# MORPHOFUNCTIONAL CHARACTERISTICS OF CUTANEOUS CONNECTIVE TISSUE SCARS IN WOMEN WITH PAST HISTORY OF CHILDBIRTH AFTER CESARIAN DELIVERY

Mishina ES[1]✉, Zatolokina MA[1], Mnikhovich MV[2], Kharchenko VV[1]

[1] Kursk State Medical University, Kursk, Russia

[2] Research Institute of Human Morphology, Moscow, Russia

The inevitable outcome of skin injuries caused by a variety of external factors is the formation of a connective tissue scar. A scar can deform when exposed to stretching, pressure or repeat surgeries and undergo structural changes leading to its dehiscence. Scar dehiscence is a common problem seen in women with a past history of cesarean delivery. There have been comprehensive studies of uterine scars formed after the C-section, but the morphology of cutaneous C-section scars has not yet been investigated. The aim of this study was to look into the morphology of connective tissue scars in multiparas with a past history of cesarean delivery. Specimens of cutaneous scars were collected from 30 women after the C-section. Within one age group, fiber thickness was directly proportional to the number of previous deliveries. Comparison of different age groups with the same number of previous deliveries revealed the thinning of collagen fibers and the increased density of type III collagen fibers. The most pronounced changes were observed in women with a history of 3 or more deliveries. We hypothesize that a connective tissue scar undergoes structural transformation, becomes thinner, and its fibers dissociate due to repeated skin stretching, which might indirectly suggest the dehiscence of the postoperative scar.

**Keywords:** connective tissue scar, skin, collagen fibers, regeneration, stretching, cesarean section

**Author contribution:** Mishina ES — study design; collection and processing of specimens; data analysis; manuscript preparation; Zatolokina MA, Mnikhovich MV, Kharchenko VV — study concept; manuscript editing. The final version of the manuscript was approved by all the authors.

**Compliance with ethical standards:** the study was approved by the Regional Ethics Committee of Kursk State Medical University (Protocol № 4 dated June 10, 2019). The study complied with the ethical standards for medical research studies involving humans. Informed consent was obtained from all study participants.

✉ **Correspondence should be addressed:** Ekaterina S. Mishina
Karla Marxa, 3, Kursk, 305041; katusha100390@list.ru

**Received:** 11.12.2020 **Accepted:** 12.01.2021 **Published online:** 22.01.2021

**DOI:** 10.24075/brsmu.2021.002

# МОРФОФУНКЦИОНАЛЬНЫЕ ОСОБЕННОСТИ СОЕДИНИТЕЛЬНОТКАННОГО РУБЦА НА КОЖЕ У ПОВТОРНОРОДЯЩИХ ЖЕНЩИН ПОСЛЕ ОПЕРАТИВНОГО РОДОРАЗРЕШЕНИЯ

Е. С. Мишина[1] ✉, М. А. Затолокина[1], М. В. Мнихович[2], В. В. Харченко[1]

[1] Курский государственный медицинский университет, Курск, Россия

[2] Научно-исследовательский институт морфологии человека, Москва, Россия

Нарушение целостности кожного покрова под действием различных факторов неизбежно приводит к образованию соединительнотканного рубца. Под воздействием динамических факторов (растяжение, давление, повторно проведенные операции) рубец подвергается деформации, вследствие чего возможна дезорганизация его структурных компонентов и последующая его несостоятельность. Наиболее частой проблемой такого плана является несостоятельность рубцов у повторнородящих женщин, в анамнезе которых имеется хирургическое родоразрешение путем кесарева сечения. В литературе представлены результаты комплексного изучения рубцов на матке после операции кесарева сечения, в то время как морфологическое исследование кожного рубца у этих же беременных не проводилось. Целью работы было изучить морфофункциональные особенности соединительнотканного рубца на коже у повторнородящих женщин после оперативного родоразрешения. Исследовали фрагмент кожного рубца у 30 женщин после кесарева сечения. У женщин в одной возрастной группе утолщение волокон было прямопропорционально числу родов. При сравнении разных возрастных групп с одинаковым числом родов наблюдали истончение коллагеновых волокон, а также увеличение плотности волокон коллагена 3-го типа. Наиболее выраженные изменения выявлены у женщин с тремя и более родоразрешениями. Можно предположить, что под влиянием кратности растяжений кожи происходит структурная перестройка соединительнотканного рубца в виде истончения и дезорганизации волокнистых структур, что может косвенно говорить о несостоятельности послеоперационного рубца.

