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## VARIA

ORIGINAL ARTICLE

# Morphological features of the dermis collagen fibers and regenerate filling the experimental skin wound cavity during its closure in different ways

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## ABSTRACT

**Aim:** The purpose was to determine the morphological features of collagen fibers of the dermis and regenerate filling the cavity of the experimental skin wound during its closure by different methods.

**Materials and Methods:** The experimental study was conducted on 60 rats of the WAG population weighing 250-300 grams. Five groups were formed (12 rats in each group). Rats of groups 1-4 underwent a 1.5 cm long skin incision on the lateral surface of the neck. The formed defect in rats of group 1 was sutured with an interrupted suture, in group 2 it was sutured with an intradermal suture, in group 3 it was closed with skin glue based on 2-octyl cyanoacrylate, in group 4 it was welded with an electrocoagulator PATONMED EKVZ-300 (Ukraine). Group 5 included intact rats that did not undergo any manipulations. On the 7th and 14th day, 6 animals were removed from the experiment in groups 1-4. In group 5, all animals were removed from the experiment on the 7th day. The material for morphological study was a skin sample from the lateral surface of the neck. Histological, histochemical, morphometric and statistical research methods were used.

**Results:** In survey microscopy, collagen fibers located in the regenerate and surrounding dermis, in cases of experimental wound closure with nodal or intradermal sutures, had different directions of location, mostly looked thickened, and were interconnected, which led to the disappearance of intercellular spaces. In cases of wound closure with 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, collagen fibers looked mostly thinned, chaotically arranged in a dense intertwining network. The density of collagen fibers in the regenerate and the surrounding dermis did not differ depending on the different methods of wound closure. The collagen content in the collagen fibers of the regenerate increased on day 14 compared to day 7 for all methods of experimental wound closure. In cases of wound closure using a nodal or intradermal suture, the collagen content was higher compared to cases when the wound was closed with 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator. In the collagen fibers of the dermis surrounding the wound, the collagen content was higher in cases of wound closure with a nodal or intradermal suture. In cases of wound closure using 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, the collagen content in the dermis surrounding the regenerated tissue corresponded to the control value.

**Conclusions:** Closing the surgical wound with a nodular or intradermal suture is likely to lead to the formation of skin scars in the future. In cases of wound closure using 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, all conditions for the manifestation of organotypic skin regeneration are formed.

**KEY WORDS:** morphology, collagen fibers, experimental skin wound, interrupted suture, intradermal suture, 2-octyl cyanoacrylate-based skin glue, welding with electrocoagulator

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## INTRODUCTION

Mechanical damage to soft tissues is an inevitable element of surgical interventions in humans. In response to mechanical trauma, complex neurohumoral mechanisms are triggered to eliminate wound damage, resulting in the formation of a skin scar or creating all the conditions for the manifestation of organotypic skin regeneration. The latter is known to be a type of regeneration when an organ defect is replaced by a regenerate that corresponds to the structure of the organ [1, 2].

Most superficial skin lesions heal with complete restoration. However, deep skin lesions often cause serious consequences in the form of scars (normotrophic, atrophic, hypertrophic, keloid), which have a significant impact on the quality of patients life [3, 4].

The process of skin scar formation can be influenced by choosing the optimal method of surgical wound closure. However, each of the known and available methods has advantages and disadvantages, indications and contraindications. To date, there is no single, generally accepted method of surgical wound closure that meets all the requirements. The evidence base for most of these methods is insufficient, and their effectiveness is limited. In this regard, this issue remains unexplored.

## AIM

The purpose was to determine the morphological features of collagen fibers of the dermis and regenerate filling the cavity of the experimental skin wound during its closure by different methods.

