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INFLUENCE OF D-ASPARAGINE ON THE ENGRAFTMENT OF A BIOPOLYMER SCAFFOLD FOR REPLACING SKIN DEFECTS

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ВЛИЯНИЕ D-АСПАРАГИНА НА ПРИЖИВЛЕНИЕ БИОПОЛИМЕРНОГО СКАФФОЛДА ПРИ ЗАМЕЩЕНИИ ДЕФЕКТОВ КОЖИ

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The influence of D-asparagine on the engraftment of a biopolymer scaffold, the main components of which were sodium alginate and gelatin, for replacing skin defects after necrectomy of burn wounds was investigated in an experimental rat model. The results of morphometric and fractal analyses of histological micro-preparations showed that the inclusion of D-asparagine in the composition of the scaffold had a positive effect on its engraftment and remodeling on day 30 after implantation. The study showed that the proposed scaffold is a promising material for replacing the skin in burn wounds.

Keywords: D-asparagine, biopolymer scaffold, skin, burn, fractal analysis

Изучено влияние D-аспарагина на приживление биополимерного скаффолда для замещения дефектов кожи после некрэктомии ожоговых ран в эксперименте у крыс. По результатам морфометрического и фрактального анализа гистологических микропрепаратов показано, что включение D-аспарагина в состав скаффолда, основными компонентами которого являются альгинат натрия и желатин, оказывает положительное влияние на его приживление и ремоделирование на 30-е сутки после имплантации.

Ключевые слова: D-аспарагин, биополимерный скаффолд, кожа, ожог, фрактальный анализ

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Early necrectomy with skin autograft primary autoplasty is the method of choice in the treatment of burn injury patients [1, 2]. However, questions remain regarding the quality of skin healing, the formation of dermal scars, and the lack of donor material in patients with extensive grade III burns [1, 3]. In this regard, the development of biodegradable hydrogel scaffolds (based on biopolymers of natural origin, such as sodium alginate and gelatin) capable of performing the role of wound coating and regenerative matrix is an actual and promising approach in solving of these problems [4, 5].

Under experimental conditions, there is significant potential for influencing the dynamics of cellular reactions, the nature of colonization, and remodeling of the scaffold after its implantation by using modulators of the proliferative activity of dermal matrix cells, including those based on a natural metabolite such as D-asparagine [6–8], which expands the possibilities for studying it in burn injury models.

The aim of the study was to determine the effect of D-asparagine on the engraftment of a biopolymer scaffold in the replacement of skin defects after burn wounds necrectomy in rats.

Material and Methods. Twenty inbred male Wistar rats weighing 270–300 g were used in this study. All interventions capable of causing pain or suffering to animals were performed under anesthesia using 20 mg/kg tiletamine hydrochloride (Zoetis Inc., USA) and 6 mg/kg xylazine hydrochloride (NITA-PHARM, Russia), both applied intramuscularly. When performing traumatic manipulations with an antimicrobial purpose, animals were injected with 2.5 mg/kg gentamicin sulfate (Agrofarm, Russia) intramuscularly.

Modeling of skin burns was performed according to the method of Cai et al. [9], by lowering a heating element with a contact surface area of 1 cm^2, a working zone temperature of 100°C, and a contact time of 20 seconds on the shaved surface of the skin. The temperature of the contact surface was controlled using a thermocouple. Under these conditions, damage to all layers of the skin was achieved, which corresponds to a grade IIIB burn.

One day after the burn, animals were randomized to two groups each with 10 rats: N1 (comparison group) with simultaneous implantation after scaffold necrectomy without D-asparagine; N2 (experimental) with simultaneous implantation after scaffold necrectomy with D-asparagine. Wound closure after scaffold implantation was performed using a self-adhesive sterile Cosmopor E dressing and sewn to the edges of the wound.