**Ключевые слова:** соединительнотканный рубец, кожа, коллагеновые волокна, регенерация, растяжение, кесарево сечение

**Вклад авторов:** Е. С. Мишина — дизайн исследования, сбор и обработка материалов, анализ полученных данных, написание текста; М. А. Затолокина, М. В. Мнихович, В. В. Харченко — концепция и редактирование текста; все авторы прочли и одобрили финальную версию статьи.

**Соблюдение этических стандартов:** исследование одобрено региональным этическим комитетом Курского государственного медицинского университета (протокол № 4 от 10 июня 2019 г.), выполнено с соблюдением этических принципов проведения научных медицинских исследований с участием человека; все участники подписали добровольное информированное согласие на участие в исследовании.

✉ **Для корреспонденции:** Екатерина Сергеевна Мишина
ул. Карла Маркса, д. 3, г. Курск, 305041; katusha100390@list.ru

**Статья получена:** 11.12.2020 **Статья принята к печати:** 12.01.2021 **Опубликована онлайн:** 22.01.2021

**DOI:** 10.24075/vrgmu.2021.002

The inevitable outcome of skin injuries caused by a variety of exposures is the formation of a connective tissue scar [1–3]. Some scars, including hypertrophic scars and keloids, can be esthetically displeasing; immature scars are prone to more serious complications. Differences in scar tissue organization and morphology are determined not only by the type of injury but also by the duration and frequency of injurious exposure [4–10]. This might explain the phenomena of wound dehiscence, postoperative hernias and other negative outcomes [11, 12]. As indications for cesarean delivery are expanding, more women of childbearing age are acquiring uterine scars, which, in turn, necessitate cesarean delivery in future pregnancies [13]. In some cases, a C-section is combined with genitoplasty or abdominal wall reinforcement to avert scar dehiscence, the most common complication of the C-section [14–17]. So, it would be clinically relevant to study cutaneous scar morphology and the reorganization of scar tissue in women with a past history of childbirth.

## METHODS

Our study recruited 30 patients of Kursk Maternity Hospital. The following inclusion criteria were applied: the absence of obstetric or gynecologic pathology; delivery by cesarean section. Exclusion criteria: preterm labor; systemic connective tissue disorders. Fig.1 illustrates the distribution of the participants by age.

The mean age of the participants was 33.1 ± 3.93 years; the mean height was 164.3 ± 6.47 cm; the mean weight, 74.57 ± 3.13 kg; the mean BMI, 0.28 ± 0.05 kg/m$^2$. The total number of pregnancies specified in medical histories was 89. The total number of deliveries was 77. Twenty-three women (76.67%) had a past history of 2 cesarean deliveries; 6 women (20%) had undergone a total of 3 cesarian sections; 1 woman (3.33%) had a history of 4 cesarean deliveries. These figures include cesarean sections performed during the study.

The participants were grouped by age and the number of deliveries. A total of 5 groups with 1 to 12 women in each group)were formed (Table 1).

Specimens of scar tissue with the adjacent flaps of normal skin sized 3 × 6 cm were collected from all study participants after the C-section. For light microscopy, the specimens were fixed in 10% neutral buffered formalin. The specimens were embedded in paraffin and sliced on a microtome following the standard protocol. Sections of 5–7 µm were stained with hematoxylin-eosin and Mallory's trichrome stain. The presence of collagen fibers in the specimens was verified by means of immunohistochemical staining (IHC) with anti-collagen type I and III monoclonal rabbit antibodies (Novocastra; Germany). Staining was performed in an automated LEICA BOND MAX IHC stainer (Leica; Germany). Digital photos geometrically and optically calibrated in ImageJ 14,7a (National Institutes of Health; USA) were further used to measure fiber thickness, the areas of collagen fibers (for each collagen type) and interfiber space in 30 fields of view (×10). The scar density coefficient was calculated using the previously proposed formula:

$K = (S_{c.f.}, \%)/(S_{i.s.}, \%)$,

where K is the scar density coefficient;

$S_{c.f.}$ is the area of collagen fibers;

$S_{i.s.}$ is the area of interfiber space.

