GREDECO

Groupe de Recherche et d'Evaluation en Dermatologie et Cosmétologie
(Group of Research and Evaluation in Dermatology and Cosmetology)

EVALUATION OF THE ANTI-CELLULITE ACTION OF AN ULTRASOUND DEVICE USING EXPLANTED HUMAN SKIN

The 20th June 2013

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SUMMARY

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Traduction libre effectuée par Christophe HOTTINGER le 26/06/2013, relue et corrigée par Russel SMITH le 3 juillet 2013.

Free translation done by Christophe HOTTINGER the 26th June 2013, reread and corrected by Russel SMITH the 3th July 2013.

I. OBJECTIVE

The objective of the study was to evaluate, using human skin explants, the **anti-cellulite** activity **and the safety of a very low frequency ultrasound device (CAVIFAST 2).**

The aesthetic claim is lipolysis or reduction of the volume of adipocites by mobilization and removal of triglycerides stored in adipocites. The effect was evaluated in our model by dosage of the glycerol released from adipocites after treatment.

The medical claim is lipoclasia or non-invasive lipoclastie due to the modification of adipocites by membrane cell disruption. This claim was analyzed by histological evaluation of skin to allow viewing the cavitational lipolysis and check the safety of the device (compliance with the essential structures of the skin such as blood and lymph vessels).

II. REMINDER

1-Definition of "cellulite":

"Cellulite" is a particular configuration of female adipose tissues. The dimpled appearance of the skin of the buttocks and thighs is due to the spatial configuration of the fat lobules separated by partitions perpendicular to the surface of the skin. These lobules are protruded into the dermis and attract the skin surface by reaction in depth. At the histological level, cellulite is a modification of the subcutaneous connective tissue (subcutis) with adipocite hyperplasia composed of adipocites rich in fat, vasodilatation and venous or lymphatic stasis.

The accumulation of fat takes effect in the adipocites from triglycerides and sugars. Lipolytic function in shown within the adipocites by a hydrolysis of the triglycerides by the triglyceride lipase, thus the liberated glycerol will reflect the intensity of the lipolysis.

2-Functioning of the CAVIFAST 2 device:

The CAVIFAST 2 device emits very low frequency ultrasounds from the skin surface that can penetrate deeply up to 4 cm in the skin. This depth corresponds to an action in the innermost layer of the skin: the hypodermis layer of fat reserves in the body. Ultrasound is emitted by the transducers also known as hand pieces. They

convert electrical energy into mechanical ultrasound energy. A transducer is composed of two pieces of piezoceramic adjusted so as not to create overheating. When activating the device, the two pieces of piezoceramic vibrate together creating ultrasound.

The cavitational lipolysis is based on the technology of ultrasonic cavitation effect using very low frequencies emitted at 38 kHz + / - 2 kHz.

The cavitational effect leads to the formation of bubbles in the water existing in the tissue of the hypodermis due to pressure and decompression of sound waves. In imploding, these bubbles weaken the membrane of fat cells and accelerate the exchanges in metabolism. Triglycerides, initially contained in the fat cells, are hydrolyzed in glycerol (lipolysis) which then passes throughout the lymphatic and circulatory system to be eliminated by the liver, kidneys and macrophages.

In addition, ultrasound, due to its hypothermic property, stimulates cell metabolism and blood flow, assisting better drainage.

III. MATERIAL AND METHOD

Fragments of regular human skin (abdominoplasties; n= 5) are obtained by plastic surgery. Transported in a swab of physiological serum, skin is cut into many pieces and rinsed in phosphate buffer pH 7.4 containing antibiotics (500 µg/ml gentamicin and fungizone). The treatment session with CAVIFAST 2 device has been done for the first donor using an intensity of treatment of 100%, 60% and 52% during 10 minutes.

Temperature controls have been performed and found a temperature of 45°C (at 60% intensity) and 65°C (at 100% intensity) therefore it was decided to use treatment intensities between 50 and 75% for the 4 other donors.

A comparison with the untreated control skin was performed to allow the evaluation of the treatment efficacy of the device.

The samples were taken in triplicate with fixing in formalin for histological evaluations and grinding for determination of the glycerol content.

IV. EVALUATION OF THE EFFICIENCY AND THE SAFETY

A) Treatment implementation

The flat transducer of CAVIFAST 2 device was used on donor #1 by folding up the skin (pinching the skin). The results are alike for part of the skin, the part in direct contact with the transducer and the opposite part; the treatments have been done for the 4 other donors without pinching the skin.

B) Histological analysis

A comparative study between treated and untreated skin was used to visualize and quantify the modification of adipose tissue. To do this, a histological staining by hemalun-eosin was performed (analysis in triplicate and serially cut).

C) Dosage of glycerol

Glycerol released was determined by enzymatic method according to Vaughan.

Briefly, the conversion of glycerol to glycerol-3-phosphate by the glycerokinase will produce ADP. ADP produced by this first step is again converted into ATP with the formation of pyruvate. Secondly, the conversion of pyruvate with the oxidizing of NADH will produce an amount of NAD + (proportional to the amount of glycerol) and lactate. The oxidation of NADH has been dosed by the spectrophotometer at 365 nm.

The final result allows to quantify the glycerol released and was expressed in µg by gramme of adipose tissue.

V. RESULTS

A) Histological analysis

The adipocites modifications of the hypodermis after treatment with CAVIFAST 2 device are identical for the five donors.

Representative photographs of these changes are reported as follows.

1- Results for donor #1

For the untreated skin, the hypodermis is composed of regular rounded shaped adipocites.

