Journal of Applied Cosmetology

ISSN 2974-6140 (online) ISSN 0392-8543 (print)

Case study

Noninvasive bipolar radiofrequency - vaginal application on swine model

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Keywords: *histology, neocollagenogenesis, neoelastogenesis, radiofrequency heating, vaginal wall*

Received: 07 February 2024
Accepted:08 May 2024

ISSN 2974-6140 (online) ISSN 0392-8543 (print).

DISCLOSURE: **ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.**

ABSTRACT

The aim of the study was to evaluate the concentration of collagen and elastin fibers, the number of fibroblasts/fibrocytes and vessels, as well as depth of action in the vaginal wall in a swine model before and after intravaginal 480 kHz bipolar radiofrequency heating created by a 360-degree intravaginal applicator (Berger and Kraft Medical). Three swine's were treated with a bipolar radiofrequency vaginal probe. Exposure to RF was administered twice, every 4 weeks, and then the vaginas were removed after slaughter, four weeks after the last RF exposure. Histology specimens were obtained from control and exposed areas. Tissue samples were stained by hematoxylin-eosin, orcein, and Mallory trichrome. The concentration of elastin and collagen fibers increased distinctly after a treatment protocol: elastin, on average, was 52.8%, and collagen, on average, was 103.6%. After the treatment protocol, we revealed a significant dispersion of fibroblasts-fibrocytes nuclei for particular animals in segmented areas, with a slightly marked falling trend. A degree of data variability cannot lead to a conclusion concerning the influence of RF heating on several vessels in evaluated areas. The depth of action of the RF heating, measured by collagen concentration, reached up to 1.3 mm of the vaginal wall thickness. Results imply that heating of the vaginal wall, created by radiofrequency, induces neocollagenogenesis and neoelastogenesis, leading to a distinctive rise of collagen and elastin fibers concentration at a depth of up to 1.3 mm of the thickness of the vaginal wall in the animal model.

INTRODUCTION

Symptoms of vulvovaginal laxity, vulvovaginal atrophy, stress urinary incontinence, and sexual dysfunction can develop as a result of aging, menopause, weight change, childbirth, and the subsequent mechanical and hormonal changes that result from these events. This happens because the quality of the connective tissue in the vulva and vagina is reduced as a result of these events. The biomechanical properties of the vaginal wall are determined by the presence of elastin and collagen fibers. Fibroblasts or fibrocytes can create These types of fibers, the cells that can construct the cellular matrix (1). Because the collagen and elastin fibers in a woman's vaginal wall tissue gradually break down as she ages, the vaginal wall tissue gradually becomes less elastic and less able to stretch. This can make it more difficult for a woman to have vaginal children. Alterations in connective tissue that come about as a result of aging, vaginal childbirth, and hormonal shifts that occur after menopause are some of the causes that contribute to this loss of elasticity and stretch (2, 3). Alterations in vaginal pH, vaginal microbiota, and the thickness of the vaginal epithelium have also been observed in addition to the alteration mentioned above (4, 5). Patients' concerns about these difficulties have prompted them to look for treatment solutions that will alleviate their symptoms and restore their vaginal and vulvar integrity (4).

Up until quite recently, the only treatment choices that were accessible to patients were surgical or conservative, which involved the use of either systemic or topical medicines. Sadly, the disappointment caused by factors such as the expense of therapies, the postoperative pain, or the transient nature of the effects of topical treatments has contributed to the creation of new therapeutic choices. Vaginal laxity and vulvovaginal atrophy can both be treated using noninvasive devices that target the vaginal wall and use either radiofrequency or laser energy as their source of energy (1–5). Presently, a number of these devices are available. When compared to fractional laser treatments for vaginal rejuvenation, nonablative radiofrequency (RF) therapies have the benefit of requiring no downtime after the procedure. Laser technologies, including CO2 and Er:YAG, are responsible for inducing changes in vaginal tissue. These changes come about as a result of

inflammation and wound healing, both of which are caused by the vaporization of extracellular water in the treated tissue. Laser devices can create microzones of tissue injury by utilizing fractional beam technology, which is then separated by intervening areas of untreated tissue, which speeds up the healing process of the damaged tissue (1, 2, 5). Previous research (6, 7) has documented and measured the effects of RF energy on collagen fiber distribution in the skin; however, there is a lack of scientific literature that details the quantitative changes that occur in collagen and elastin due to intravaginal RF treatments (8, 9). RF devices produce an electrical field within the tissue, which promotes the migration of charged particles on the molecular level, producing heat (4, 6, 8, 10). The quantity of heat generated in the tissue is directly proportional to the amount of current flowing through it as well as the duration of time that the device is in contact with the tissue. RF devices can be monopolar, bipolar, or multipolar, which results in variations in the way an electric current travels from the device through the tissue and back to a grounding pad or between the electrodes. By activating heat shock proteins and the commencement of the inflammatory cascade, RF can trigger fibroblasts to make collagen at tissue temperatures ranging from 40 to 45 degrees Celsius. Temperatures above 45 degrees Celsius have been shown to cause thermal injury and pain at the skin level. However, vaginal tissue can tolerate temperatures up to 47 degrees Celsius without showing any obvious signs of thermal injury (11, 12).

The histologic effects of RF treatment on tissue were investigated. It was discovered that RF might lessen the skin's laxity, increase the skin's mechanical strength, and stimulate neocollagenogenesis and elastogenesis (6, 7). Moreover, radiofrequency energy was investigated using a vaginal ovine model equipped with a Viveve monopolar applicator. It was revealed that the enhanced collagen production observed in the first month and the increased fibroblasts continued even after three months. In the ovine model, there was no investigation of the changes in elastin (8). In a similar vein, the vaginal swine model was investigated not too long ago. This study used a domestic pig model to quantitatively analyze the ability of volumetric radiofrequency heating delivered by the Exilis Ultra 360 monopolar intravaginal applicator (BTL Industries Inc) to enhance vaginal tissue. Neocollagenesis and neoelastinogenesis were identified, along with a rise in the quantity of collagen and elastin, as a response to therapy (9). Furthermore, the number of fibroblasts and fibrocytes that were present was shown to have increased. Our research aimed to quantify the changes in collagen and elastin fiber concentration, as well as the number of fibroblasts/fibrocytes and blood vessels, in the vaginal wall of a swine model before and after intravaginal radiofrequency heating. The heating was administered in the form of a series of consecutive vaginal applications. The vagina of a domestic swine was employed as a model because it is equivalent to the physiological response of human vaginal tissue (13), in addition to having an architecture that is analogous to that of a woman's vagina. In addition, the swine model appears to have certain benefits over the ovine model due to regional anatomic variations in the distribution of collagen along the vaginal wall of the ovine (10). Using a bipolar RF device and a novel treatment regimen, in which we considered the physiological dynamics of collagen and elastin formation after tissue heating, set our research apart from the studies listed above (8, 9).

MATERIALS AND METHODS

The protocol for the study was approved by the University of Natural Science’s Local Ethics Committee for Animal Experiments in Poznań, Poland (Resolution nr. 36/2021). It was carried out under the supervision of the Faculty of Biotechnology and Animal Husbandry at the West Pomeranian University of Technology in Szczecin, Poland.

The experiment was carried out using an animal model consisting of three domestic pigs housed in a building designed to kill animals. The animals used in the experiment were 3,3 and 5 years old, respectively, and had

been weaned from their last litter of piglets for two weeks before the tests. They were each kept in separate housing and provided a complete grain meal for pigs.

During the inspection by the veterinarian, none of the three pigs showed any signs of illness, and the veterinarian found no abnormalities or deficiencies in any part of the genital tract.

RF radiation was applied to the vaginal tissue of the animals twice every four weeks, and the vaginas were removed following slaughter, which occurred four weeks after the most recent RF application. This technique had no choice but to be used because of the physiological dynamics of collagen and elastin formation after heating the tissue.