Then, the cellular makeup of the sampled scar tissue was analyzed. Based on karyological features (×40), we identified fibroblast cell lineages and proinflammatory cells. Immunophenotyping tests were not performed. Cell count was done per 100 cells in several (at least 10) non-overlapping fields of view. Then, mean values were calculated. Statistical analysis was carried out in Statistika 10.0 (Stat Soft; Russia). The normality of data distribution was tested using the Kolmogorov-Smirnov and the Shapiro–Wilk tests. The significance of differences was assessed using the Mann–Whitney *U* test for independent samples. Differences were considered significant at $p \leq 0.05$.

## RESULTS

Fiber analysis revealed that all the studied connective tissue structures were composed of mature granulation tissue. In groups I and III (see Table 1), the fibers lied closest to each other and were organized in one direction. In all groups, the fibers were fairly thick, with dense intrafibrillar structures. However, the major fibers in groups II and V (see Table 1) were composed of multiple thin branching fibers. Cross-sectionally, all fibers were mostly round. The morphometric analysis revealed that collagen fibers in the studied fibrous tissue differed significantly in their thickness between the groups. The thickest fibers were observed in the specimens obtained from women of young reproductive age with a past history of 3 deliveries. The thickness of collagen fibers was 1.2 lower in women of the same age with a past history of 2 deliveries (7.8 ± 0.11 µm). In women of late reproductive age, the thickness of collagen fibers was directly proportional to the number of previous deliveries, decreasing from 6.1 ± 0.12 to 4.7 ± 0.1 µm for women with a history of fewer deliveries.

The analysis of the ratio of collagen fibers area to the area of interfiber space in postoperative scars revealed that connective tissue fibers were more densely arranged in women with a past history of 2 deliveries at young reproductive age. The lowest ratio was observed in women with a history of 3 to 4 deliveries. These morphometric characteristics are shown in Fig. 2.

The IHC study of scar fragments revealed a decrease in collagen content in patients with a history of 2 and 3 deliveries at late reproductive age. Type I collagen was the most prevalent in the scar tissue of younger women (Table 2).

The dynamics of inflammation and tissue repair was analyzed by means of cell count in the scar tissue. The analysis revealed that collagen synthesis was the dominant process in the scar tissue of all study participants at the end of gestation. However, the fact that immature fibroblast lineage cells dominated the cellular composition of scars in women of late reproductive age led us to hypothesize that collagen synthesis was still ongoing. The dynamics of scar tissue cellular composition is illustrated in Fig. 3.

## DISCUSSION

With regard to the dynamics of cutaneous scar tissue structure, the roughness and thickness of collagen fibers were directly

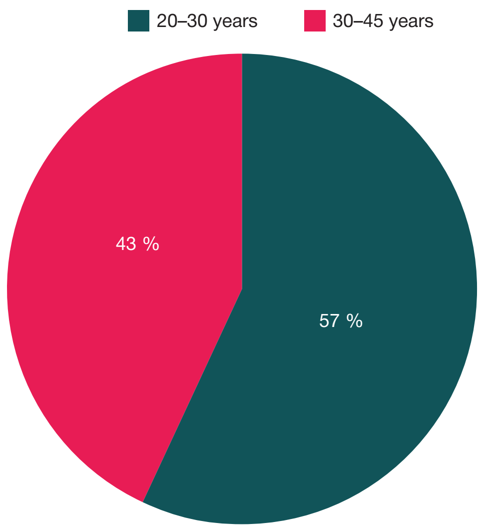


**Fig. 1.** The distribution of the participants by age

**Table 1.** The distribution of the participants by age and the number of cesarean deliveries

| Age | Number of deliveries | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| 20–30 years | Group I | Group III | |
| 31–40 years | Group II | Group IV | Group V |