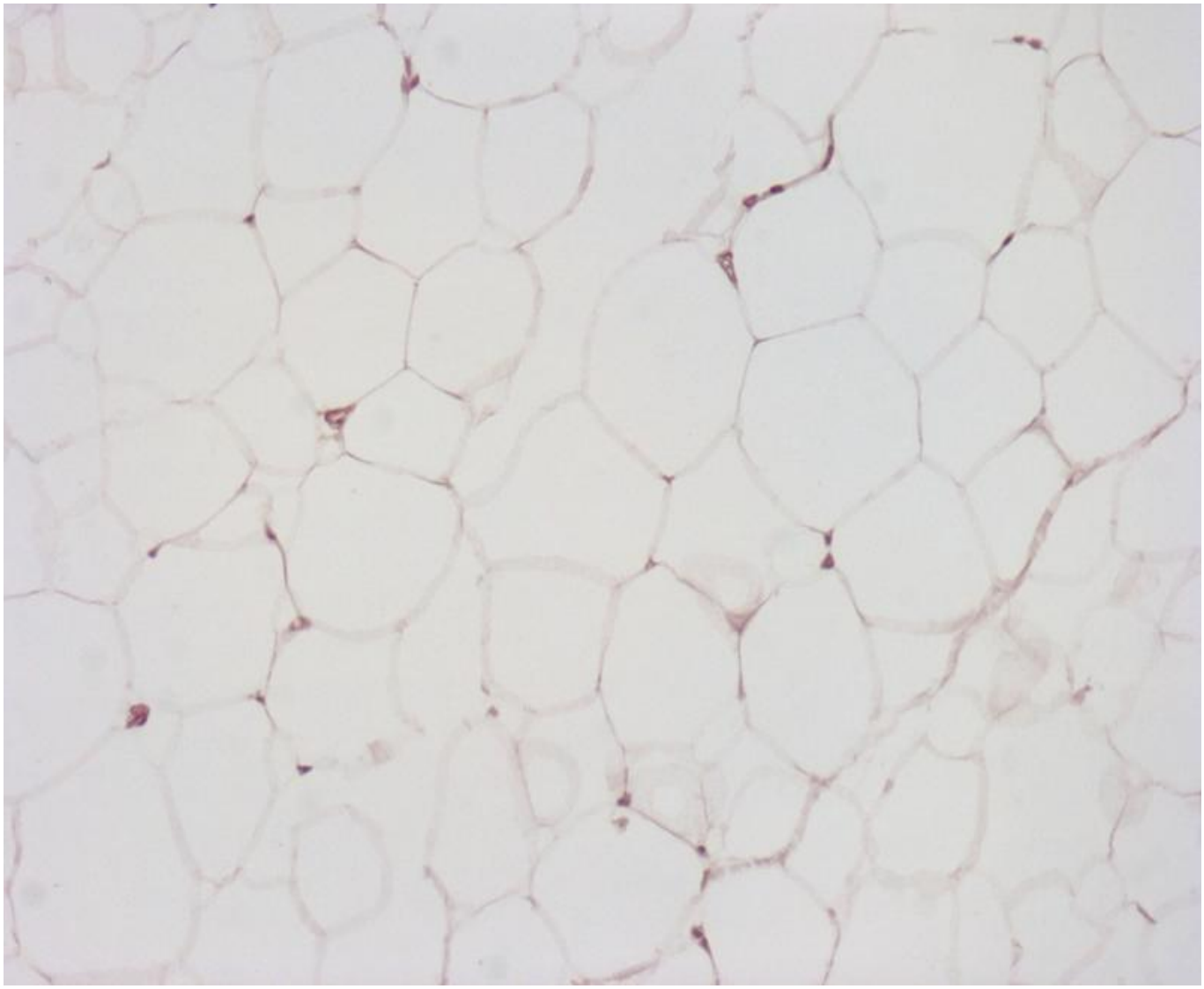


Figure 1: skin before treatment (x200)

After treatment (intensity 100, 62 and 50%), same level changes have been observed at the hypodermis level confirming the mechanism of cavitational lipolysis.

Indeed a very jagged contour of the adipocites is observed with some of them showing membrane's disruption; consequently increasing the size of the adipocites.

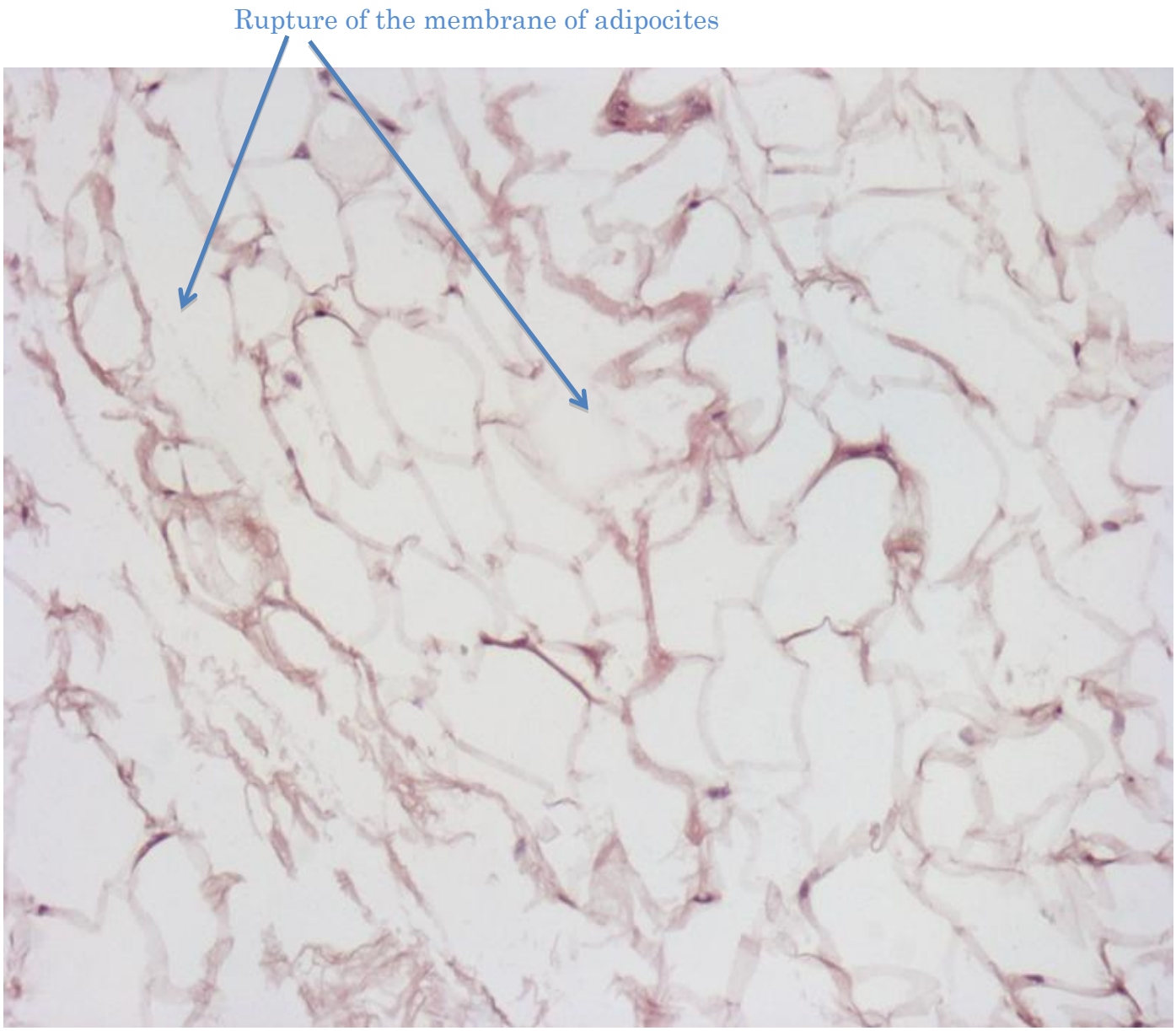


Figure 2: skin after treated by CAVIFAST 2 (x200)

At 60 and 52% treatment intensity the collagen bundles in contact with the hypodermis are not distorted. A comparison between the skin before and after treatment is performed in the following two photographs:

