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## Morphological features of pathological skin scarring under the conditions of diabetes mellitus in different ways of wound closure

### ІНФОРМАЦІЯ

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**Key words:** oxidative stress; antioxidant protection; markers of inflammation; microbial contamination; nonspecific immunity; reparative ability; diabetes mellitus; surgical threads; skin glue; efficiency.

### АНОТАЦІЯ

**Summary.** Diabetes mellitus (DM) is recognized worldwide as one of the most important non-communicable diseases, as well as one of the most serious health problems of the 21st century. The need to prevent the formation of pathological scarring of tissues after surgery, injuries, burns was and remains an urgent scientific and practical task. Therefore, the optimization of methods of wound healing and prevention of pathological scars on the background of diabetes is of considerable scientific interest for practical medicine and dentistry.

**The aim of the study** – to find out the features of pathological scarring in experimental diabetes and to establish the effectiveness of preventive use of skin glue.

**Materials and Methods.** Experimental studies were performed on 130 white outbred adult male rats weighing from 240 to 320 g. The animals were kept in the vivarium of I. Horbachevskiy Ternopil National Medical University Ministry of Health of Ukraine, in accordance with sanitary and hygienic norms and requirements of GLP.

**Results and Discussion.** The results of biochemical studies obtained by us indicate an increase in the activity of free radical processes in the experimental wound process. However, different intensities of these reactions have been found in healthy and diabetic animals, in particular, with different methods of fixing wound edges.

**Conclusions.** The use of surgical glue to suture the edges of surgical wounds in animals with induced diabetes is characterized by less inflammatory response, faster epithelialization of the wound surface and the transformation of granulations into young connective tissue, as well as reducing scarring of the damaged area than using surgical sutures.

**Introduction.** Diabetes mellitus (DM) is recognized worldwide as one of the most important non-communicable diseases, as well as one of the most serious health problems of the 21st century (Andreeva VV et al., 2020; Liu J. et al., 2020). Over the last decade, the prevalence of diabetes has increased rapidly due to increasing average age, aggravated heredity, eating disorders, sedentary lifestyles and increasing body mass index in line with increasing urbanization (Zhang N. et al., 2017). In total, slightly less than half a billion people live with diabetes in the world, and their number is projected to increase by 25 % in 2030 and by 51 % in 2045 (Saeedi P. et al., 2019). In Ukraine in 2016,

the total number of patients with diabetes was 1 million 223 thousand 607 people; in 2019, 207,383 patients with diabetes were registered taking insulin preparations, of which 66.8 % were diagnosed with type 2 diabetes (Information Department of the Verkhovna Rada of Ukraine, 2016; Association of Innovative Medicines Manufacturers, Kyiv School of Economics, 2020). Thus, in Ukraine the number of patients with diabetes is about 3.0 % of the total population, which is less than in other European countries (in Germany – 10.2 %, Sweden – 7.3 %, France – 6.2 %, Spain – 9.9 %) (WHO, 2016).

Diabetes and its complications are one of the most important causes of death (Mirzaei M. et al.,

2020). According to some authors, the presence of diabetes is associated with increased mortality from infections, cardiovascular disease, stroke, chronic kidney disease, chronic liver disease and cancer (Bragg F. et al., 2017). As a consequence of hyperglycemia in patients with diabetes there is an increased risk of concomitant diseases (Maffi P., Secchi A., 2017), and one of the important consequences of diabetes is impaired ability to repair (Zhang P. et al., 2017). Healing disorders in diabetes are the result of complex pathophysiology involving vascular, neuropathic, immune and biochemical components (Spampinato S.F. et al., 2020). Hyperglycemia correlates with blood vessel stiffness, which leads to slow blood circulation and microvascular dysfunction, causing a decrease in tissue oxygenation (Dinh T., Elder S., Veves A., 2011). The changes in blood vessels observed in patients with diabetes also explain the decrease in the migration of leukocytes into the wound, which becomes more vulnerable to infections (Spampinato S.F. et al., 2020). Timely prevention of infectious complications provides an easier course of the wound process, much less likely to develop severe purulent-septic processes and scar formation (Strilchuk L., 2018).

The need to prevent the formation of pathological scarring of tissues after surgery, injuries, burns was and remains an urgent scientific and practical task (Bogomolova EB, 2017; Chernyakov AV, 2017; Gaidina TA et al., 2018; Ogawa R., Akaishi S., 2016; Ogawa R., 2017; Lee H.J., Yang Y.J., 2018; Grabowski G. et al., 2020). Despite the constant improvement of methods of prevention and tactics of postoperative management of patients, the formation of rough deforming scars is one of the serious problems of modern medicine. Scars are stored for life. Cosmetic and functional defects that occur cause psychological discomfort, disrupt the social adaptation of patients after injuries and operations, and significantly affect the quality of life of patients (Bock O. et al., 2016; Lee Peng G., Kerolus J.L., 2019).