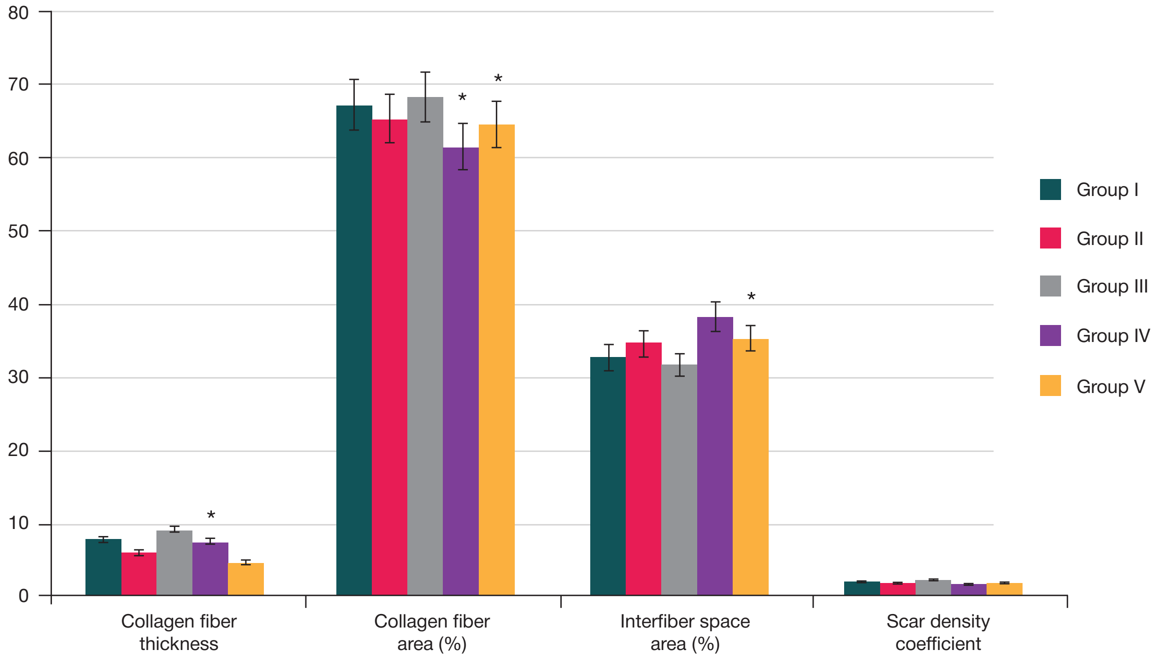


**Fig. 2.** The dynamics of morphometric characteristics of fibrous tissue. * — differences are statistically significant relative to the previous group ($p \leq 0.05$)

**Table 2.** The distribution of collagen types in the scar tissue between different groups of patients

| Group | Type I collagen density | Type II collagen density | Type 1 to 3 collagen ratio |
|---|---|---|---|
| I | 23.4 ± 1.1* | 20.7 ± 1.2 | 1.1 ± 0.32 |
| II | 19.6 ± 0.76 | 18.9 ± 1.3 | 1.03 ± 0.1 |
| III | 23.4 ± 1.1 | 25.9 ± 1.2* | 0.9 ± 0.07 |
| IV | 14.9 ± 1.3* | 10.7 ± 0.4* | 1.4 ± 0.05 |
| V | 17.9 ± 1.3 | 16.6 ± 0.5 | 1.07 ± 0.05 |

**Note:** * — differences are statistically significant relative to the previous group ($p \leq 0.05$).

proportional to the number of previous deliveries (groups I and III). The densest scars with the narrowest interfiber space were observed in young women with a history of 3 deliveries. Comparison of different age groups with the same number of past deliveries revealed collagen fiber thinning. These structural changes were the most pronounced in groups IV and V, i.e. in women with a past history of 3 or more deliveries. One of the main characteristics of a mature scar is its thickness and