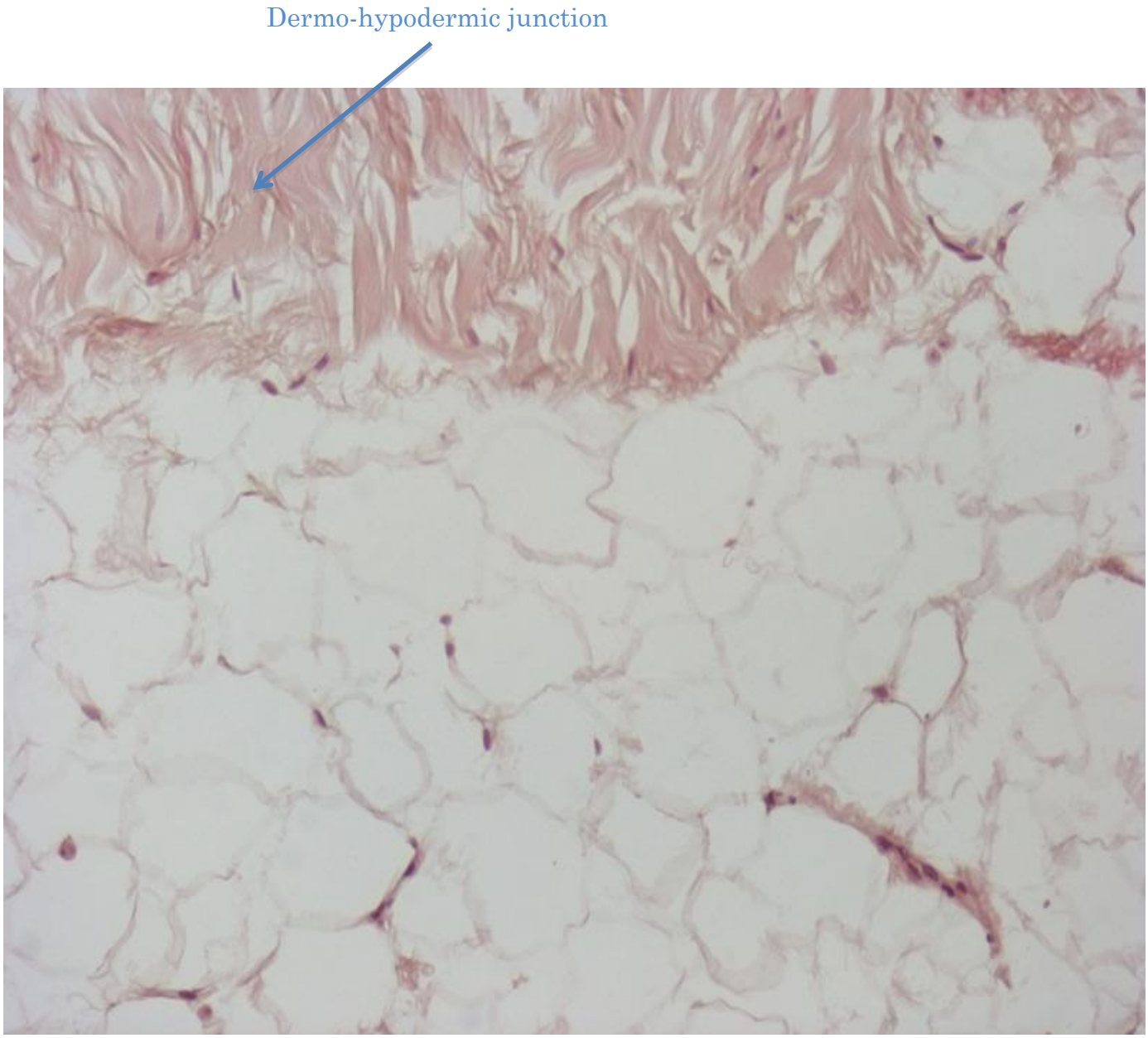


Figure 3: skin before treatment (x200)

Absence of alteration of the dermo-hypodermic junction

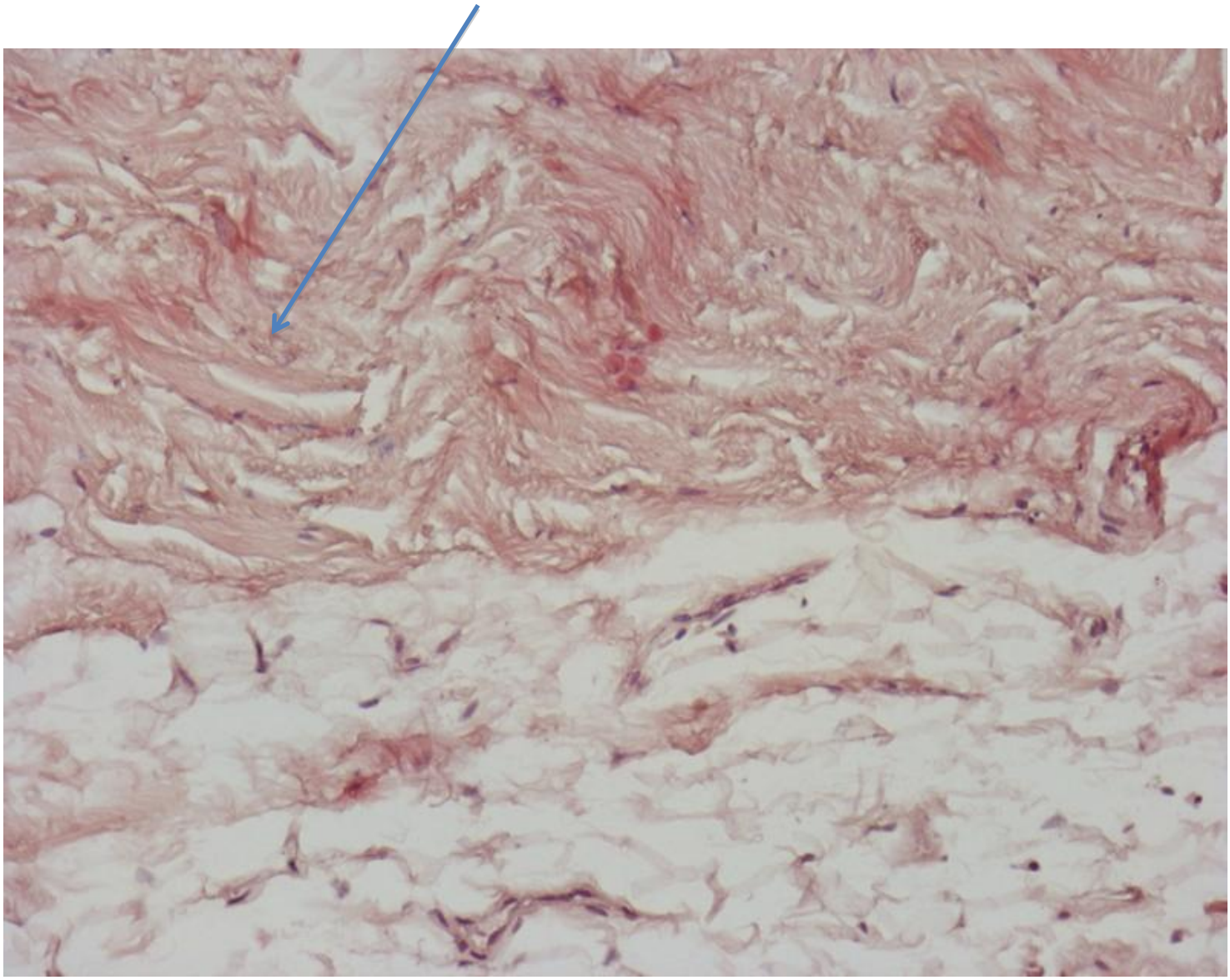
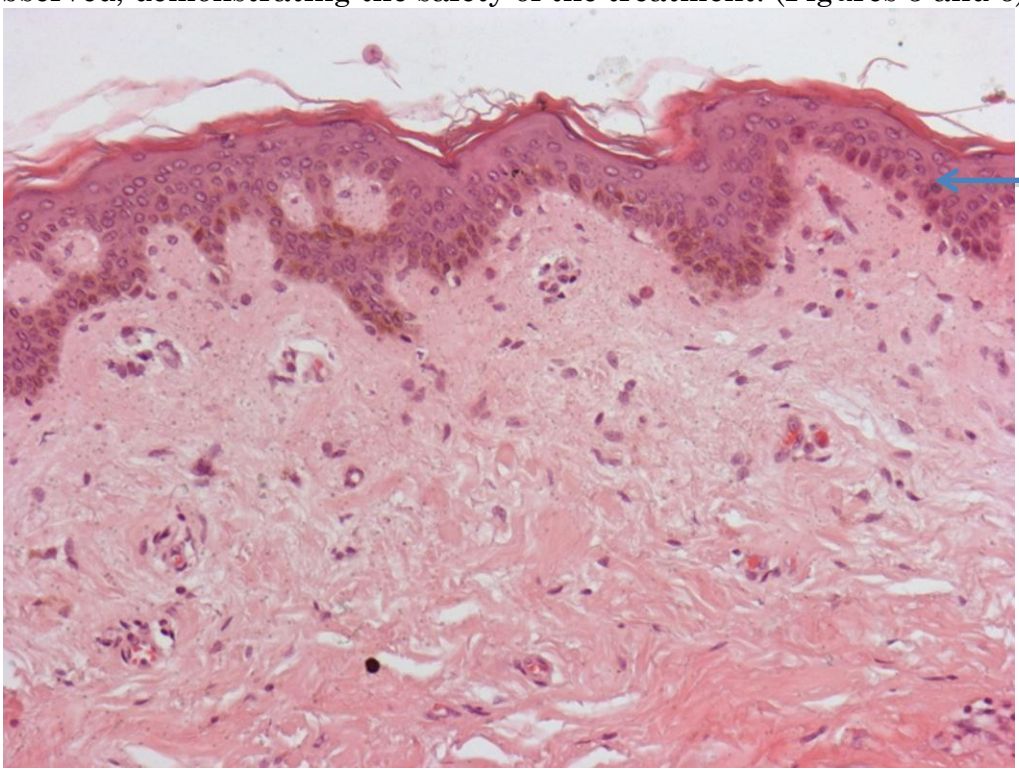
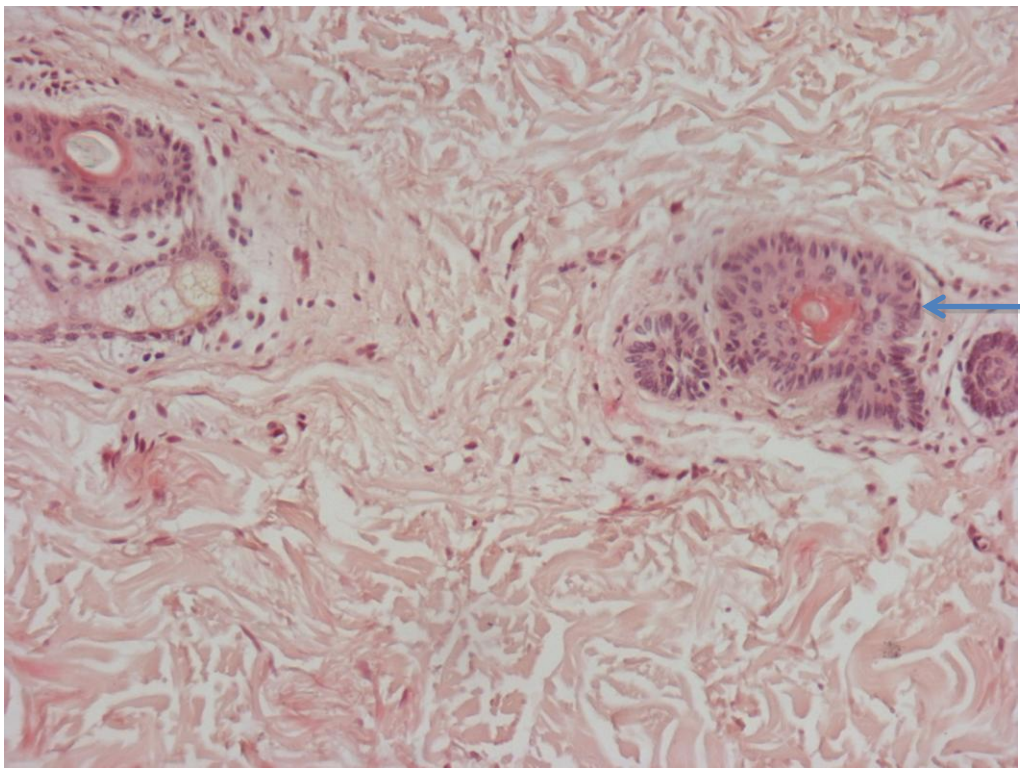