Using radial energy distribution, a bipolar radiofrequency of 480 kHz was delivered using an intravaginal applicator that rotated 360 degrees (manufactured by Berger and Kraft Medical). Due to the length of the vaginal probe (Fig. 1), the vagina was sectioned off into two distinct areas, each measuring three centimeters. The control zone was located in the distal zone (C), while the exposed zone (EX) was located in the proximal zone. The therapy parameters were set in accordance with the suggestion provided by the manufacturer, and they were the same for each zone in terms of both the time and energy settings. We used the same vaginal probe that was used throughout the experiment to properly delineate the exposure and control zones (Fig.1). This allowed us to precisely measure the influence that the RF had on the vaginal wall. This strategy was chosen as a result of the fact that there were no discernible macroscopic changes seen in the vaginal wall that was being evaluated.

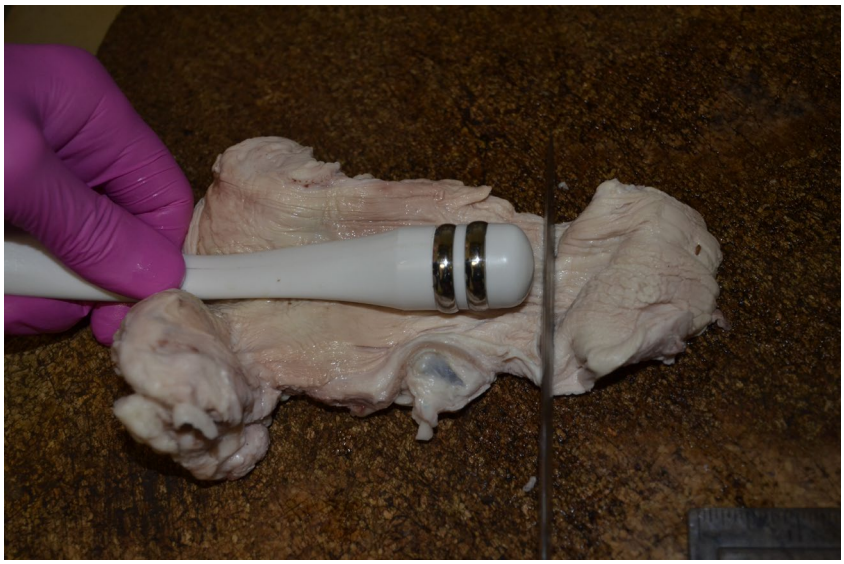

Fig. 1. *An illustrative picture showing the method of determining the zones exposed to radiofrequency from which the samples were taken.*

Following the slaughter, the vaginas were extracted in their entirety and then treated with formalin. After removing samples from areas exposed to radiofrequency energy and from the control group, the samples were embedded in paraffin wax and then sectioned using a microtome. Mallory Tri-chrome (Bio Optica, Italy, cat. no.: 04-020802, and orcein Bio Optica, Italy, cat. No.:04-055802, Sirius red picrate, Italy, cat. No.:04-121873) and fibroblasts nuclei using standard procedure hematoxylin and eosin were used to stain the samples in preparation for the histological examination. This was done to The H&E staining was done with reagents that were prepared in the laboratory immediately before the staining process (Hematoxylin: 0.3g of eosin in 100mL of distilled water for staining use; Eosin: 50g of chloral hydrate, 1g of citric acid, 0.2g of sodium iodate or potassium; Eosin: 50g of alum. potassium, 50g of chloral hydrate, 1g diluted solution: laboratory, immediately

before staining (Hematoxylin: 50g alum. potassium, 50g of chloral hydrate, 1g of citric acid, 0.2g of sodium iodate or potassium; Eosin: 0.3g eosin in 100mL distilled water, for staining use) Hematoxylin: 50g alum. potassium, 50g chloral hydrate, 1g citric acid, 0.2g sodium I solution that has been diluted: a solution that has been diluted: (20 mL of eosin combined with 80 mL of distilled water).

It was hypothesized that each control sample would be obtained from the same pig located in a zone that was not exposed to radiofrequency radiation. The KS 300 (Zeiss) scanner was used to do computational analysis on each specimen, and the results were analyzed in relation to the chosen region of interest. The region that was examined included the layers of the vaginal wall that are directly beneath the lamina propria of the vaginal epithelium (up to 1.3 mm of the thickness of the vaginal wall). We employed a magnification of 400 times to examine the specimen. Less powerful magnifications would take in information about layers outside of the zone of interest, which would skew the test results.

When the threshold values were determined, the recorded images were segmented by calculating the total surface areas of the objects to be segmented. The addition of individual pixel areas was the method utilized for performing quantitative measurements. A logarithmic scale was adopted since the values of the segmented areas in pixels were unusually high. This led to the need for the logarithmic scale.

RESULTS

Histological assessment of the specimens stained with H-E revealed no perceptible differences between exposed zones and the control. To evaluate this more specifically, we utilized staining with Orcein and Mallory trichrome.

The segmentation results for orcein staining

Fig. 2 and 3 show segmentations of the specimens stained with orcein, a marker of elastin fibers. This methodology was performed in a zone of exposure (EX) and compared to the control zone (C) (Fig. 4).