## MATERIALS AND METHODS

The experimental study was conducted on 60 rats of the WAG population weighing 250-300 grams. Five groups were formed (12 rats in each group). Rats of groups 1-4 underwent a 1.5 cm long skin incision on the lateral surface of the neck. The formed defect in rats of group 1 was sutured with an interrupted suture, in group 2 it was sutured with an intradermal suture, in group 3 it was closed with skin glue based on 2-octyl cyanoacrylate, in group 4 it was welded with an electrocoagulator PATONMED EKVZ-300 (Ukraine). Group 5 included intact rats that did not undergo any manipulations. On the 7th and 14th day, 6 animals were removed from the experiment in groups 1-4. In group 5, all animals were removed from the experiment on the 7th day.

The material for morphological study was a skin sample from the lateral surface of the neck. Microslides were stained with hematoxylin and eosin. For the histochemical detection of collagen fibers and their evaluation, deparaffinized skin sections on slides were stained with red sirius in a solution of concentrated picric acid for 20 minutes at 25°C (0.5 g of Direct Red 80 (Magnacol Ltd., UK) in 500 ml of picric acid) [5, 6], dehydrated, and embedded in balsam (Merck, Germany). Microphotographs were taken using a microscope, Olympus C3040ZOOM camera, and Olympus DP-Soft 3.2 software (Olympus, Japan) (at ×400, 2270×1700 pixels).

Densitometry was performed using ImageJ 1.46 software (ver. 64-bit Java 1.8.0_172, Wayne Rasband, USA). To do this, the color RGB images were deconvolved to obtain three 8-bit photos by identifying a significant signal in a color close to red sirius (region of interest – ROI). Then, by searching for the threshold of positively colored structures in the regions, the collagen fibers density in the ROI was determined, i.e., the specific collagen density in percent (%) was obtained [7]. For each micrograph, 3-5 measurements were obtained. Taking into account the heterogeneity of granulation and connective tissues, their difference in density and thickness of collagen fibers, densitometry was performed in the regions at the regenerate site and in the dermis surrounding the regenerate.

The color features of collagen fibers in the regenerate and the surrounding dermis of the regenerate were evaluated by calculating the brightness coefficient in the Lab color model using the computer program "Analysis of color properties of raster images" [8].

The statistical study was performed in the program Origin ver. 8.0 (Origin Lab, USA) and StatPlus ver. 7.0 (AnalystSoft Inc., USA). The normality of the distribution was checked by the Kolmogorov-Smirnov criterion, and descriptive statistics of the variation series were analyzed. The measurement results were recorded in terms of mean and error. The difference was considered statistically significant at $p<0.05$.

## RESULTS AND DISCUSSION

One of the stromal elements of the skin with underlying soft tissues is connective tissue, which performs diverse and complex functions aimed at maintaining homeostasis. The fibrous structures of the extracellular matrix of connective