Figure 4: skin treated by CAVIFAST 2 (x200)

The treatment with the device using treatment intensities from 50 to 100% does not cause alteration of epidermis or superficial dermis or middle dermis. Furthermore, no alteration of the annexes or of the blood and lymph vessels was observed, demonstrating the safety of the treatment. (Figures 5 and 6)





2- Results for donor #2

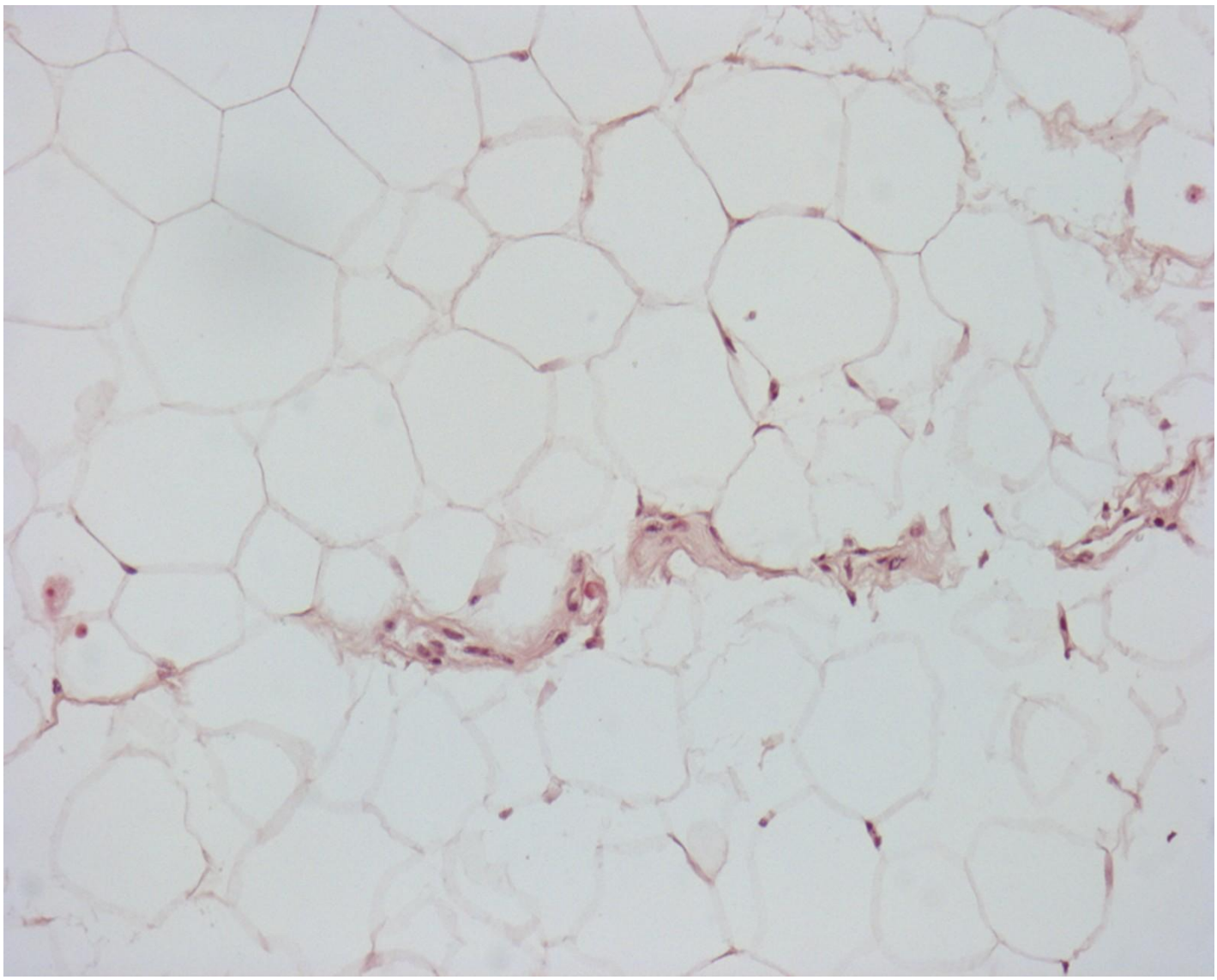


Figure 7: skin before treatment (x200)