The hydrogel was prepared under aseptic conditions, based on the following components: sodium alginate – 2.6 g; gelatin type A – 0.2 g; D-asparagine (only in N2) – 0.013 g, vinyltriethoxysilane – 0.07 g; DMEM/F-12 culture medium powder (without L-glutamine, L-leucine, L-lysine, L-methionine, calcium, magnesium chloride, magnesium sulfate, or phenolic red) – 1.48 g, and the rest highly purified water up to 100 ml per mixture. The resulting liquid was poured into Petri dishes to form a 3-mm layer and cooled to +4 °C. To polymerize the mixture, 10 ml of a solution containing 3 % aqueous hydrogen peroxide and 0.1 g calcium gluconate powder was carefully layered on top of the cooled mixture. The resulting mixture was frozen at –25 °C, and after defrosting and washing, the scaffold was ready for use.

On day 30 after implantation, a skin area of 15×15 mm^2 was taken, including the border of the scaffold area with intact tissue. The biomaterial was subjected to fixation in a zinc-formalin fixator with zinc sulfate, followed by embedding in paraffin using isopropanol and mineral oil. Histological staining of micro-preparations obtained from paraffin blocks with a thickness of 4 μm was performed according to the protocol for hematoxylin-eosin, as well as according to Mallory-Slinchenko.

Microscopy was performed using a Micmed-5 microscope (Lomo, Russia) and images were obtained using a Levenhuk M300 Base ocular camera (Levenhuk, Tampa, FL, USA). Morphometric analysis of images was performed using the program «Image J1. 51j8» (NIH, USA).

For fractal analysis of the fibrillar structure of the extracellular matrix, images were converted to binary form, skeletonized, and evaluated using the FracLac 2.5 plugin (NIH) for «Image J1.51j8». The «box-counting method» was used to determine the value of the fractal dimension and lacunarity [10].

Statistical processing was performed using the program «Statistica13» (StatSoft Inc., USA). When using the Shapiro-Wilk test, an alternative hypothesis was adopted about the deviation from the normal distribution of features in the studied groups, and, therefore, nonparametric statistics were used. The results of the descriptive part of the analysis are presented using the median (Me) and interquartile range (Q1–Q3). Binary data were described with absolute values and percentages. To analyze the conjugacy tables of nominal features, the Fisher's exact test was applied. The Mann-Whitney test was used to assess the intergroup differences between the two independent groups. In all cases, at $p<0.05$, the null hypothesis of the absence of intergroup differences was rejected.

Results and Discussion. Visual assessment of wound defect healing showed in the experimental group that the epithelialization completion time was 21.5 (19–23) days, which was 7.5 % longer than in the comparison group of 20 (18.5–21) days (p=0.80). This trend may be due to a degree of slowdown in the proliferative activity of cells under the influence of D-asparagine during the initial period after scaffold implantation. This hypothesis is supported by the fact that *in vitro* D-asparagine reduced the proliferative activity of dermal fibroblasts during a 48-hour incubation [6].

Morphological analysis of skin micro-preparations indicated the completion of the formation of a continuous layer of the epidermis by day 30 after scaffold implantation in both groups (Fig. A, B). At this time, in group N1, the epidermis was thinner with a rough disorganization of its layers, an indistinct border of the transition of the epidermis to the dermis, and the absence of dermal papillae. The papillary and reticular layers of the dermis were practically undifferentiated, while in the area of the basal and spiny epidermal layers, diffuse spongiosis and intracellular edema of cells with the formation of many small subepidermal blisters were observed. In the dermis, there were pronounced signs of inflammatory infiltration with numerous giant and epithelioid cells and a large number of large, variable-diameter vessels (Fig. A). It was important that the micro-preparations clearly traced the cellular structure of the scaffold in all the studied areas, which is typical for the implantation of «cell-free» alginate-gelatin scaffolds [4].

In the experimental N2 group, the thickness of the epidermis by the end of the experiment of 11 (10–15) μm was 45.5 % greater than in the comparison group of 6 (5–7.8) μm (p=0.002). The differentiation of the epidermis into basal, spiny, and granular layers was observed in the micro-preparations of the N2 animals. In the basal and spiny layers, moderate focal spongiosis with small subepidermal blisters (possibly scaffold cells) was detected (Fig. B). In contrast to the comparison group, in the experimental group, the transition of the epidermis to the dermis was clearly traced, single dermal papillae were visualized (the cellular structure of the scaffold was visible), single giant and epithelioid cells were identified, and a moderate number of large, thin-walled vessels was observed, while there was no division into papillary or reticular layers of the dermis.