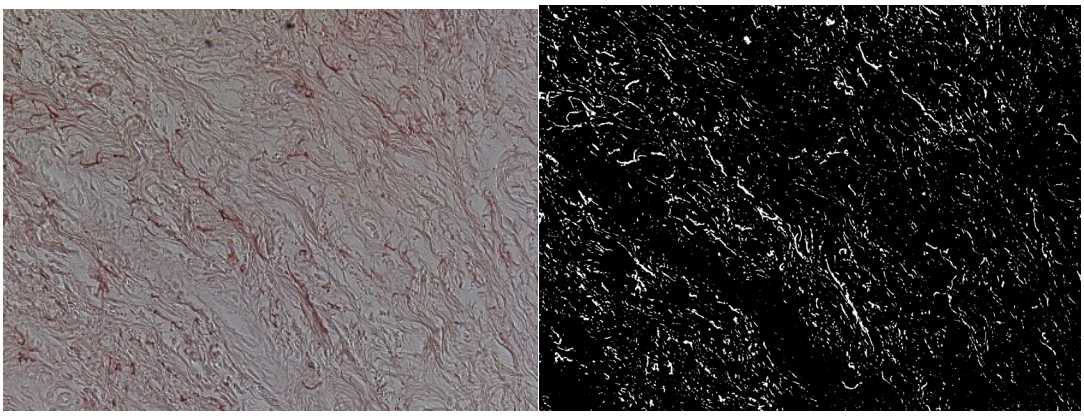

Fig. 2. *An example of the segmentation in the control zone.*

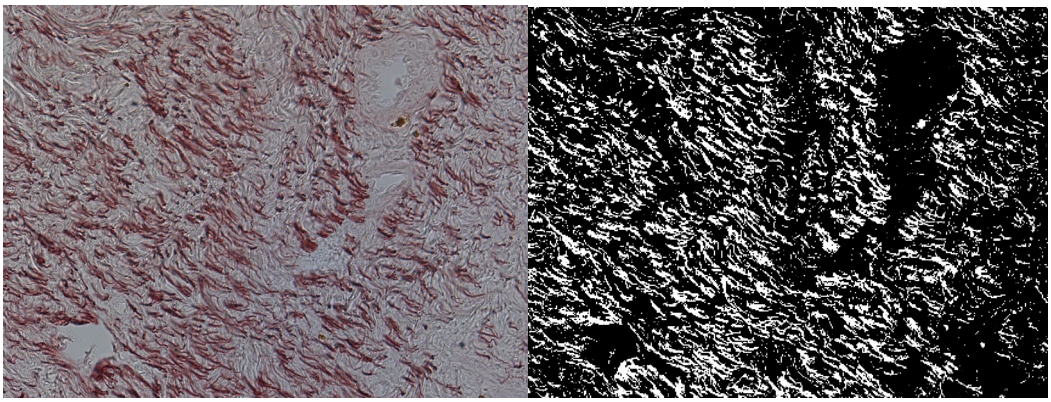

Fig. 3. *An example of the segmentation in the exposed zone.*

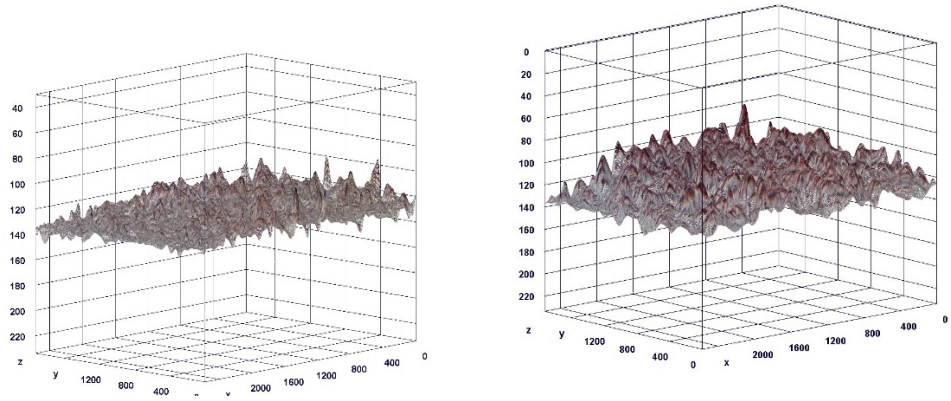

Fig. 4. *Visualization of the differences in elastin fiber concentration between the control and exposure zone from Fig. 2 and 3 (computer program IMAGEL).*

The obtained values are presented in Table I and II, as well as Fig. 5-8.

Table I. *The segmentation results for orcein staining.*

	Object I	Object II	Object III
	Area	Area	Area
C	5.699318	5.322496	5.641315
EX	5.825657	5.97895	5.856866

Table II. *The percentage of elastin fibers concentration for particular objects.*

Object I	72.4%
Object II	25.4%
Object III	60.7%

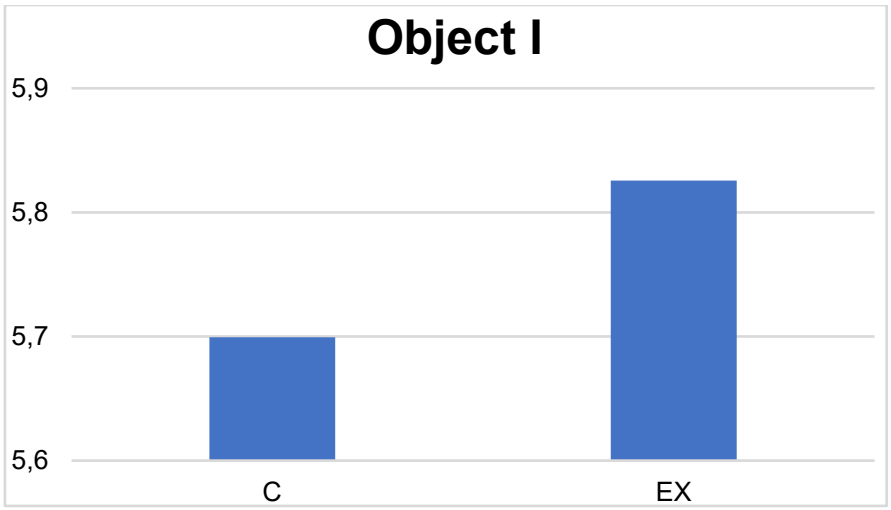

Fig. 5. *In the control zone of the vaginal wall, an orcein activity as a marker of elastin fibers is lower in comparison to the exposed zones.*

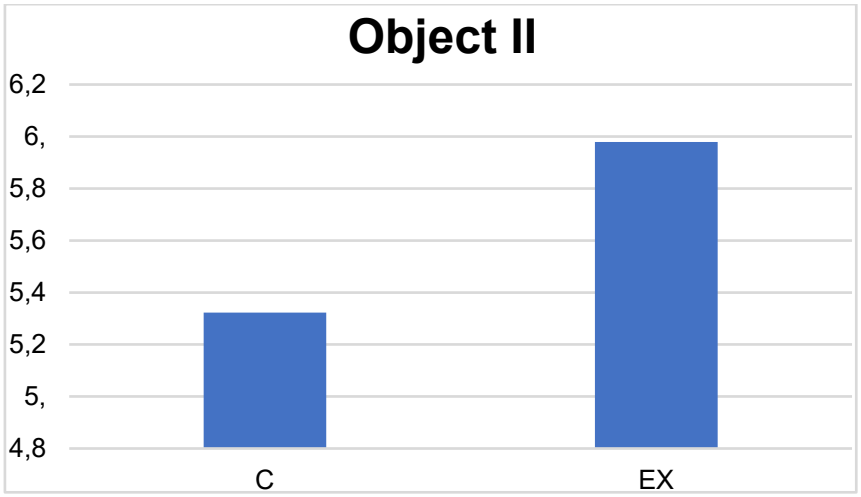

Fig. 6. *In the exposed zones of the vaginal wall, the activity of orcein is higher.*

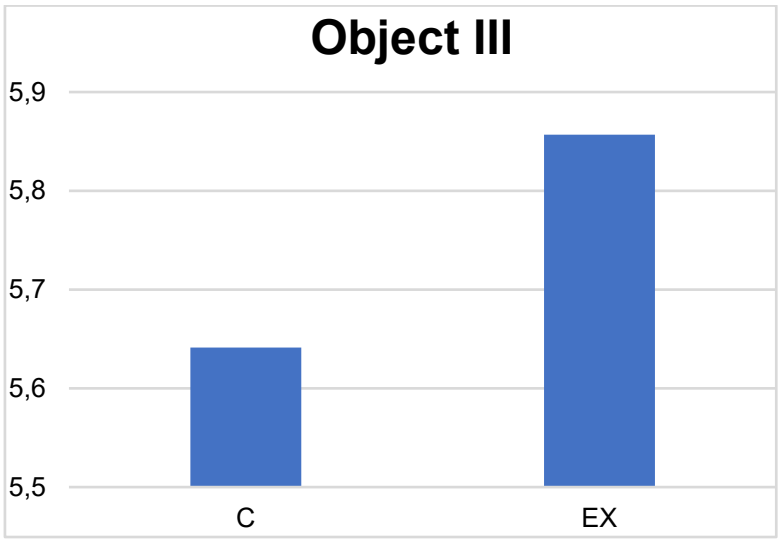

Fig. 7. *In the exposed zones of the vaginal wall, the activity of orcein is higher.*

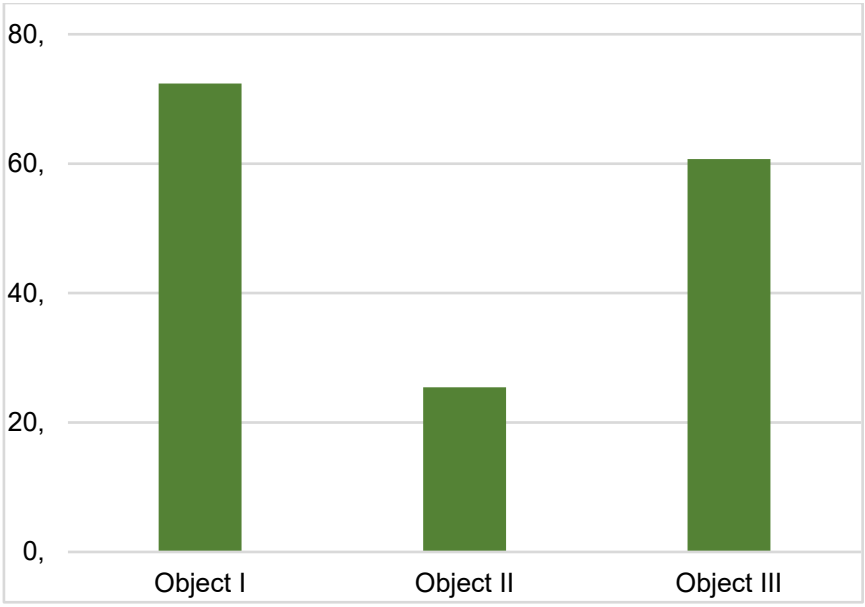

Fig. 8. *An increase of an orcein staining as a marker of elastin fibers in percentages for particular objects.*

The segmentation results for Mallory trichrome staining.