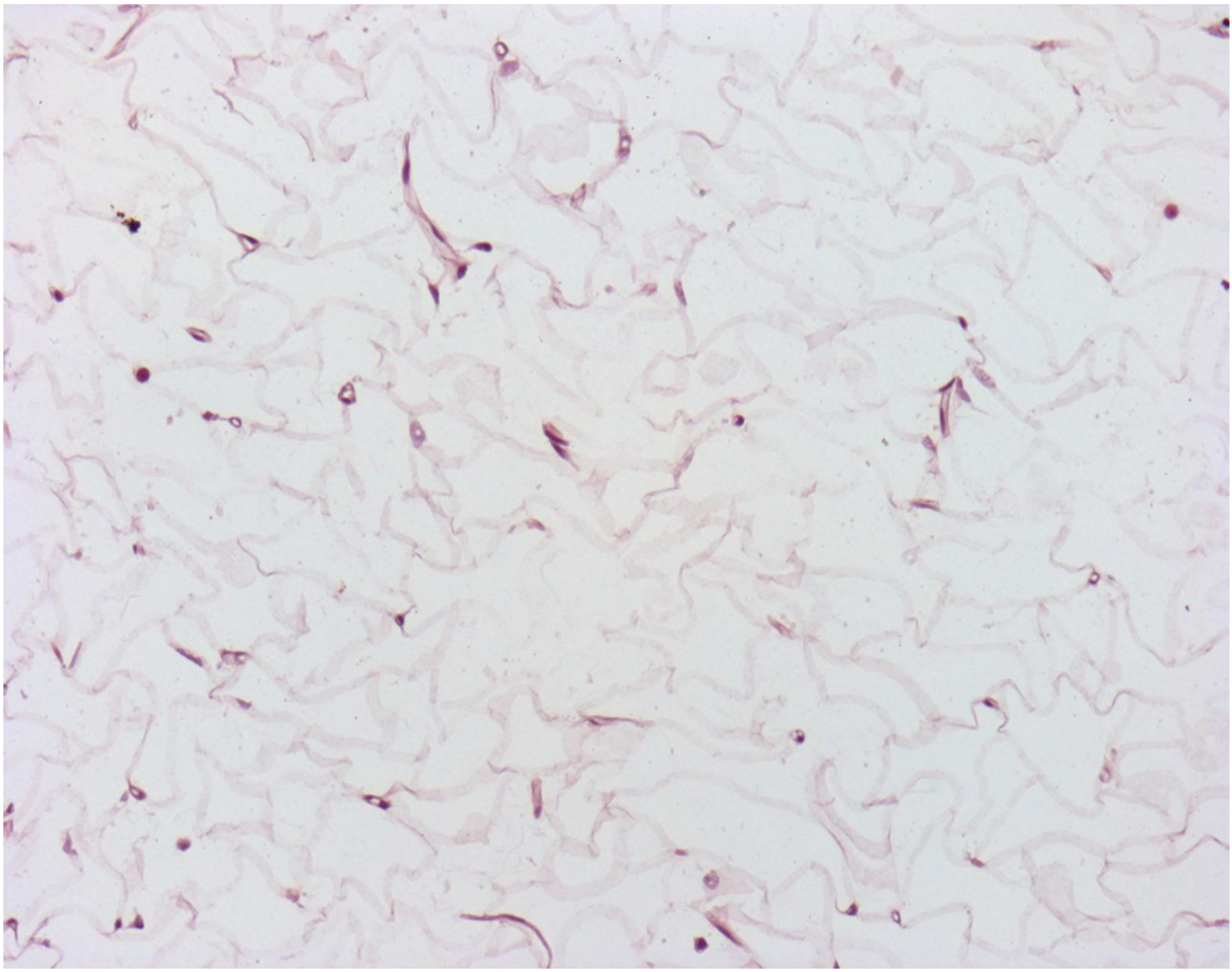


Figure 8: skin treated by CAVIFAST 2 (x200)

3- Results for donor #3

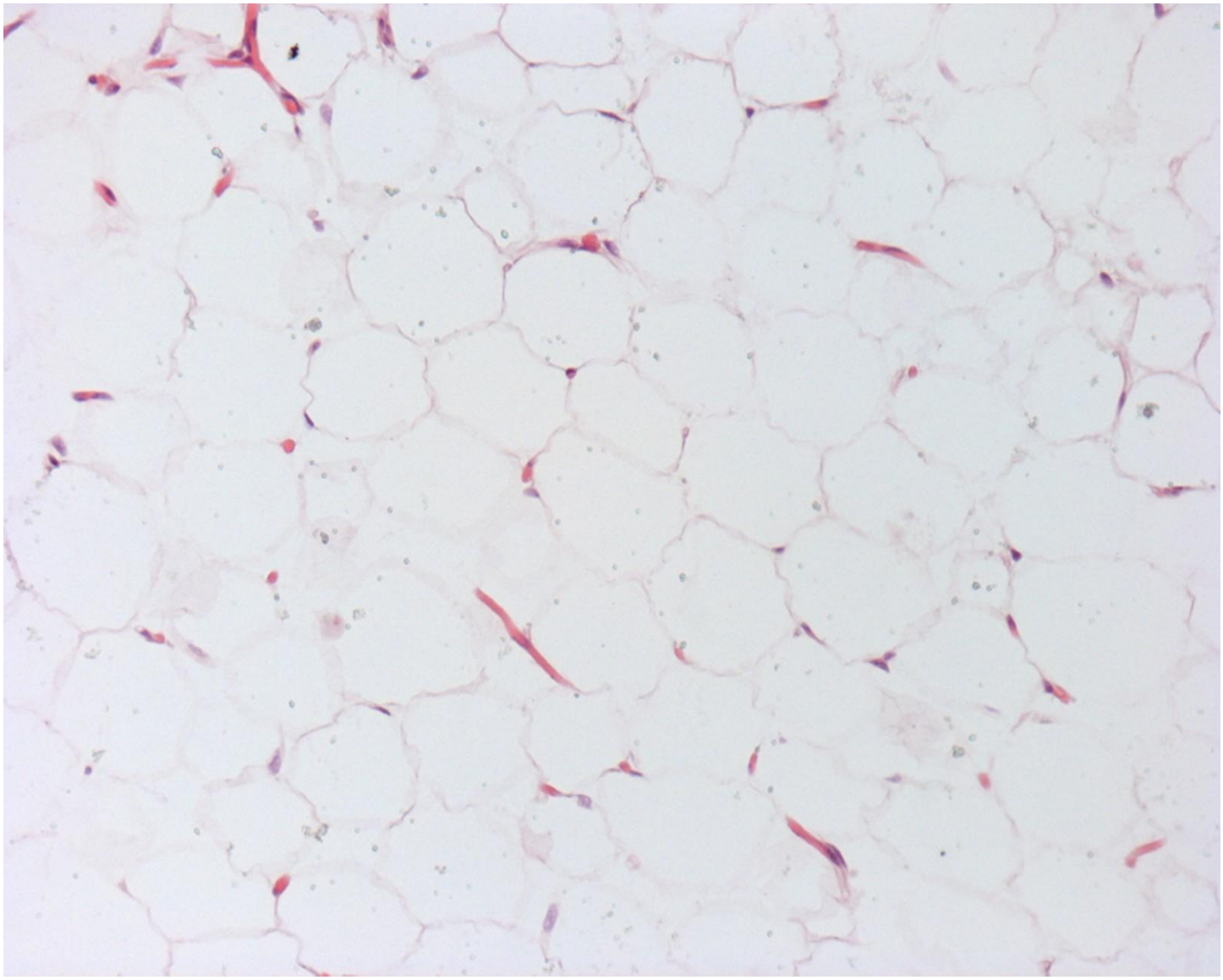


Figure 9: skin before treatment (x200)