In the central zone at the site of the wound defect in all experimental animals, no hair follicles or sebaceous glands were detected. Single sebaceous glands and hair follicles were detected only on the periphery of the scaffold (Fig. C, D). In the N2 group, hair follicles were detected on 80 % of micro-preparations compared with 30 % in the comparison group (p=0.037). Sebaceous glands were detected on 80 % and 10 % of micro-preparations of the N2 and N1 groups, respectively (p=0.002).

Detailed study of micro-preparations of groups N1 (Fig. D) and N2 (Fig. E) revealed a tendency to remodel the structure of the fibrillar component of the extracellular matrix in the area of scaffold implantation, which was confirmed by fractal analysis. In the experimental group N2, the value of the fractal distribution (on a scale from 0 to 2 units) was 1.77 (1.73–1.83) units, which was 2.91 % more (p=0.042) than in the comparison group N1 of 1.72 (1.71–1.77) units. Additionally, the value of the lacunarity index in the experimental group N2 was 0.285 (0.200–0.300) units, which was 5 % less (p=0.281) than in the comparison group N1 of 0.30 (0.270–0.327) units. These changes in the fibrillar component of the extracellular matrix in the area of implantation of the scaffold with D-asparagine may indicate a complication of the fractal structure and a decrease in the degree of its disorganization, in relation to the comparison group [11].

