate binuclear hepatocytes

Table 2

Distribution density of binuclear hepatocytes in Wistar rats 30 days after the subcutaneous implantation of PLA matrices, *Me* (Q_1; Q_3)

Group	Количество двуядерных клеток на 1 мм^2
SO animals, n = 10	4,600 (4,450; 4,800), n_1 = 100
Implantation of PLA matrix, n = 10	5,300 (4,700; 5,500), n_1 = 100

Note: n_1 is the number of calculated fields of vision.

According to our findings, subcutaneous implantation of PLA matrices has a functional hepatoprotective effect by day 30 after administration to laboratory rats. The mechanisms of the established phenomenon are not clear, but they are likely to be complex. Postoperative regeneration in subcutaneous adipose tissue may be important, as it leads to the release of EGFR and other growth factors activating liver regeneration into the blood [26]. In addition, lactate released during the degradation of PLA matrices can have a regulatory effect on migration and activity of T lymphocytes [27, 28], which are recruited as conductor cells of proliferative inflammation and subsequent regenerative processes in parenchymal organs [29].

Indeed, after subcutaneous implantation of PLA matrices, a pronounced leukemoid reaction was detected, due to an increase in the number of lymphocytes (by 1.7 times compared with the controls, $p < 0.02$) (Table 3). The absence of significant neutrophilia indicated sterile inflammation taking place in the subcutaneous tissue around the PLA material.

Table 3

Hemogram (10^9 / l), RBC count, and hemoglobin concentration (g / l) in the blood of Wistar rats 30 days after the subcutaneous implantation of PLA matrices, *Me* (Q_1; Q_3)

Group	Total WBC count	Lymphocytes	Monocytes	Neutrophils	Erythrocytes, 10^{12} / l	Hemoglobin, g / l
SO animals, n = 10	6.4 (4.6; 9.2)	3.6 (2.8; 6.1)	0.5 (0.2; 0.7)	2.5 (1.2; 2.8)	9.41 (7.93; 9.52)	146 (123; 147)
Implantation of PLA matrix, n = 10	8.7 (6.7; 13.3)	6.1* (5.2; 9.0) $p < 0.02$	0.4 (0.3; 0.6)	2.2 (1.3; 3.4)	9.43 (7.92; 9.94)	150 (148; 154)

* significant differences according to the Mann – Whitney test.

The systemic effect of PLA matrices on biochemical markers of hepatocyte cytolysis is largely similar to the effect of subcutaneous injection of hyaluronic acid implants in healthy women during cosmetic procedures [30]. Various chemical substances have a similar effect when injected subcutaneously, which suggests a non-specific mechanism of their long-term effect besides the specific impact of monomers (lactic or glucuronic acid). This mechanism may be linked to the controlled onset of low-grade local productive inflammation, which activates the compensatory – adaptive and adaptive reactions of the host organism.

CONCLUSION

Biodegradable scaffolds based on PLA and copolymers are of fundamental and clinical interest for soft and solid tissue bioengineering [31, 32], including the liver [33]. These scaffolds can be used as carriers of stem cells [34] and as a means of delivery and release of drugs and biological molecules [35].

According to the data obtained, bioabsorbable nonwoven PLA matrices are biocompatible with subcutaneous tissue and have a distant protective effect on the capacity of the liver in laboratory animals. Nonwoven PLA matrices can be used as cell carriers. The areas of application may include treating damage induced by cytostatic agents, radiation injuries, and chronic liver diseases. However, the specific mechanisms underlying the revealed effect that subcutaneous implantation of PLA matrices had on hepatocytes require further study.