To visualize collagen fibres, Mallory trichrome-stained was performed. The same method of segmentation of staining was used (Fig. 9-11).

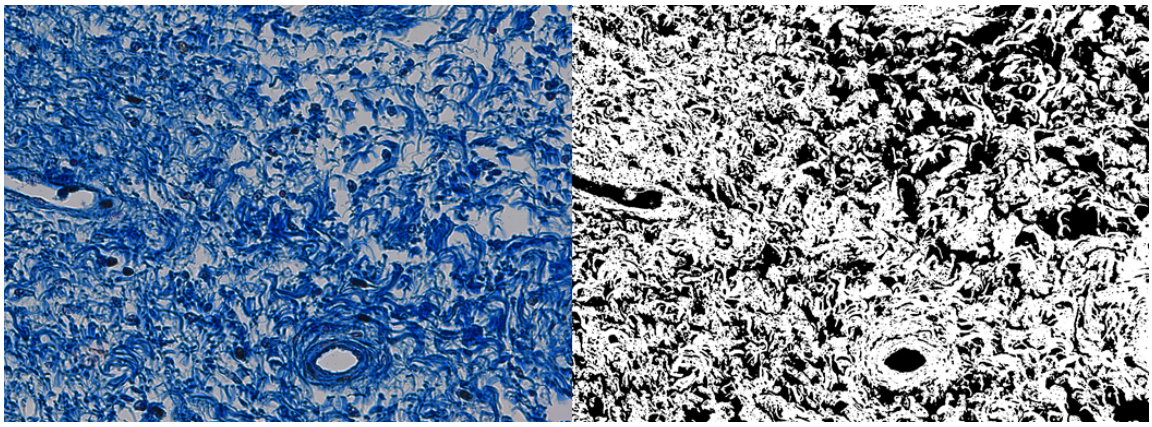

Fig. 9. *An example of the segmentation of the control zone.*

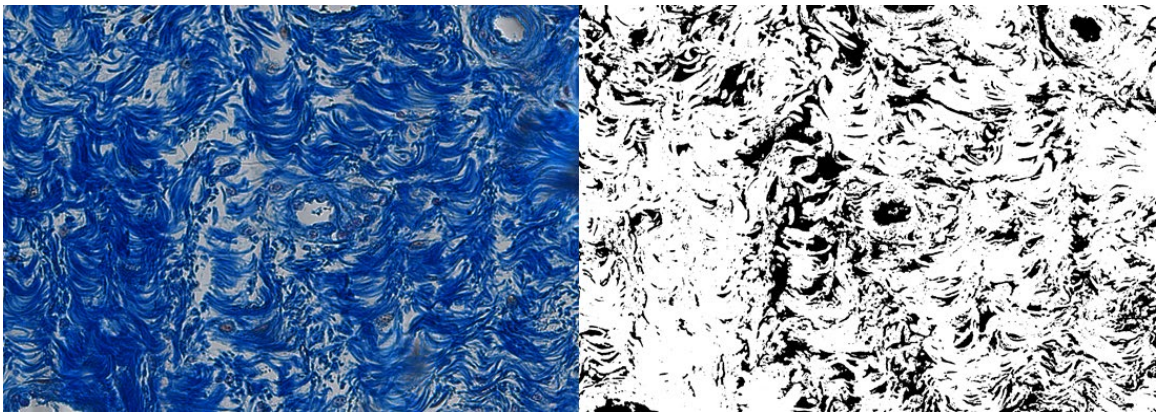

Fig. 10. *An example of the segmentation of the exposed zone.*

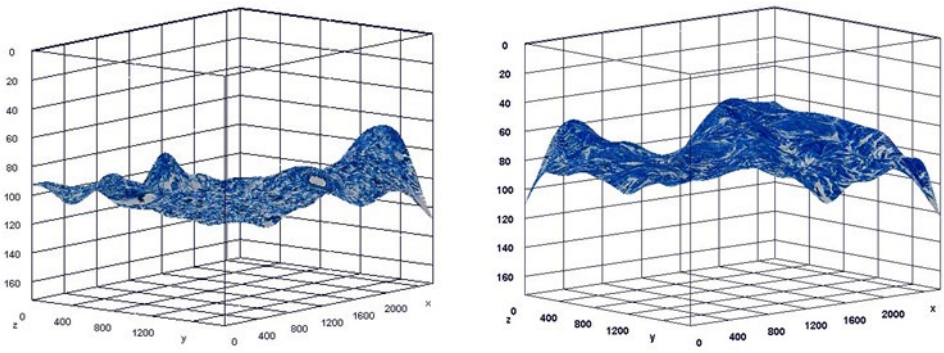

Fig. 11. *Visualization of the differences of collagen fibers between control and exposure zones from Fig. 9 and 10 (computer program IMAGEL).*

In Tables III and IV, as well as Fig. 12-15, the obtained results have been summarized.

Table III. *The segmentation results for Mallory staining.*

	Object I	Object II	Object III
	Area	Area	Area
C	6.523814	6.59573	6.650788
EX	6.563968	6.660167	6.663562