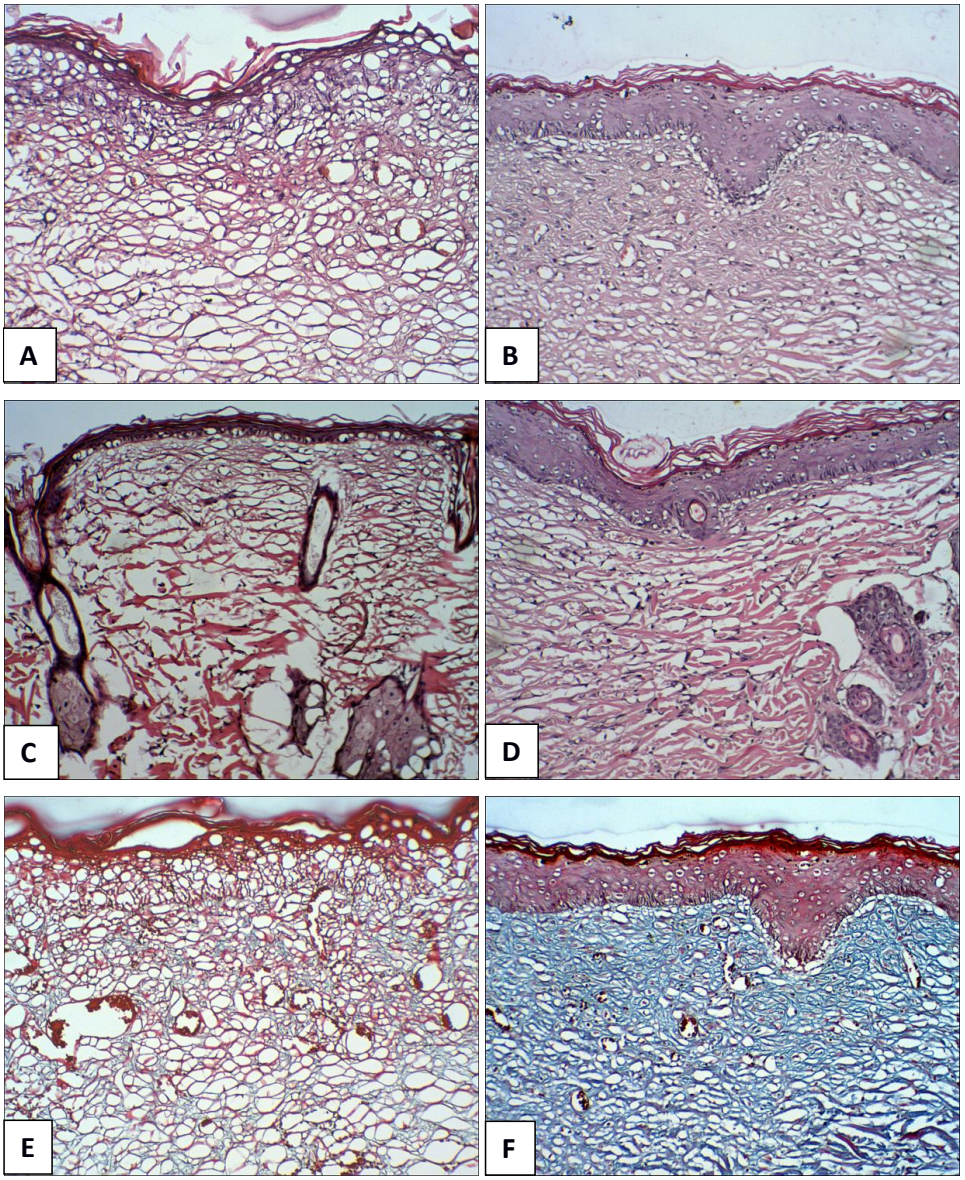

Fig. Biopolymer scaffold on the 30th day after implantation: A and C – comparison group N1; B and D – experimental group N2; hematoxylin-eosin staining, x200 magnification; E – comparison group N1; F – experimental group N2, Mallory-Slinchenko staining, magnification x200

Conclusions. The study showed that the inclusion of D-asparagine in the composition of the scaffold, the main components of which were sodium alginate and gelatin, had a positive effect on its engraftment and remodeling. On day 30 after the scaffold implantation to close the area after necrectomy of burn wounds in rats, D-asparagine provided activation of the proliferative activity of cells, with differentiation of the epidermis. In addition, the study revealed that the proposed scaffold is a promising material for replacing the skin in burn wounds.

Experimental animal procedures. The study was performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (Strasbourg, 1986; ed. Strasbourg, 2006) and in accordance with international legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU). The maintenance and use of animals for experimental purposes was performed in accordance with the international rules «Guide for the Care and Use of Laboratory Animals – 8th edition, 2011». The study protocol was approved by the Local Ethical Committee.

Disclosures: The authors declare no conflict of interest.

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THE ROLE OF DYSMETABOLIC MECHANISMS IN THE DEVELOPMENT OF NEURODEGENERATIVE PROCESSES IN AN EXPERIMENTAL METABOLIC-COGNITIVE SYNDROME MODEL

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ИЗУЧЕНИЕ РОЛИ ДИСМЕТАБОЛИЧЕСКИХ МЕХАНИЗМОВ В РАЗВИТИИ НЕЙРОДЕГЕНЕРАТИВНЫХ ПРОЦЕССОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ МОДЕЛИРОВАНИИ МЕТАБОЛИКО-КОГНИТИВНОГО СИНДРОМА

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This article describes the role of dysmetabolic mechanisms in the development of neurodegenerative processes in an experimental animal model of metabolic-cognitive syndrome. Cognitive decline and neurodegenerative changes in experimental animals were revealed in the form of decreased cortical thickness in the temporal and parietal lobes of the brain. Administration of polyphenol preparations prevented morphological and functional changes in animals. Neuroprotection in individuals with metabolic syndrome can be individualized using drugs with different polyphenol compositions.

Keywords: neurodegeneration, metabolic syndrome, cognitive impairment, polyphenols

Описаны результаты изучения роли дисметаболических механизмов в развитии нейродегенеративных процессов при экспериментальном метаболико-когнитивном синдроме. Выявлены когнитивное снижение и нейродегенеративные изменения у экспериментальных животных в виде уменьшения толщины коры в височно-теменных долях головного мозга. При коррекции полифенольными препаратами наблюдалось нивелирование морфофункциональных из-