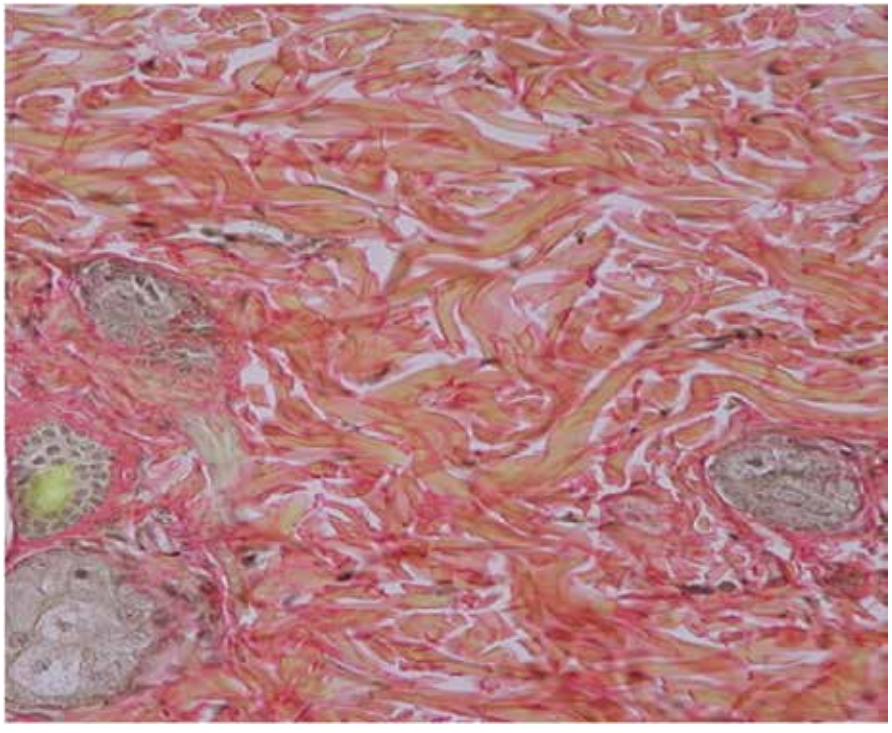

**Fig. 1.** Collagen and non-collagen fibers, skin appendages in group 5. Histochemical staining of collagen fibers: red sirius, picric acid, Weigert's hematoxylin, × 200.