Table IV. *The percentage of collagen concentration for particular objects.*

Object I	109%
Object II	100%
Object III	102%

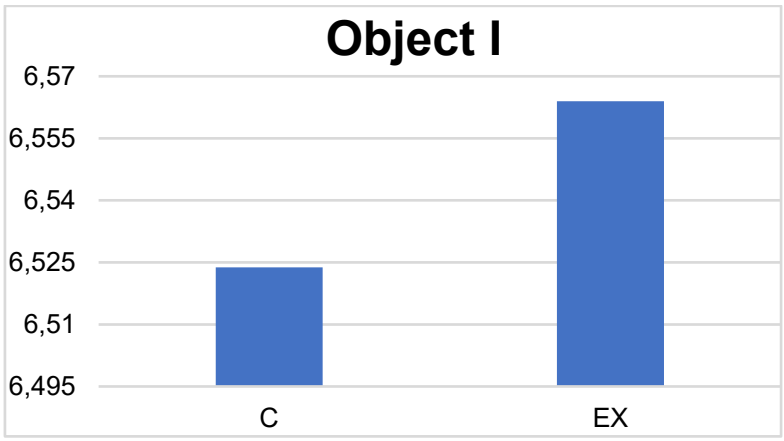

Fig. 12. *A comparison between the control zone and the exposed zones.*

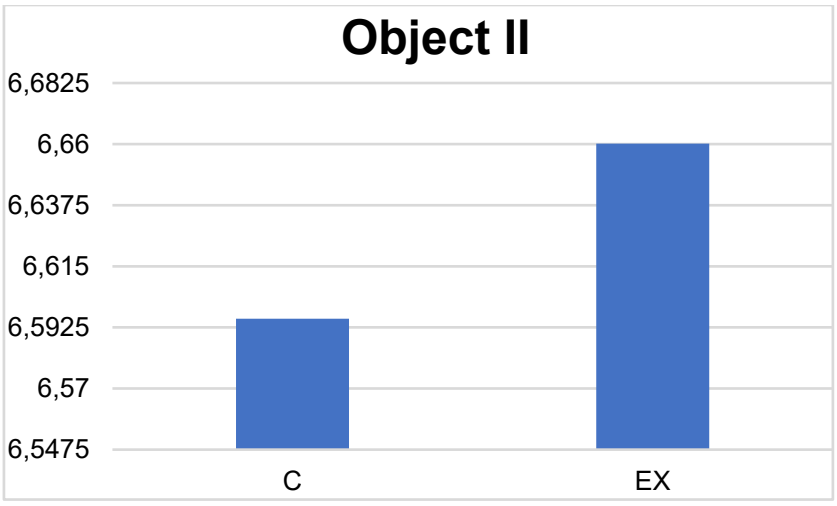

Fig. 13. *A comparison between the control zone and the exposed zones.*

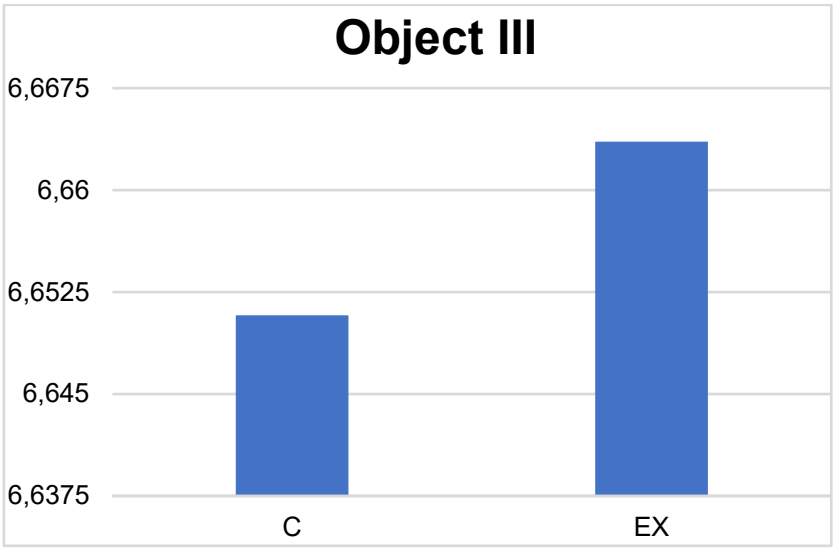

Fig. 14. *A comparison between the control zone and the exposed zones.*

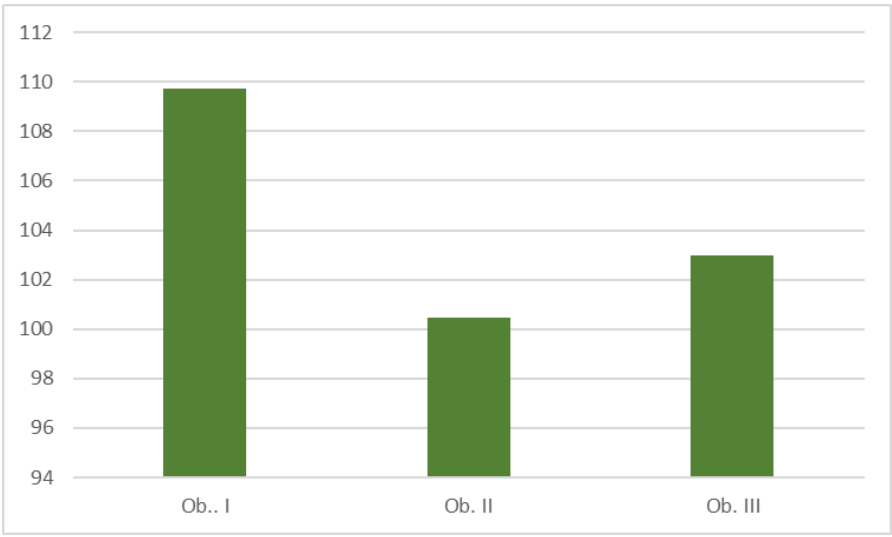

Fig. 15. *A rise of collagen fibers identified by Mallory staining was quantitively similar for particular objects.*

We also attempted to identify the number of fibroblasts/fibrocytes by counting cell nuclei after staining with hematoxylin in the same areas where we assessed the concentration of elastic fibers (Fig. 16, 17).

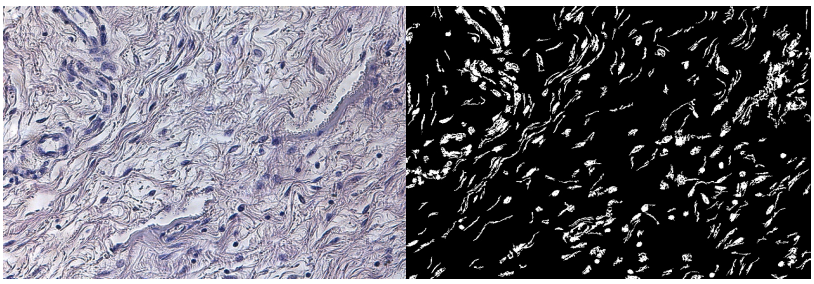

Fig. 16. *The segmentation of a control zone stained with hematoxylin.*

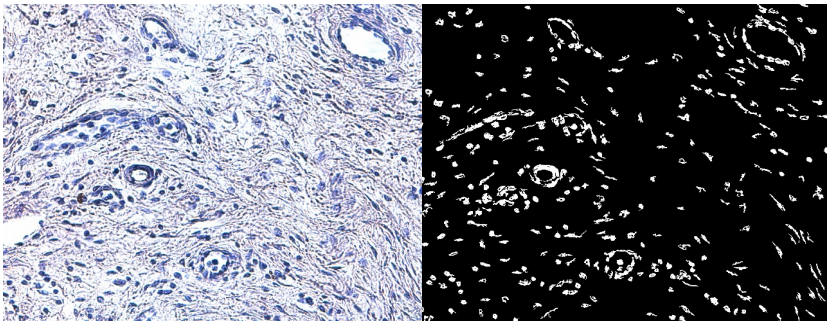

Fig. 17. *The segmentation of an exposed zone stained with hematoxylin.*

Artifacts were found during the segmentation process, which can distort the study's final results. Therefore, we used shut-off filtering as part of the segmentation program and rejected the segmented nuclei belonging to the cells of the vascular walls (Fig. 18).

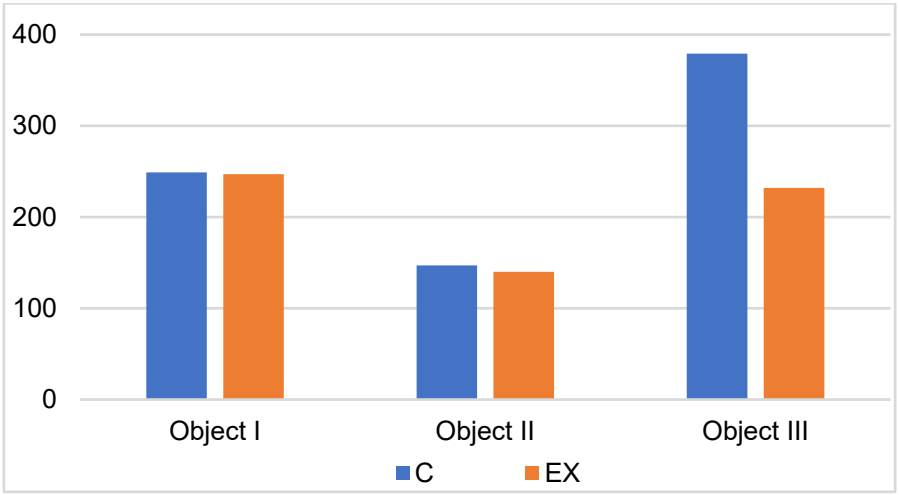

Fig. 18. *The results of segmenting the fibroblasts/fibrocyte nuclei for particular objects showed a significant dispersion in specific areas.*

According to the measured data, there is a pronounced discrepancy between investigated objects, which makes it impossible to interpret them unequivocally. However, it can be suggested that during our experiment, there was only an apparent increase of collagen and elastin fibers with no perceptible proliferation of fibroblasts/fibrocytes.