REFERENCES

1. Jafari M., Paknejad Z., Rad M.R., Motamedian S.R., Eghbal M.J., Nadjmi N. et al. Polymeric scaffolds in tissue

engineering: a literature review. *J. Biomed. Mater. Res. B Appl. Biomater.* 2017;105(2):431–459. DOI: 10.1002/jbm.b.33547.
2. Jukkala-Partio K., Laitinen O., Vasenius J., Partio E.K., Toivonen T., Tervahartiala P. et al. Healing of subcapital femoral osteotomies fixed with self-reinforced poly-L-lactide screws: an experimental long-term study in sheep. *Arch. Orthop. Trauma Surg*. 2002;122(6):360–364. DOI: 10.1007/s00402-001-0379-y.
3. Zhou J., Han S., Dou Y., Lu J., Wang C., He H. et al. Nanostructured poly(L-lactide) matrix as novel platform for drug delivery. *Int. J. Pharm*. 2013;448(1):175–188. DOI: 10.1016/j.ijpharm.2013.03.038.
4. Wulf K., Arbeiter D., Matschegewski C., Teske M., Huling J., Schmitz K.P. et al. Smart releasing electrospun nanofibers-poly: L. lactide fibers as dual drug delivery system for biomedical application. *Biomed. Mater*. 2020;16(1):015022. DOI: 10.1088/1748-605X/abbec8.
5. Biomaterials science: an introduction to materials in medicine; 2nd ed.; ed. by B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons. San Diego: Elsevier Academic Press; 2004:851.
6. Do A.V., Khorsand B., Geary S.M., Salem A.K. 3D printing of scaffolds for tissue regeneration applications. *Adv. Healthc. Mater.* 2015;4(12):1742–1762. DOI: 10.1002/adhm.201500168.
7. Daristotle J.L., Behrens A.M., Sandler A.D., Kofinas P.A Review of the Fundamental Principles and Applications of Solution Blow Spinning. *ACS Appl. Mater. Interfaces*. 2016;8(51):34951–34963. DOI: 10.1021/acsami.6b12994.
8. Tverdokhlebov S.I., Stankevich K.S., Bolbasov E.N., Khlusov I.A., Kulagina I.V., Zaytsev K.V. Nonwoven polylactide scaffolds obtained by solution blow spinning and the *in vitro* degradation dynamics. *Advanced Materials Research*. 2014;872:257–263.
9. Bolbasov E.N., Popkov A.V., Popkov D.A., Gorbach E.N., Khlusov I.A., Golovkin A.S.et al. Osteoinductive composite coatings for flexible intramedullary nails. *Mater Sci. Eng. C Mater. Biol. Appl.* 2017;75:207–220. DOI: 10.1016/j.msec.2017.02.073.
10. Menshikov V.V., Delektorskaya L.N., Zolotnitskaya R.P., et al. Laboratory methods of research in the clinic: handbook; ed. by V.V. Menshikov. Moscow: 1987:368 (in Russ.).
11. Avtandilov G.G. Diagnostic medical ploidometry. Moscow: Medicine; 2006:192 (in Russ.).
12. Clark D., Nakamura M., Miclau T., Marcucio R. Effects of aging on fracture healing. Curr. Osteoporos Rep. 2017;15(6):601–608. DOI: 10.1007/s11914-017-0413-9.
13. Loi F., Córdova L.A., Pajarinen J., Lin T.H., Yao Z., Goodman S.B. Inflammation, fracture and bone repair. Bone. 2016;86:119–130. DOI: 10.1016/j.bone.2016.02.020.
14. Yurova K.A., Khaziakhmatova O.G., Malashchenko V.V., Norkin I.K., Ivanov P.A., Khlusov I.A., et al. The role of γC cytokines (IL-2, IL-7, AND IL-15) in regulation of activation-induced cell death of memory T cells. A brief review. *Cell and Tissue Biology*. 2020;62(5):305–315 (in Russ.). DOI: 10.31857/S0041377120050090.
15. ElHawary H., Baradaran A., Abi-Rafeh J., Vorstenbosch J., Xu L., Efanov J.I. Bone healing and inflammation: Principles of fracture and repair. Semin. Plast. Surg. 2021;35(3):198–203. DOI: 10.1055/s-0041-1732334.
16. Fayers W. Biological methods of treatment with tumor necrosis factor: preclinical studies. In Biologic therapy of cancer: translated from Engli-sh; edited by Vincent T. DeVita, Samuel Hellman, Steven A. Rosenberg. Moscow: Medicine; 2002:309–343 (in Russ.).
17. Milenkovic L., Rettori V., Snyder G.D., Beutler B., McCann S.M. Cachectin alters anterior pituitary hormone release by a direct action in vitro. *Proc. Natl. Acad. Sci. U S A.* 1989;86(7):2418–2422. DOI: 10.1073/pnas.86.7.2418.
18. Hormone therapy: trans. from German; edited by H. Schambach, G. Knappe, W. Carol. Moscow: Medicine, 1988:416 (in Russ.).
19. Goldberg E.D., Dygai A.M., Udut V.V., Naumov S.A., Khlusov I.A. Regularities of the structural organization of life support systems in normal and in the development of the pathological process. Tomsk, 1996:283 (in Russ.).
20. Yang W.H., Park H., Grau M., Heine O. Decreased blood glucose and lactate: Is a useful indicator of recovery ability in athletes? *Int. J. Environ. Res. Public Health.* 2020;17(15):5470. DOI: 10.3390/ijerph17155470.
21. Selye H., Lemire Y., Bajusz E. Induction of bone, cartilage and hemopoietic tissue by subcutaneously implanted tissue diaphragms. Wilhelm Roux Arch. Entwickl. Mech. Org. 1960;151(5):572–585. DOI: 10.1007/BF00577813.
22. Voitenko N.G., Makarova M.N., Zueva A.A. Variability of blood biochemical parameters and establishment of reference intervals in nonclinical studies. Part 1: rats. *Laboratory animals for science.* 2020;(1):47–53 (in Russ.). DOI: 10.29296/2618723X-2020-01-06.
23. Lifshits V.M., Sidelnikova V.I. Biochemical analysis in the clinic: handbook. Moscow: MIA; 1998:303 (in Russ.).
24. Khlusov I.A., Khlusova M.Yu. Method of distant stimulation of hepatocyte regeneration: RF Patent for invention No. 2590859 of 15.06.2016 (published 10.07.2016, Issue No. 19) (in Russ.).
25. Severin M.V., Yushkov B.G., Yastrebov A.P. Tissue regeneration under extreme effects on the body. Yekaterinburg: USMI, 1993:187 (in Russ.).
26. Bader A. Method of tissue regeneration: RF Patent for invention No. 2392314 of 20.06.2010 (in Russ.).
27. Haas R., Smith J., Rocher-Ros V., Nadkarni S., Montero-Melendez T., D'Acquisto F. et al. Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PLoS Biol.* 2015;13(7):e1002202. DOI: 10.1371/journal.pbio.1002202.
28. Pucino V., Bombardieri M., Pitzalis C., Mauro C. Lactate at the crossroads of metabolism, inflammation, and autoimmunity. *Eur. J. Immunol.* 2017;47(1):14–21. DOI: 10.1002/eji.201646477.
29. Babaeva A.G. Regeneration and immunogenesis system. Moscow: Medicine; 1985:256 (in Russ.).
30. Khlusov I.A., Igumnov V.A., Chukhnova D.L., Mitasova O.L., Zaitsev K.V., Abdulkina N.G., et al. Local and system effects of hyaluronic acid implants in conditions of corrections of age-related changes in skin. *Bulletin of Siberian Medicine*. 2013;12(1):61–68 (in Russ.).
31. Grémare A., Guduric V., Bareille R., Heroguez V., Latour S., L'heureux N. et al. Characterization of printed PLA scaf-