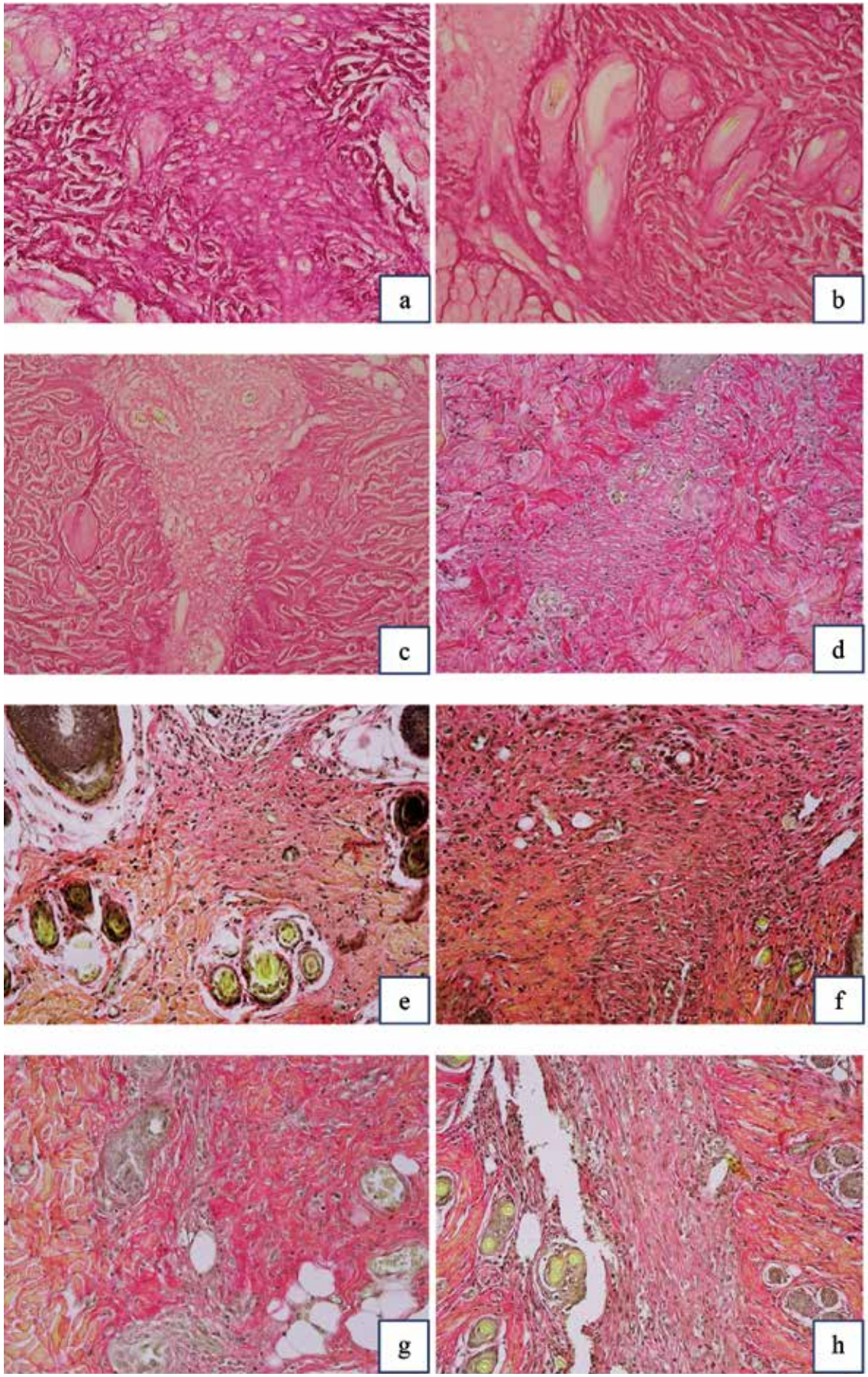


**Fig. 2.** Formed regenerate in the dermis on the left at day 7 and on the right at day 14 (a, b – group 1; c, d – group 2; e, f – group 3; g, h – group 4). Histochemical staining of collagen fibers: red sirius, picric acid, Weigert's hematoxylin, × 100.

tissue include collagen, elastic, and reticular fibers. Among the latter, a significant proportion is accounted for by collagen fibers, which consist mainly of collagen. There are at least 29 distinct kinds of collagen known to science [9]. The qualitative and quantitative characteristics of collagen fibers vary in granulation tissue of different degrees of maturity, which is known to transform into connective tissue over time [2, 10, 11]. The process of collagen fibers production is a complex and multistage process influenced by genetic and non-genetic factors. This process begins in fibroblasts and continues in the intercellular tissue [12].

The formation of granulation tissue in the dermis of rats of groups 1-4 was recorded in the area of the modeled defect, which in some visual fields turned into connective tissue. In groups 1-5, the method of histochemical staining allowed us to identify collagen fibers in the dermis in group 5 (Fig. 1), in the formed regenerate in groups 1-4 (Fig. 2), which were represented by clearly defined, red-colored fibrillar elements, and non-collagen fibers of yellow color (positive regions for picric acid).

In survey microscopy in group 5, the distribution of collagen fibers in the dermis of the skin was uniform, diffuse, with multidirectional orientation and the presence of intercellular space (Fig. 1). In groups 1-4, the newly formed collagen fibers were structurally different from the corresponding fibers of intact skin in group 5. In the area of the modeled defect in groups 1-2, collagen fibers had different directions of location, were thinned and thickened, and there were frequent fields of view with a predominance of thickened collagen fibers that were interconnected, as a result of which no intercellular spaces were noted. In groups 3-4, in the areas of the modeled defect, collagen fibers were predominantly thinned, chaotically arranged in the form of a dense intertwining network. In groups 1-4, the structural features of collagen fibers in the dermis surrounding the regenerate were similar to those already described in the regenerate area. The morphological features of collagen fibers in the dermis surrounding the regenerate and in the regenerate, identified by the authors in groups 1-2, were similar to those described by numerous scientists in the formation of pathological skin scars. It is known that collagen fibers are the main structural component of a skin scar, which determines its relief and density [2, 13].

Subsequently, a morphometric study was conducted to measure the density of collagen fibers in the dermis in group 5, in the dermis surrounding the regenerate (Fig. 3) and in the regenerate in groups 1-4 (Fig. 4).

In group 1, there were no significant ($p>0.05$) differences in the density of collagen fibers on day 14 compared to day 7 in the regenerate zone and in the dermis surrounding the regenerate. In the dermis surrounding the regenerate on day 7, the density of collagen fibers was lower ($p<0.05$) compared to the index of group 5, and on day 14, the density of collagen fibers did not differ ($p>0.05$) from the index of group 5.

In group 2, the collagen fibers density in two study sites did not have significant ($p>0.05$) differences on day 14 compared to day 7. As in group 1, on day 7, a lower ($p<0.05$) density of collagen fibers in the perifocal area was also found compared to the index of group 5, and on day 14, no differences ($p>0.05$) were found.