An examination of deeper layers > 1.3mm to assess the working range of radiofrequency.

To assess the depth of action of RF Sectum, we studied deeper layers of the vaginal walls by measuring collagen concentration as described above (Fig. 19, 20).

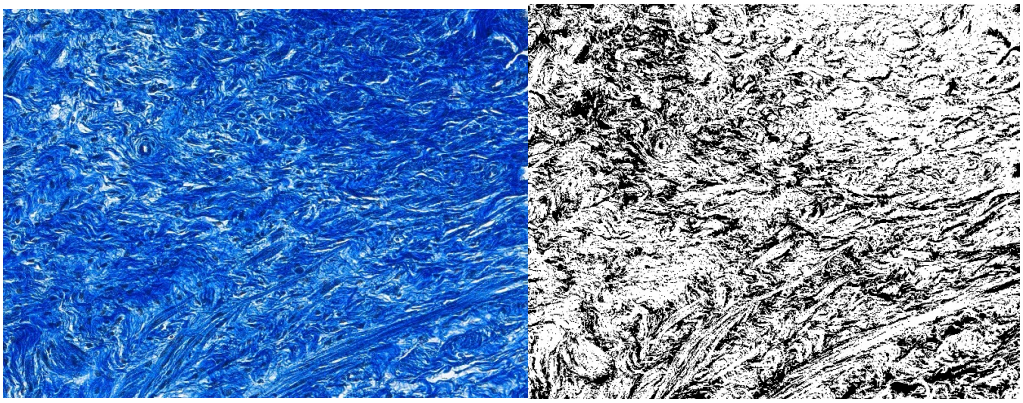

Fig. 19. *The segmentation of a specimen in the control zone.*

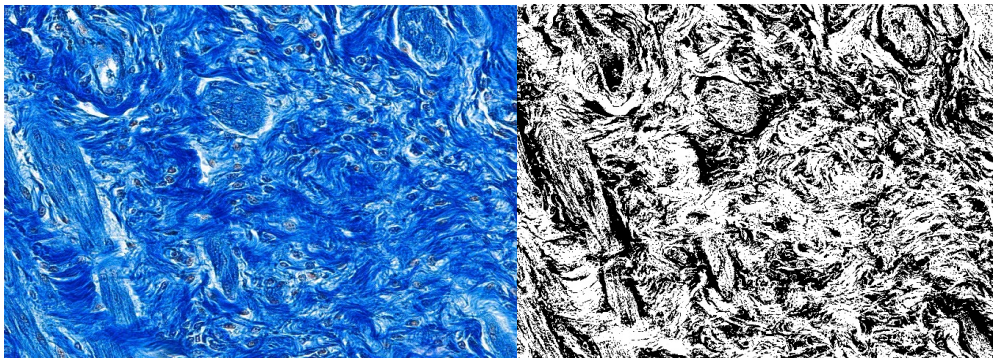

Fig. 20. *The segmentation of a specimen in the exposed zone.*

The obtained values are presented in Table V and VI, as well as Fig. 21-24.

Table V. *The percentage of collagen concentration for all investigated objects.*

	Object I	Object II	Object III
C	6.557893	6.622702	6.591465
EX	6.51468	6.651847	6.668145

Table VI. *The percentage of collagen concentration for all investigated objects.*