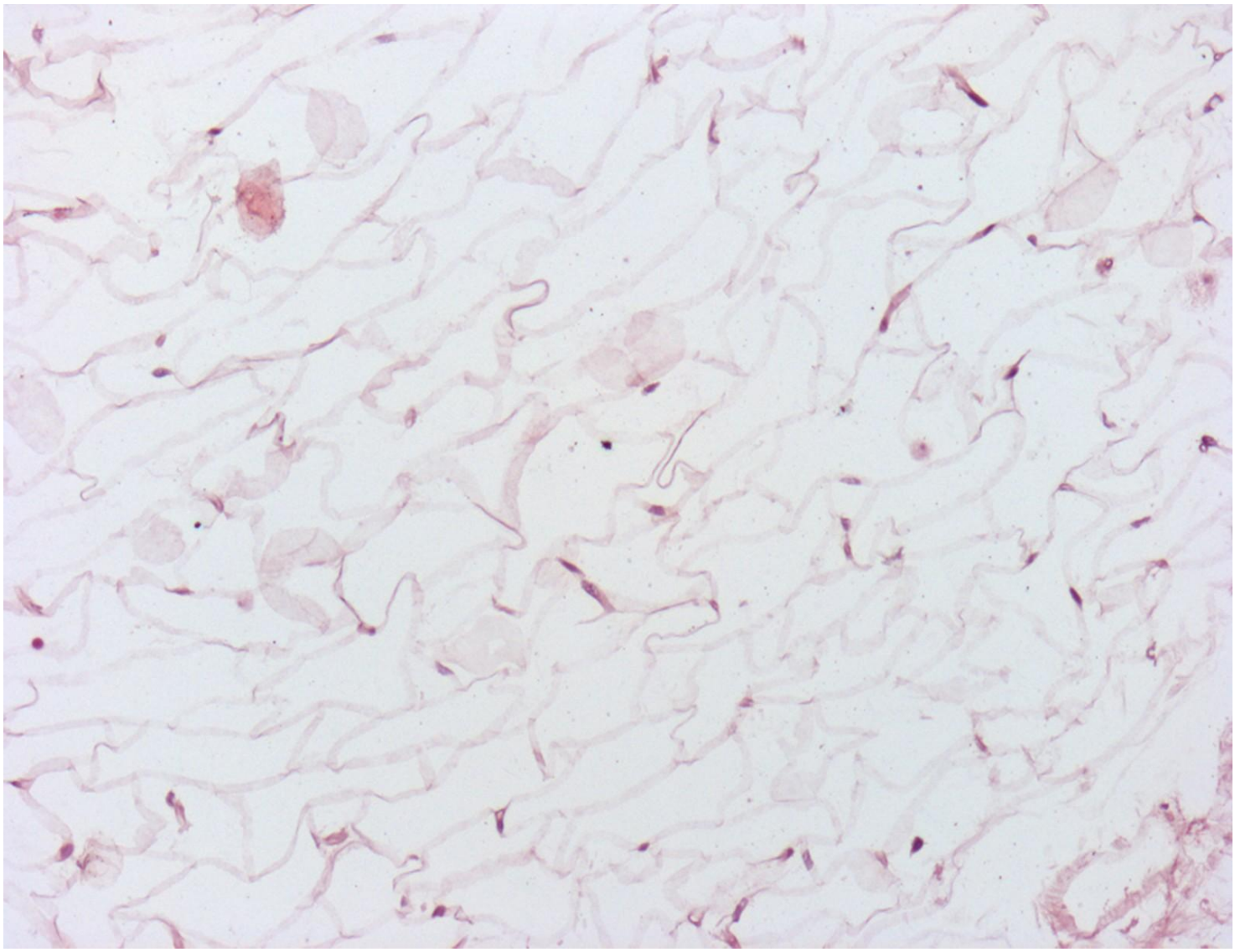


Figure 10: skin treated by CAVIFAST 2 (x200)

4- Results for donor #4

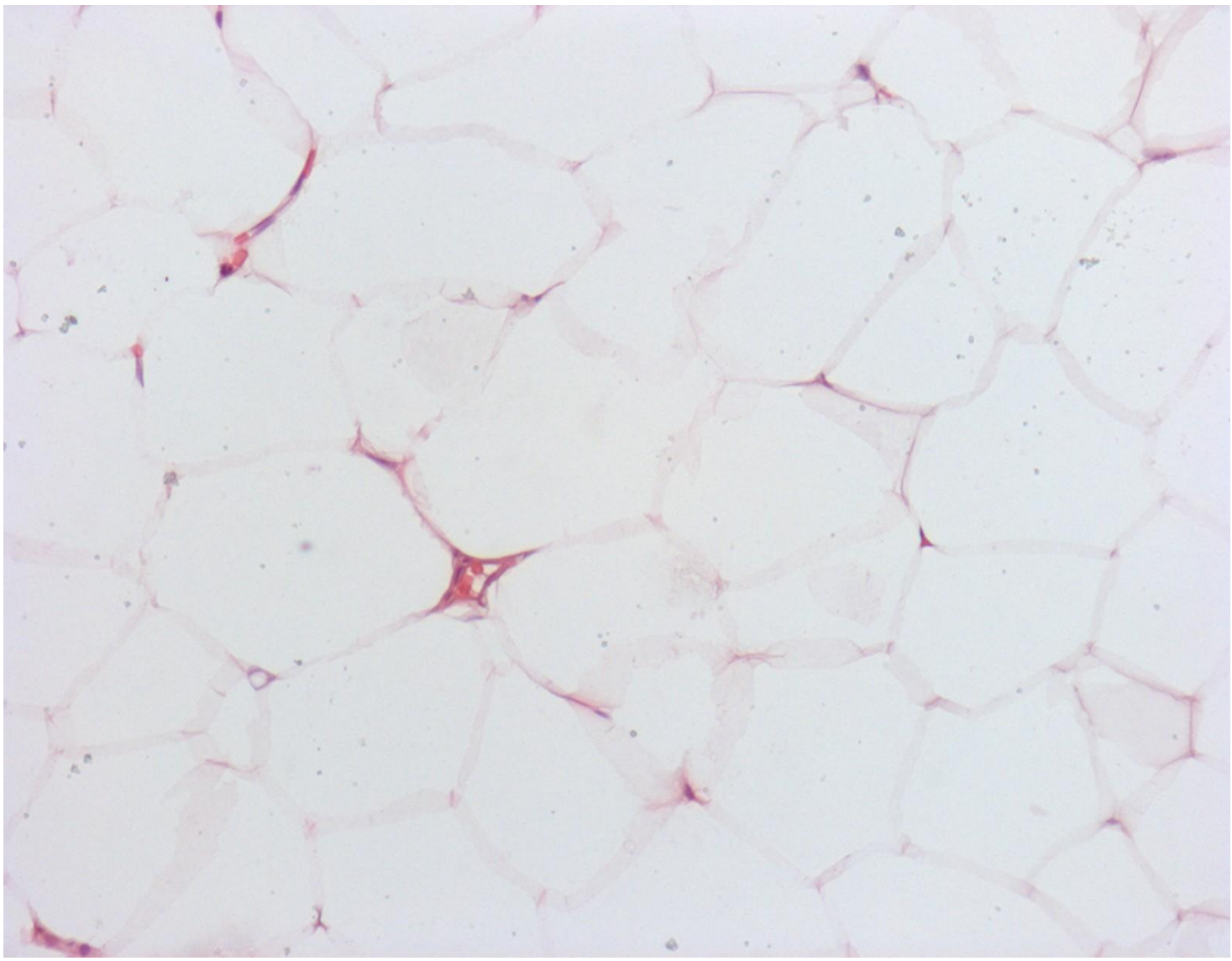


Figure 11: skin before treatment (x400)