Modern medicine includes a wide variety of invasive and non-invasive methods of treatment and prevention of pathological scars. There are publications in the literature on the influence of methods of fixing wound edges, suture material on scar formation (C. Dennis, S. Sethu, J. Nayak et al., 2016; Forsch, Randall & Little, Sahoko & Williams, Christa., 2017; F Selvi, S. Cakarar, T. Can et al., 2016). However, despite significant achievements, there is still a large number of unsatisfactory results of postoperative wound healing, in particular against the background of diabetes (Andreeva VV et al.,

2020; Ding Y., Cui L., Zhao Q., 2017; Zaitsev P. P. et al., 2019).

Therefore, the optimization of methods of wound healing and prevention of pathological scars on the background of diabetes is of considerable scientific interest for practical medicine and dentistry.

**The aim of the study** – to find out the features of pathological scarring in experimental diabetes and to establish the effectiveness of preventive use of skin glue.

**Materials and Methods.** Experimental studies were performed on 130 white outbred adult male rats weighing from 240 to 320 g. The animals were kept in the vivarium of I. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine, in accordance with sanitary and hygienic norms and requirements of GLP. All experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Ministry of Health of Ukraine No. 690 of 23.09.2009 The Commission on Bioethics of I. Gorbachevsky Ternopil National Medical University Ministry of Health of Ukraine violations of moral and ethical norms during the research work were not detected. All animals were divided into 4 experimental groups: healthy white rats, which were sutured to the surgical wound; healthy white rats, fixation of the edges of the surgical wound is carried out by applying skin glue; animals with diabetes mellitus, which are sutured to the surgical wound; animals with diabetes mellitus, fixation of the edges of the surgical wound is carried out by applying skin glue. For control, all the results were compared with those of intact animals.

Diabetes mellitus was simulated with streptozotocin (Sigma, USA) (intraperitoneally – 65 mg / kg) with previous (15 minutes) injection of nicotinamide (intraperitoneally – 230 mg / kg) on the background of obesity, which was caused by 4-week keeping of animals on a high-fat diet. The development of type 2 diabetes was confirmed by determining the concentration of fasting glucose in peripheral blood. High glucose levels persisted for 7 weeks.

Animals of all groups (I–IV) under thiopental anesthesia (40 mg/kg body weight of rats) were made full-layer rectilinear incisions 2 cm long in the anterior-lateral region of the abdomen. To fix the edges of the surgical wound, the animals of the I and III experimental groups were sutured with Vicryl

5/0 thread (ETHICON, Inc. and Johnson & Johnson company (USA). Fixation of the edges of the surgical wound in animals of the II and IV experimental group was performed by applying skin glue "Dermabond"(ETHICON, Inc. a Johnson & Johnson company (USA). Rats were removed from the experiment for 3, 7, 28 days after surgery under thiopental anesthesia (90 mg/kg body weight of rats).

Under the conditions of experimental diabetes mellitus, the use of skin glue is accompanied by a greater thickness of the epidermis of the skin of rats (30.9 %,  $p < 0.001$ ) in relation to the use of surgical sutures on the 7th day of the wound process. At day 28, the thickness of the epidermis under the conditions of application of surgical glue is probably not different from the data of the intact group, when using surgical threads, its thickness is less by 9.12 % ( $p < 0.05$ ); in the damaged area of the dermis after using skin glue is dominated by fibroblasts (15.38 %,  $p < 0.05$ ) and a lower number of leukocytes (43.63 %,  $p < 0.001$ ) relative to the data when using surgical sutures.

**Results and Discussion.** Under the conditions of induced diabetes, surgery and suturing of the wound edges with biological skin glue for 3 days observed scab crust and manifestations of epithelialization of the lesion. At the microscopic level, unevenly thickened marginal areas of the epidermis with an average thickness ( $46.52 \pm 2.32$ )  $\mu\text{m}$  gave

rise to a thin epithelial regenerate that covered the wound. In some cases, there were gaps. In the granulation tissue below it, the percentage of fibroblasts was 1.65 times higher than that of the third experimental group (table).

On day 7 of the experiment, animals of the fourth group were characterized by complete epithelialization of the scar area with thin epidermis with an average thickness ( $14.75 \pm 0.34$ )  $\mu\text{m}$ , which is significantly lower than intact, but exceeds this parameter in the third experimental group. Two layers can be distinguished in the granulation tissue of the surgical wound sutured with biological glue under the conditions of induced diabetes. Manifestations of collagenization, connective tissue formation were observed in the deep, and thin fibrils with numerous neutrophils and erythrocytes between them are still present in the upper part. However, the number of fibroblastic cells is significantly higher than in the third experimental group.