C	100
A	100.077
B	99.322
Cex	99.653

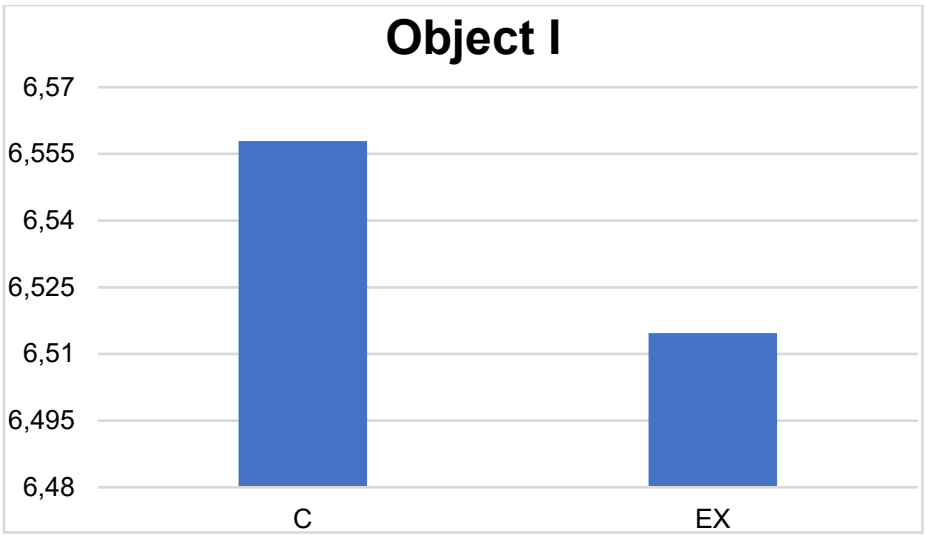

Fig. 21. *A comparison of zones for an object I.*

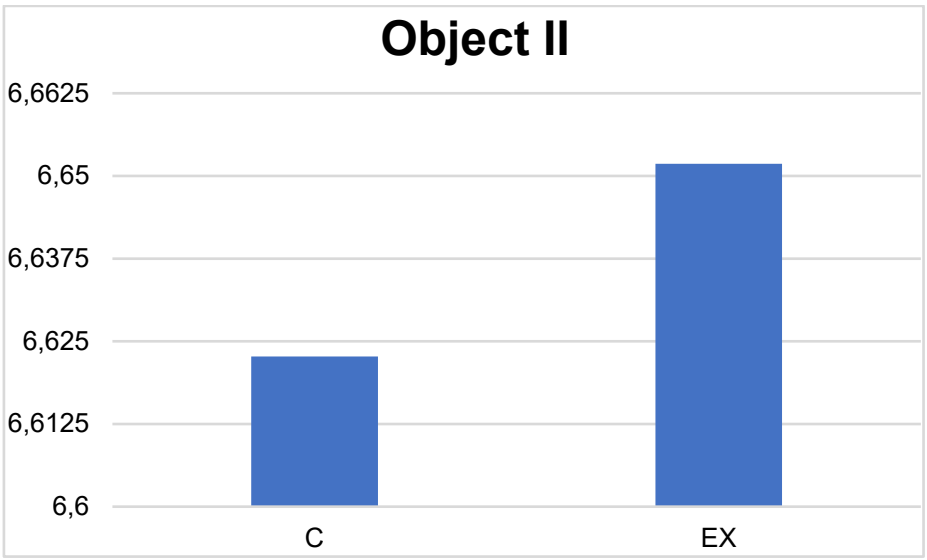

Fig. 22. *A comparison of zones for an object II.*

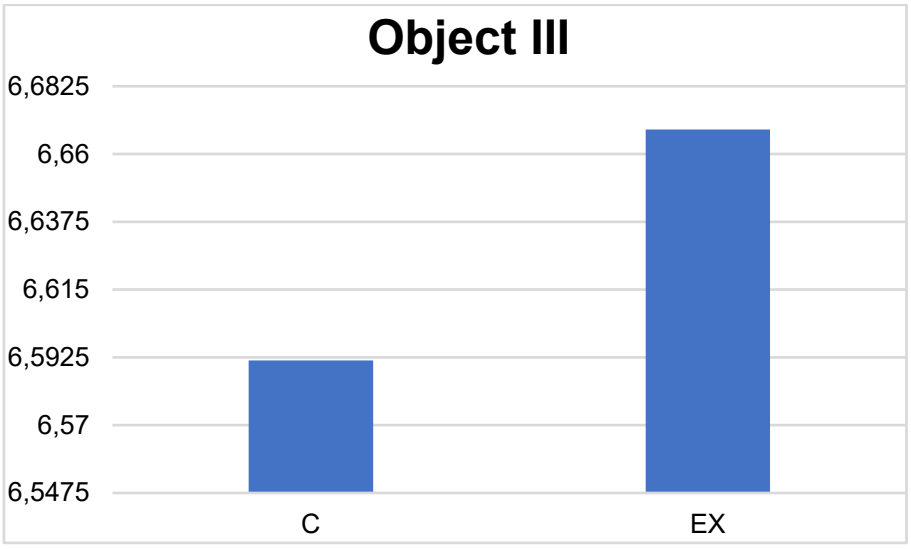

Fig. 23. *A comparison of zones for an object III.*

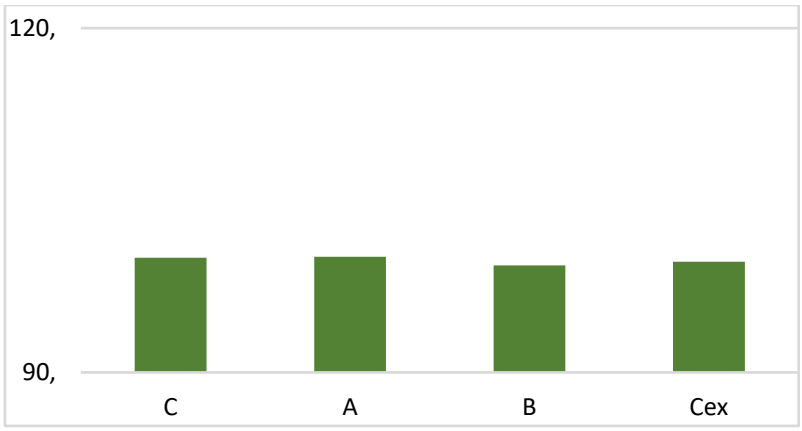

Fig. 24. *The percentage of collagen concentration for all investigated objects.*

According to the data, there are no perceptible changes in collagen concentration in deeper (> 1.3 mm) layers of vaginal walls.

An assessment of a vascularization of a vaginal wall.

To evaluate the changes in vessel concentration, we utilized Mallory staining, which allowed us to distinguish the vessels' lumen (Fig. 25-27). Measurements were performed at the levels just under the lamina propria and deeper. Similarly to the previous measurements, quantitative measures were conducted by summating extracted areas (vessel lumen) in pixels. Since the segmented areas in pixels were extremely high, a logarithmic scale was used.

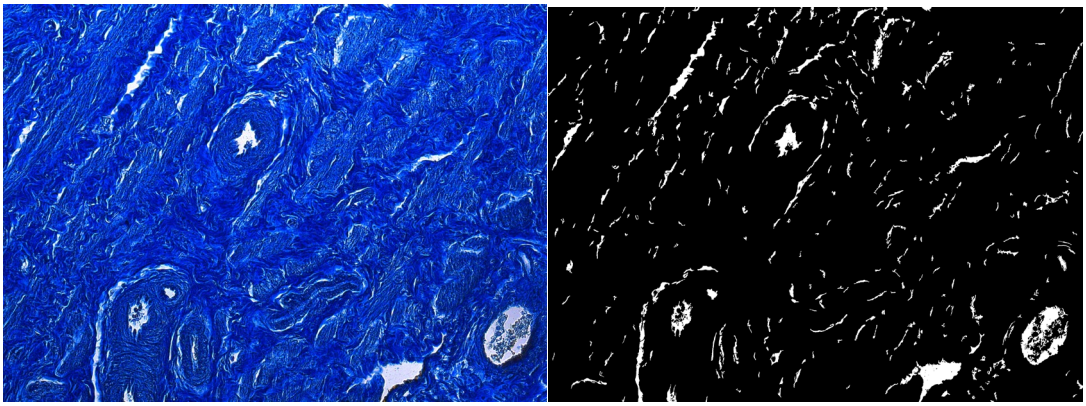

Fig. 25. *The segmentation of a specimen in the control zone.*

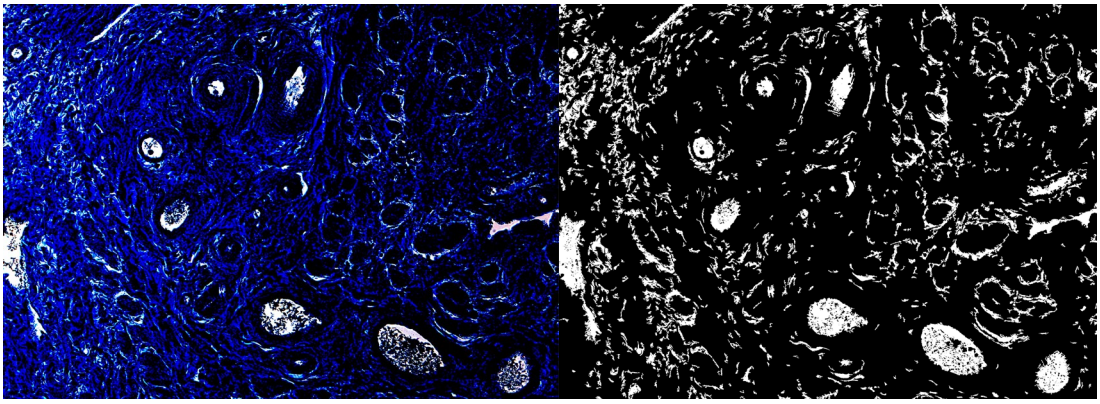

Fig. 26. *The segmentation of a specimen in an exposed zone just under lamina propria- shallow layer.*

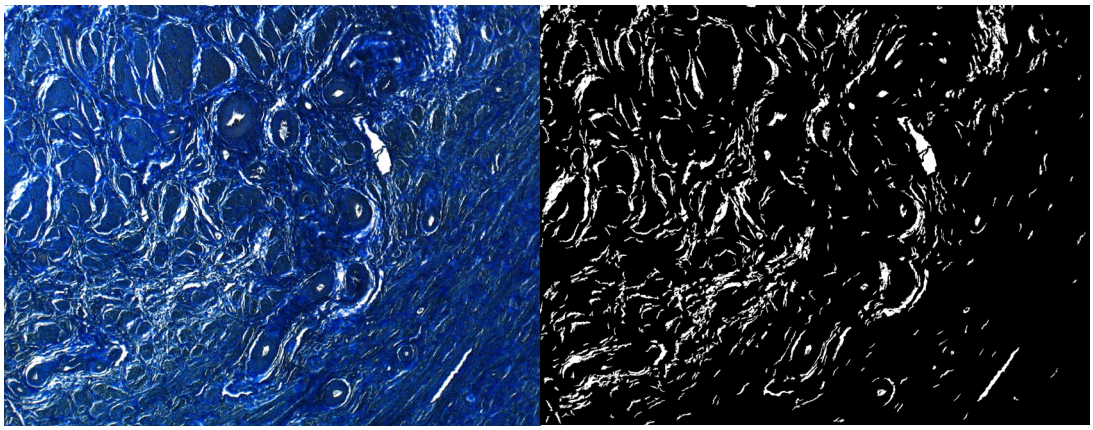

Fig. 27. *The segmentation of a specimen in an exposed zone in a deeper layer > 1.3 mm.*

Data for particular objects

A comparison of several vessels of a control zone and an averaged number of vessels in exposed zones with a simultaneous comparison of shallow (S) and deeper (D) layers for Objects I, II, and III (Fig. 28-30).