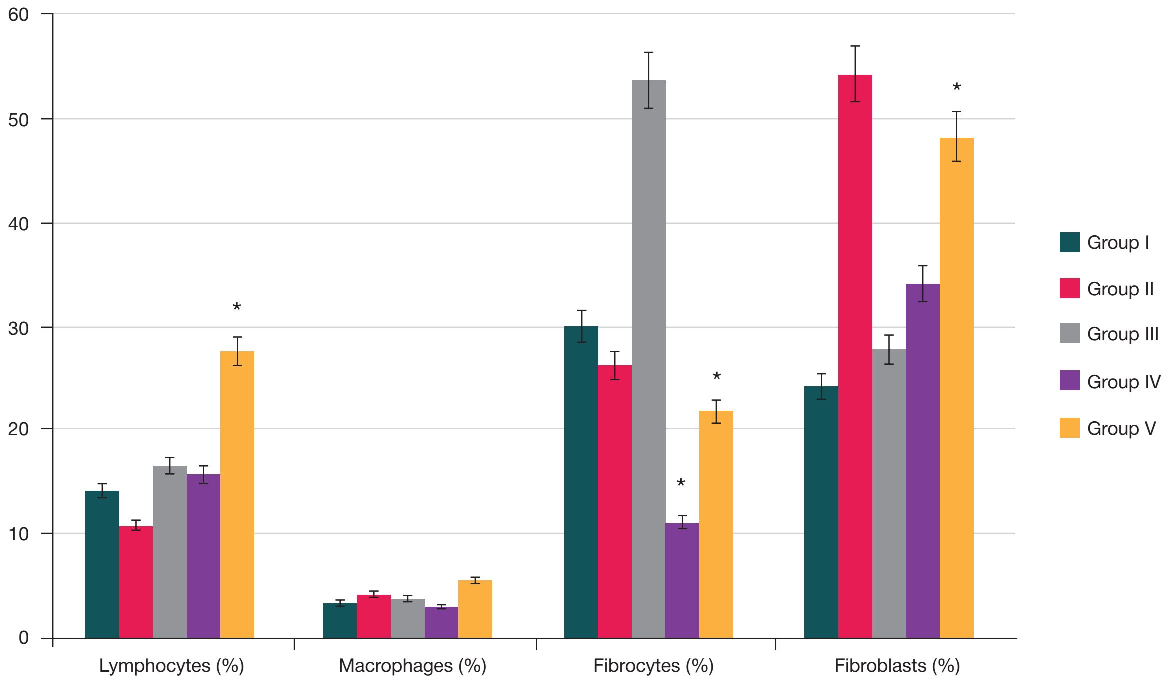


**Fig. 3.** The dynamics of scar tissue cellular composition. * — differences are statistically significant relative to the previous group ($p \leq 0.05$)

structural heterogeneity [18]. The scar density coefficient calculated in the study reflects fiber thickness and the size of the interfiber space. The greater is the scar density coefficient, the thicker is the scar and the less pronounced is the interstitial edema, which can expand the interfiber space. The obtained data are indirectly suggestive of scar tissue dehiscence after the third delivery in young patients. The analysis of collagen types distribution demonstrated that the greatest number of fibrous structures made up of type III collagen was observed in young women; in older women, scar tissue was mainly composed of type I collagen. These data are consistent with the data on the mechanisms of collagen synthesis in uterine scars. Immunohistochemically, connective tissue is characterized by moderate expression of type III collagen [19]. Cellular mechanisms play a significant role in the formation of a mature scar. One of the most important criteria of a mature scar is the completion of the inflammation phase, i.e. a decline in lymphocyte and granulocyte counts [20]. The analysis of scar tissue cellular composition demonstrated that the morphology of the examined scars was consistent with mature granulation tissue dominated by fibroblast lineage cells.

## CONCLUSION

Low inflammation levels, the cessation of collagen synthesis and the prevalence of fibroblast lineage cells are prerequisites for a sound scar. Microscopically, a sound scar is characterized by dense bundles of collagen fibers separated by small interfiber spaces. Repeated stretching causes fibrous structures to become thinner and dissociate. This morphological picture can be an indirect indication for abdominal wall and suture reinforcement with synthetic materials.

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