On day 28, slightly hyperemic scar was observed in group IV animals, which rose slightly above the skin surface. Histologically, minor hemorrhages were observed under a well-differentiated epidermis with an average thickness ( $40.21 \pm 2.12$ )  $\mu\text{m}$  (as close as possible to the intact index). The border between the epidermis and the dermis was characterized by a small number of papillae. In the dermis, the number of cellular elements was lower than in

**Table.** The ratio of fibroblastic and leukocyte cells in the dermis of intact rats, in the damaged area of the dermis of healthy rats and rats with induced diabetes mellitus using surgical sutures or skin glue ( $M \pm m$ )

A group of animals	A type of cell	Cell ratio, %		
		Term		
		3 days	7 days	28 days
Intact	Fibroblasts	89.36 $\pm$ 2.43		
	Leukocytes	10.64 $\pm$ 0.35		
I group	Fibroblasts	21.67 $\pm$ 1.01	30.56 $\pm$ 1.02	71.92 $\pm$ 2.19
	Leukocytes	78.33 $\pm$ 2.61	69.44 $\pm$ 2.54	28.08 $\pm$ 1.15
II group	Fibroblasts	30.23 $\pm$ 1.08***	41.39 $\pm$ 1.67***	87.44 $\pm$ 2.08***
	Leukocytes	69.77 $\pm$ 1.08***	58.61 $\pm$ 1.73***	12.56 $\pm$ 0.67***
III group	Fibroblasts	12.75 $\pm$ 0.23	23.84 $\pm$ 0.98	66.39 $\pm$ 2.59
	Leukocytes	87.25 $\pm$ 3.02	76.16 $\pm$ 2.57	33.61 $\pm$ 1.26
IV group	Fibroblasts	21.03 $\pm$ 0.96###	32.79 $\pm$ 2.08###	76.60 $\pm$ 3.08#
	Leukocytes	78.97 $\pm$ 2.14#	67.21 $\pm$ 1.88##	23.40 $\pm$ 0.69###

Note 1. \* the values that are statistically significantly different from the indicators of the animals of the first group are marked(\*\*\* –  $p < 0.001$ ).

Note 2. # values that are statistically significantly different from the indicators of animals of the third group are marked(# –  $p < 0.05$ ; ## –  $p < 0.01$ ; ### –  $p < 0.001$ ).

group III and with a significantly greater predominance of fibroblasts over leukocytes. It should be noted that among the cells of the leukocyte lineage were present mainly plasma cells and macrophages.

Our studies indicate that the choice of suture material affects the restoration of wound epithelium, because in healthy rats and animals with diabetes mellitus, which was applied skin glue, epithelialization is completed on day 7, after applying sutures – on day 28. Thus, histologically and morphometrically it was found that the use of skin glue

to fix the edges of surgical wounds in both healthy experimental animals and animals with induced diabetes leads to better results than when using surgical sutures (fig. 1, 2).

The results of biochemical studies obtained by us indicate an increase in the activity of free radical processes in the experimental wound process. However, different intensities of these reactions have been found in healthy and diabetic animals, in particular, with different methods of fixing wound edges.



**Fig. 1.** Histological condition of the connective tissue of the skin of the animal of the fourth experimental group for 3 days. Hematoxylin and eosin staining. Photomicrograph. Magnification: x100.

Symbols: 1 – thickened marginal area of the epidermis; 2 – epidermal regenerate, 3 – dermis.



**Fig. 2.** Histological condition of the skin of the animal of the fourth experimental group at 28 days. Hematoxylin and eosin staining. Photomicrograph. Magnification: x200.

Symbols: 1 – epidermis; 2 – dermis, 3 – sebaceous gland, a fragment of the hair follicle.

**Conclusions.** The use of surgical glue to suture the edges of surgical wounds in animals with induced diabetes is characterized by less inflammatory response, faster epithelialization of the wound

surface and the transformation of granulations into young connective tissue, as well as reducing scarring of the damaged area than using surgical sutures.

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## Морфологічні особливості патологічного рубця шкіри за умов цукрового діабету при різних способах зашивання рани

**Резюме.** Цукровий діабет в усьому світі визнано одним із найважливіших неінфекційних захворювань, а також однією з найсерйозніших проблем здоров'я ХХІ ст. Актуальним науково-практичним завданням була і залишається необхідність запобігання утворенню патологічного рубцювання тканин після операцій, травм, опіків. Тому оптимізація методів загоєння ран і профілактики патологічних рубців на тлі цукрового діабету становить значний науковий інтерес для практичної медицини та стоматології.

**Мета дослідження** – з'ясувати особливості патологічного рубцювання при експериментальному цукровому діабеті та встановити ефективність профілактичного застосування шкірного клею.

**Матеріали і методи.** Експериментальні дослідження проводили на 130 білих безпородних дорослих щурах-самцях масою від 240 до 320 г. Тварини утримувалися у віварії Тернопільського національного медичного університету імені І. Я. Горбачевського МОЗ України відповідно до санітарно-гігієнічних норм і вимог.

**Результати досліджень та їх обговорення.** Результати біохімічних досліджень, які ми отримали, свідчать про підвищення активності вільнорадикальних процесів в експериментальному рановому процесі. Проте виявлено різну інтенсивність цих реакцій у здорових і хворих на цукровий діабет тварин, зокрема при різних способах фіксації країв рани.

**Висновки.** Застосування хірургічного клею для зшивання країв операційних ран у тварин з індукованим цукровим діабетом характеризується меншою запальною реакцією, швидшою епітелізацією ранової поверхні та трансформацією грануляції у молоду сполучну тканину.

**Ключові слова:** окиснювальний стрес; антиоксидантний захист; маркери запалення; мікробне забруднення; неспецифічний імунітет; репаративна здатність; цукровий діабет; нитки хірургічні; клей для шкіри; ефективність.

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