In group 3, the density of collagen fibers in the regenerate area and in the perifocal area on day 14 did not differ ($p>0.05$) compared to day 7. The density of collagen fibers in the perifocal area on days 7 and 14 did not differ ($p>0.05$) from the corresponding indicator of group 5, that is, the density of collagen fibers in the connective tissue was close to normal.

In group 4, the results of the comparative analysis were similar to group 3. Thus, the density of collagen fibers in the regenerate and peripheral area did not differ ($p>0.05$) on day 14 compared to day 7. On days 7 and 14, the density of collagen fibers in the perifocal dermis did not differ ($p>0.05$) from the control group, i.e., group 5.

Thus, the analysis conducted by the authors did not reveal any peculiarities in the density of collagen fibers in the regenerate and the dermis surrounding the regenerate in cases of different methods of closing surgical wounds in the experiment.

Collagen fibers are known to be composed of collagen protein [13]. The staining method we used resulted in different shades of red, which, in our opinion, indicates different collagen content in them. We analyzed the color properties of collagen fibers by calculating the brightness coefficient, the average values of which are shown in Figures 5 and 6.

Figure 5 shows that in all groups, the brightness coefficient of collagen fibers of the regenerate decreased ($p<0.05$) on day 14 compared to day 7, which indicates an increase in the collagen content in collagen fibers and is a sign of wound healing. Collagen is a key component of the extracellular matrix that plays critical roles in the regulation of the phases of wound healing [14]. On days 7 and 14 in groups 3-4 compared to groups 1-2, the brightness coefficient had a higher ($p<0.05$) value, indicating a lower collagen content in collagen fibers. Excessive collagen content in collagen fibers, which we found in groups 1-2, can lead to the development of skin scars in the future [15].

The brightness coefficient in the collagen fibers of the dermis in groups 3-4 did not differ ($p>0.05$) from the control indicator, i.e., the indicator of group 5 (Fig. 6). However, in groups 1-2, this indicator decreased ($p<0.05$) compared to that of group 5, indicating a higher collagen content in collagen fibers. It was interesting that on day 14 compared to day 7, the index in groups 3-4 did not change ($p>0.05$), and in groups 1-2 it decreased ($p<0.05$). Thus, in groups 1-2, excessive collagen content in collagen fibers was found in the dermis surrounding the wound, which may also be important in the development of a skin scar.

It is known that not only granulation and connective tissues that fill the skin defect, but also the surrounding dermis are involved in the formation of skin scars [2, 13].

## CONCLUSIONS

1. In survey microscopy, collagen fibers located in the regenerate and surrounding dermis, in cases of experimental wound closure with nodal or intrader-