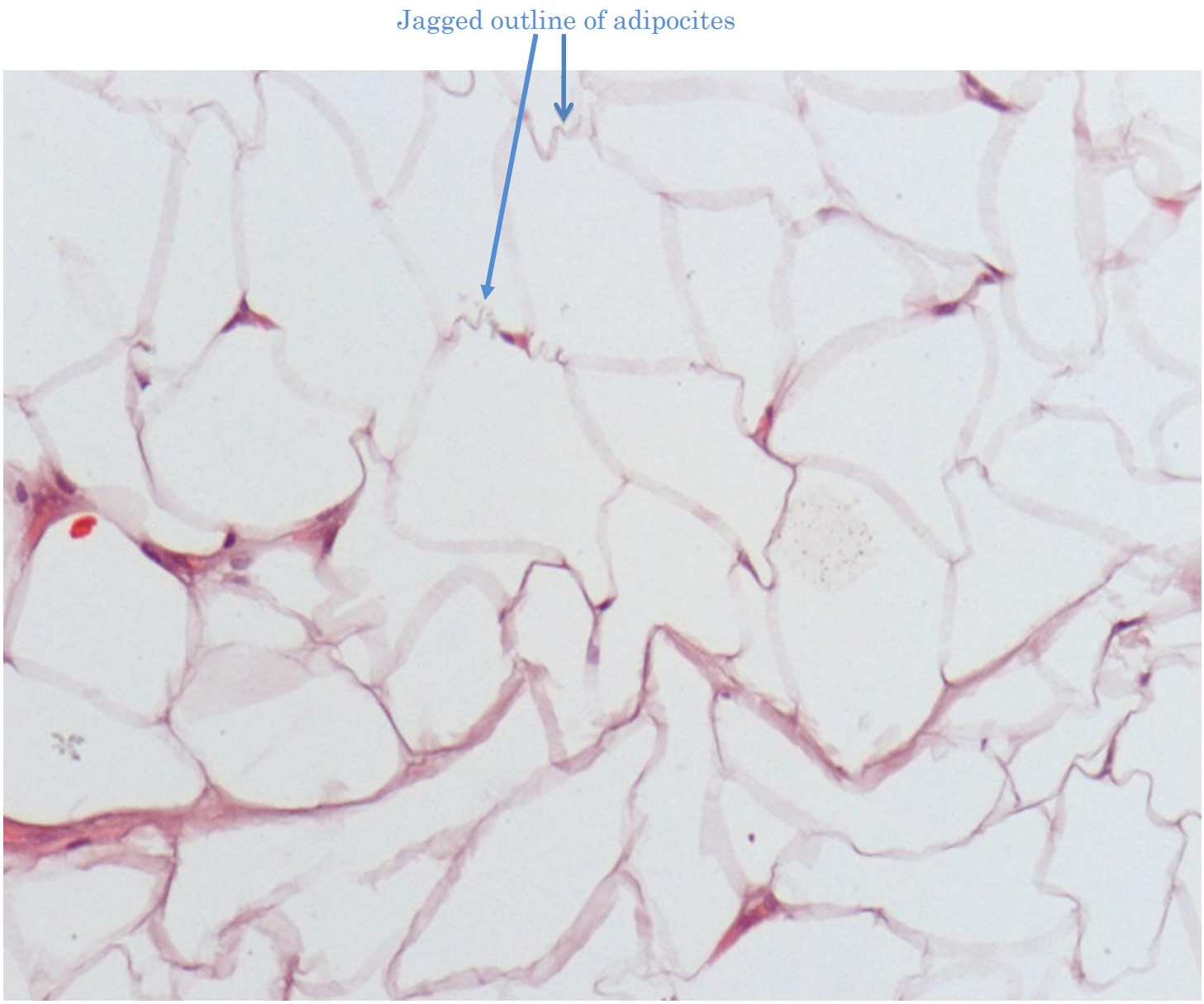


Figure 12: skin treated by CAVIFAST 2 (x400)

5- Results for donor #5

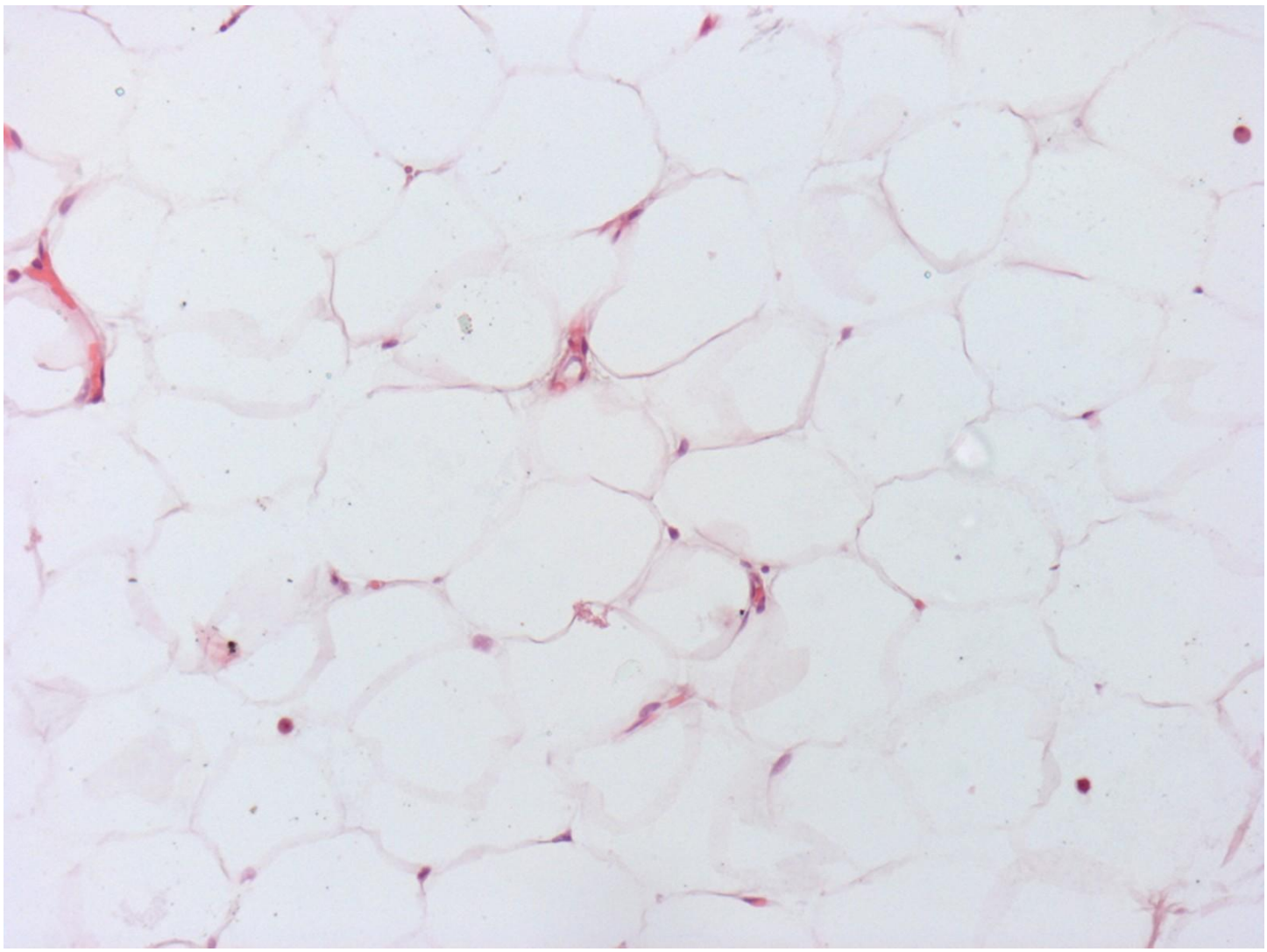


Figure 13: skin before treatment (x200)