folds for bone tissue engineering. *J. Biomed. Mater. Res. A.* 2018;106(4):887–894. DOI: 10.1002/jbm.a.36289.
32. Gangolphe L., Leon-Valdivieso C.Y., Nottelet B., Déjean S., Bethry A., Pinese C. et al. Electrospun microstructured PLA-based scaffolds featuring relevant anisotropic, mechanical and degradation characteristics for soft tissue engineering. *Mater Sci. Eng. C Mater. Biol. Appl.* 2021;129:112339. DOI: 10.1016/j.msec.2021.112339.
33. Wang M., Pei H., Zhang L., Guan L., Zhang R., Jia Y. et al. Hepatogenesis of adipose-derived stem cells on poly-lactide-co-glycolide scaffolds: in vitro and in vivo studies. *Tissue Eng. Part C Methods.* 2010;16(5):1041–1050. DOI: 10.1089/ten.TEC.2009.0244.
34. Diomede F., Gugliandolo A., Cardelli P., Merciaro I., Ettorre V., Traini T. et al. Three-dimensional printed PLA scaffold and human gingival stem cell-derived extracellular vesicles: A new tool for bone defect repair. *Stem. Cell Res. Ther.* 2018;9(1):104. DOI: 10.1186/s13287-018-0850-0.
35. Eğri S., Eczacıoğlu N. Sequential VEGF and BMP-2 releasing PLA-PEG-PLA scaffolds for bone tissue engineering: I. Design and in vitro tests. *Artif. Cells Nanomed. Biotechnol.* 2017;45(2):321–329. DOI: 10.3109/21691401.2016.1147454.

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