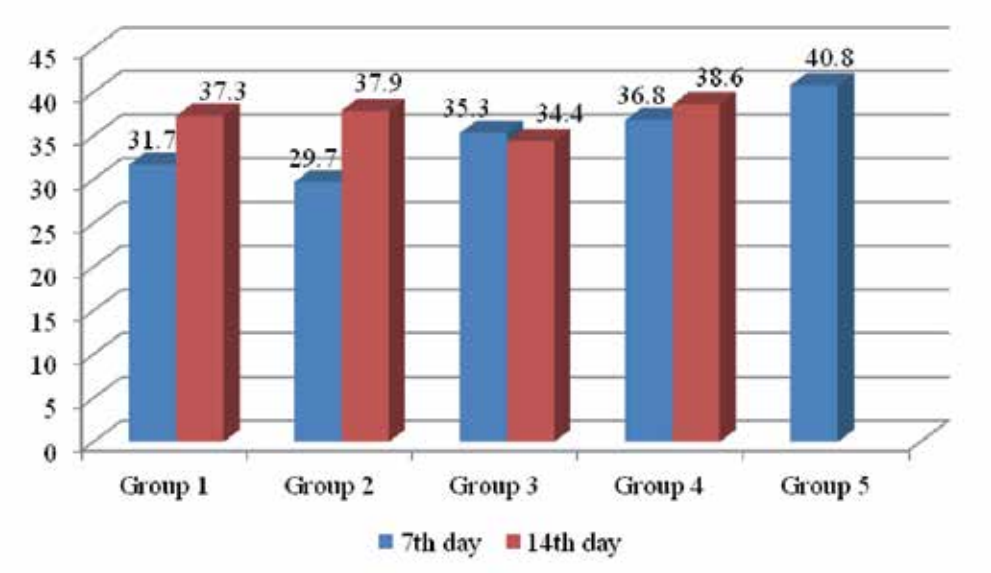


**Fig. 3.** Results of measuring the density (%) of collagen fibers in the dermis on day 7 in group 5, in the dermis surrounding the regenerate on days 7 and 14 in groups 1-4.