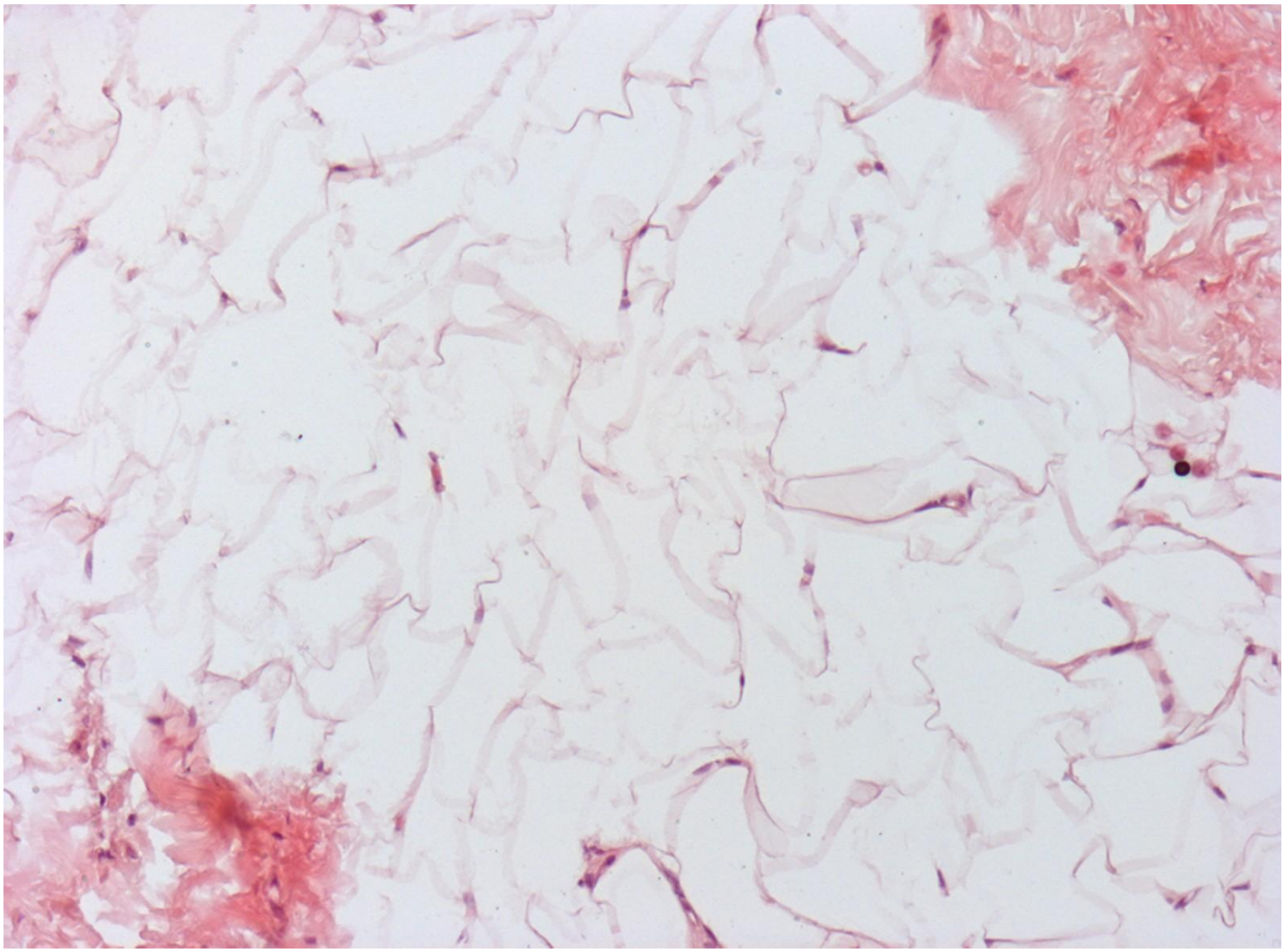


Figure 14: skin treated by CAVIFAST 2 (x200)

B) Glycerol dosage

Results are reported in Table **I (**average**) and I bis** (individual values) and represented as histograms.

We have demonstrated in this model of skin explant a statistically significant increase in the amount of glycerol released by the abdominoplaty skins (n = 5) after completion of an ultrasound session (CAVIFAST 2) with a rate of glycerol 45.54 µg/g for hypodermis versus 23.9 µg/g for the untreated skin (p = 0.01), showing an increase of 90%. The rate of excretion is almost doubled after treatment.

Table I:

Biochemical dosage of glycerol based on hypodermis fragments of skins of each donor

(Average ± ET, n = 5)

	µg/g of adipose tissue.
Skin before treatment	**23,9** ± 9,0
Skin + **CAVIFAST 2**	**45,54** ± 11,8 * p = 0,01

*: Statistically significant **difference** compared with the skin before treatment (paired Student's t test, p <0.05)

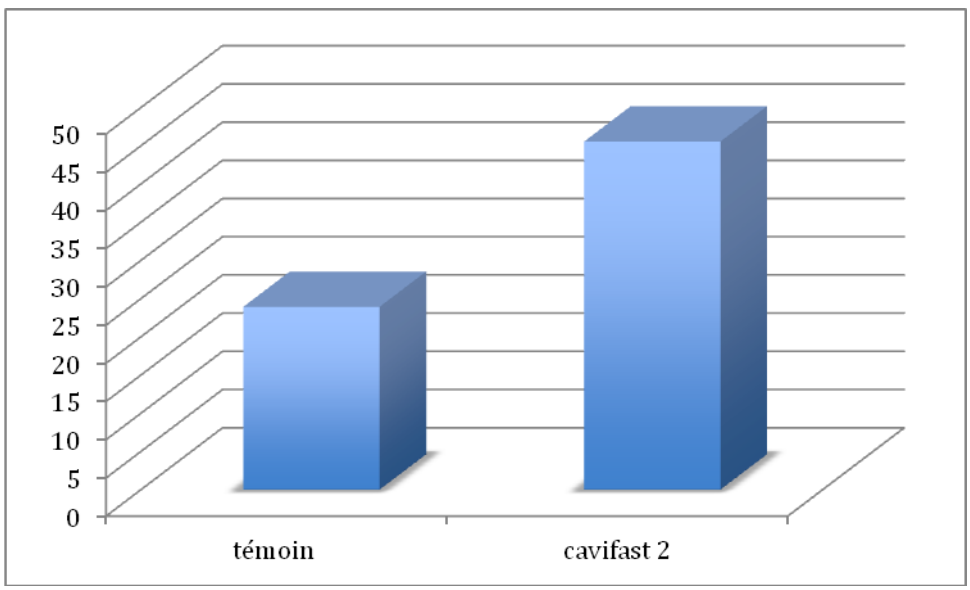
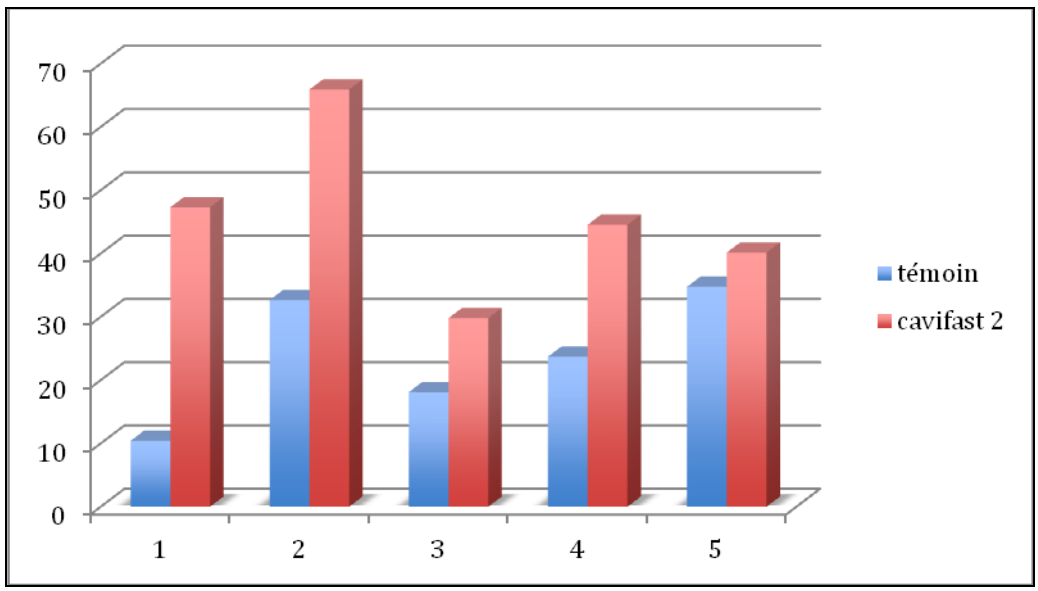


Table I bis:

Biochemical dosage of glycerol (individual results)

	Normal (témoin)	CAVIFAST 2
Skin #1	10,4	47,3
Skin #2	32,64	65,9
Skin #3	18,1	29,8
Skin #4	23,7	44,55
Skin #5	34,75	40,16



VI.CONCLUSION

In this model of human skin explants, the **anti-cellulite activity** and the **safety** of the very low frequency ultrasound device (CAVIFAST 2) has been visualized and quantified.

The cavitational lipolysis observed at hypodermis level for all donors associated with a nearly double increase of glycerol released, confirms the anti-cellulite activity of the device CAVIFAST 2.

The respect of the essential structures of the skin such as epidermis, dermis and vessels demonstrates the perfect safety of the device.

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