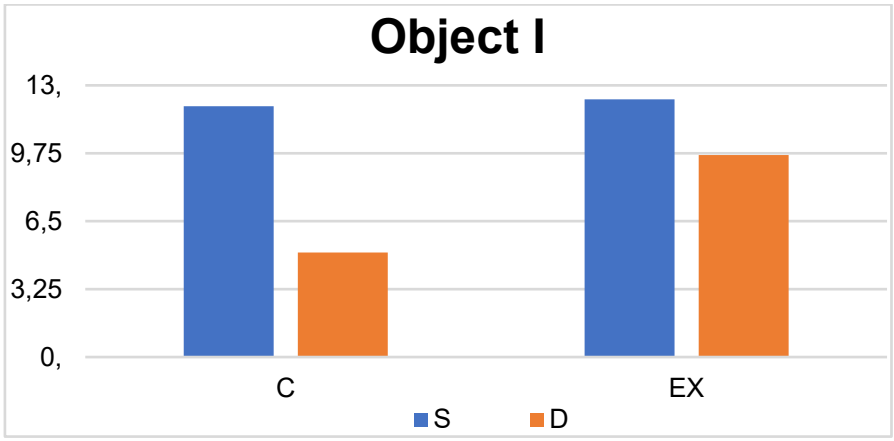

Fig. 28. *A comparison of several vessels in a control zone and an averaged number of vessels in exposed zones with a simultaneous comparison of shallow (S) and deeper (D) layers for Object I.*

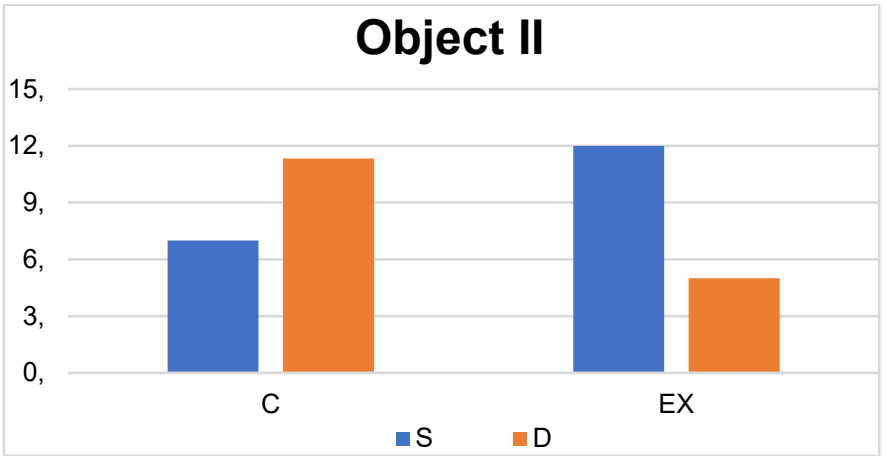

Fig. 29. *A comparison of the number of vessels in a control zone and an averaged number of vessels in exposed zones with a simultaneous comparison of shallow (S) and deeper (D) layers for Object II.*

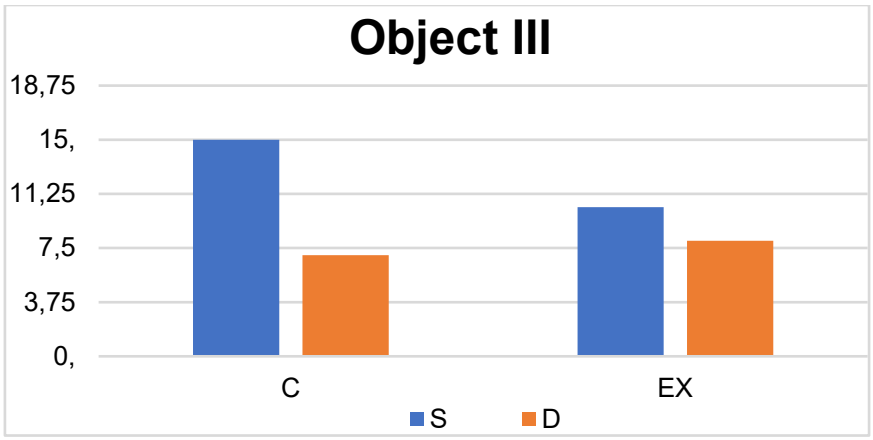

Fig. 30. *A comparison of several vessels of a control zone and an averaged number of vessels in exposed zones with a simultaneous comparison of shallow (S) and deeper (D) layers for Object III.*

The measured data show significant diversity for all zones. We can state that irrespective of exposure to RF, the layers just under lamina propria are better vascularized compared to the deeper layers. A degree of data variability cannot lead to a conclusion concerning the influence of RF heating on several vessels in the evaluated areas.

DISCUSSION

There has been a rise in the number of energy-based devices for the treatment of sexual dysfunction, vulvovaginal laxity, and genitourinary syndrome of menopause (GSM) during the previous ten years (1, 2, 4, 5). Very recently, the use of radiofrequency for the treatment of vaginal laxity, sexual dysfunction, and GSM has demonstrated promising treatment results (4, 11, 12, 14).

We researched the vaginal wall of domestic pigs to evaluate the impact of a 480 kHz bipolar radiofrequency that was generated by the 360 intravaginal applicator (Berger and Kraft Medical). When histological photos of the vaginal walls were evaluated, they were stained with hematoxylin and eosin. Examining these pictures revealed that the exposed portions showed no discernible changes. A study that was conducted in a similar manner and headed by Kent (9) indicated that the lamina propria looked to be more compact with a denser arrangement of fibers at the follow-up point. Taking into consideration the findings of previous research (8, 9), we also attempted to determine the concentration of elastin and collagen, the quantity of fibroblasts and fibrocytes, and the number of vessels in control and exposed areas of the vaginal wall. These areas were stained with orcein as a marker of elastin fibers and vessels, Mallory trichrome as a marker of collagen fibers, and hematoxylin and eosin.

The KS 300 (Zeiss) scanner, which segmented and counted the area of interest displayed in detail above, was used to determine the strength of the reaction. All of the segmented sections were examined, and the results showed a significant increase in the concentration of elastin and collagen fibers in the areas that had been subjected to radiofrequency for up to 1.3 mm of the thickness of the vaginal wall. In the sections of the vaginal wall that were exposed to radiofrequency energy, there was an increase of 52.8% in the concentration of elastin and 103.6% in the concentration of collagen. According to the findings, there are no discernible shifts in the collagen concentration in deeper layers of the vaginal wall (layers with a thickness of more than 1.30 millimeters).

At the same time, we discovered a considerable dispersion in the amount of fibrocytes and fibroblast nuclei for certain objects in segmented areas, and we saw a little notable down-ward tendency in this quantity. This

discovery is distinct from the results provided by other research (8, 9), and it may indicate that while the experiment is being conducted, fibroblasts are induced to create collagen and elastin fibers without their detectable proliferation. In addition, we can assume that a slightly marked downward trend in their amount may result from an increasing amount of the intercellular matrix composed of collagen and elastin, which separates fibroblasts and fibrocytes from one another. This is because the intercellular matrix moves fibroblasts and fibrocytes further apart.

A measurement of the number of vessels showed a large variability for all zones, both in control and exposure regions. Because of this, it is impossible to arrive at a conclusion regarding the influence of radiofrequency generated by RF on several vessels in evaluated areas.

CONCLUSION

The current study demonstrated that increasing the amount of collagen and elastin fibers in the vaginal wall could be achieved through the use of bipolar radiofrequency heating delivered by an intravaginal applicator (Berger and Kraft Medical). We can extrapolate these results to the human vagina in terms of rejuvenation and improvement of the vaginal wall quality due to the similarities between the vaginal tissues of pigs and humans. The findings of this study provide further evidence for the therapeutic efficacy of the treatment and offer a roadmap for future research. However, since this is only a preliminary investigation with a limited number of subjects, further research will be necessary to validate our findings and assess the long-term consequences of combination treatment.

Author Contributions:
Conceptualization, P.K.; methodology, M.P. and R.K.; software, A.K.K.; validation, M.B., and M.Ł.; formal analysis, J.U., A.K. and D.B.-W.; investigation, N.Z.; resources, D.G. and A.K.; data curation, A.B. and D.B.-W.; writing, original draft preparation, P.K.; writing, review and editing, M.K.; visualization, M.P. and R.K.; supervision, A.C.P.; project administration, N.Z.
Declarations of interest: none

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