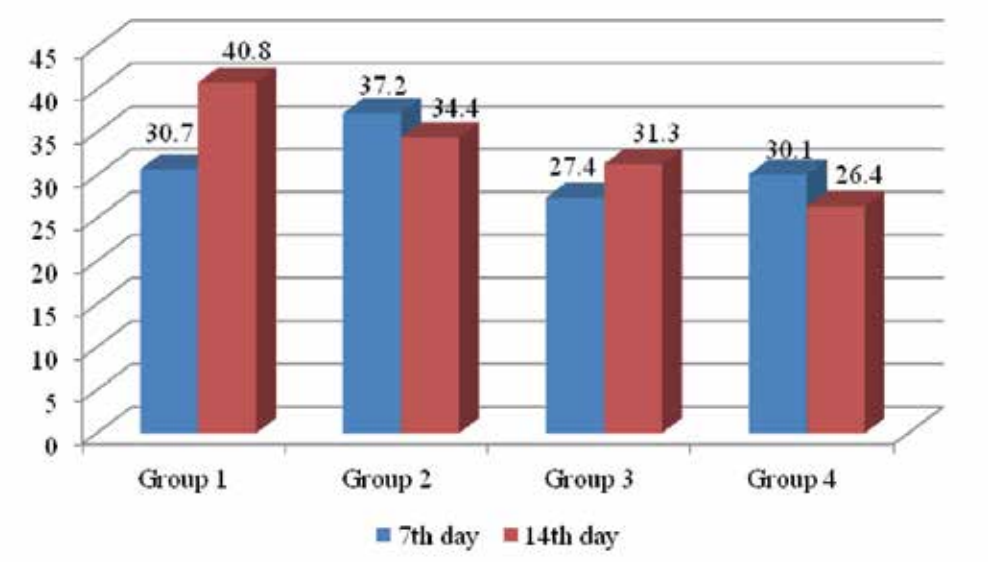


**Fig. 4.** Results of measuring the density (%) of collagen fibers in the regenerate on day 7 and 14 in groups 1-4.

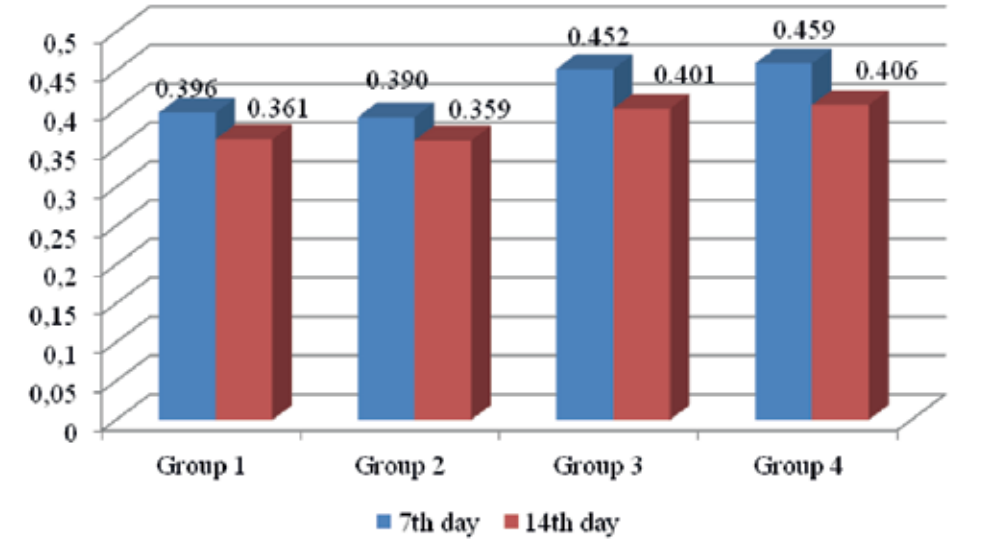


**Fig. 5.** Average brightness coefficient values in collagen fibers of the regenerate in groups 1-4.

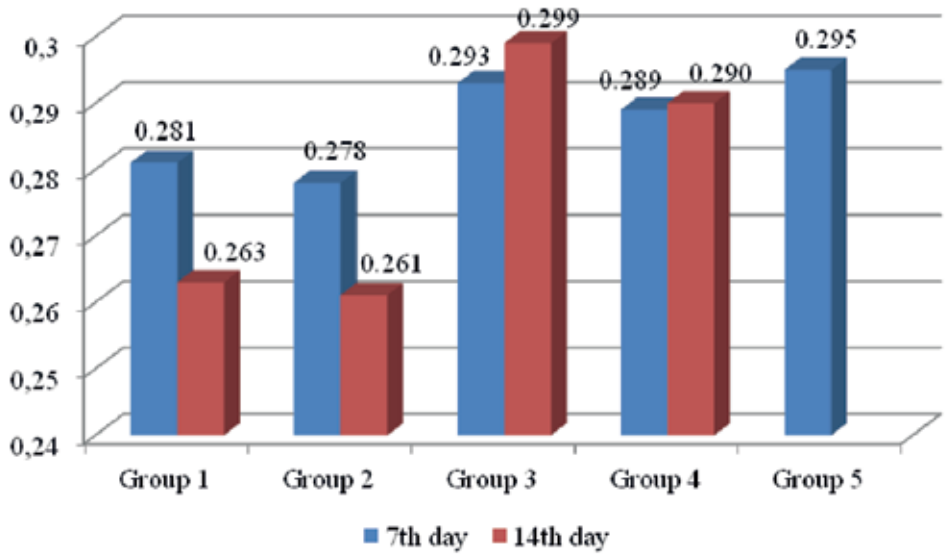


**Fig. 6.** Average brightness coefficient values in collagen fibers in dermis in group 5 and in dermis surrounding the regenerate in groups 1-4.

mal sutures, had different directions of location, mostly looked thickened, and were interconnected, which led to the disappearance of intercellular spaces. In cases of wound closure with 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, collagen fibers looked mostly thinned, chaotically arranged in a dense intertwining network. The density of collagen fibers in the regenerate and the surrounding dermis did not differ depending on the different methods of wound closure.

2. The collagen content in the collagen fibers of the regenerate increased on day 14 compared to day 7 for all methods of experimental wound closure. In cases of wound closure using a nodal or intradermal suture, the collagen content was higher compared to cases when the wound was closed with 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator.
3. In the collagen fibers of the dermis surrounding the wound, the collagen content was higher in cases of wound closure with a nodal or intradermal suture. In cases of wound closure using 2-octyl cyanoacrylate--based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, the collagen content in the dermis surrounding the regenerated tissue corresponded to the control value.
4. Closing the surgical wound with a nodular or intradermal suture is likely to lead to the formation of skin scars in the future. In cases of wound closure using 2-octyl cyanoacrylate-based skin glue or by welding with the PATONMED EKVZ-300 electrocoagulator, all conditions for the manifestation of organotypic skin regeneration are formed.

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**CONFLICT OF INTEREST**
The Authors declare no conflict of interest

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