Evaluation of the skin redermalization effects by injection combination of hyaluronic and succinic acids by shear wave elastography (2D-SWE).

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Aims and objectives

The elasticity of the skin is one of the most important parameters of its health or aging, as well as evaluating the effectiveness of procedures with intradermal injections - redermalization (RD). There are many terms of aesthetic medicine and cosmetology that characterize exactly the mechanical properties of the skin: elasticity, rigidity, stiffness, density, tone, flableness, plasticity and others.

However, objective instrumental methods for quantitative measurement of these fundamental parameters of the skin are rarely used in everyday practice [1-3]. Osmetologists and clients themselves use mostly subjective data to assess the condition of the skin.

The biomechanical tests of human skin help to qualitatively and quantitatively assess the effectiveness of dermatological products and procedures, the detection of skin diseases, the planning of surgical and dermatological interventions and treatment. The skin reacts to mechanical effects, primarily to stretching, by a series of adaptive reactions (increase in the mitotic activity of the cells of the dermis and epidermis, the synthesis of collagen) aimed at restoring skin homeostasis [4, 5, 6, 7].

The mechanical properties of the skin primarily depend on the layer of the dermis, which is saturated with connective tissue - the extracellular matrix (ECM). It is represented by three-dimensional interlacing of protein fibers (collagen, reticulin, elastin) and sol, which consists of water, ions, hyaluronic acid (HA) and other macromolecules.

HA has a high hydrophilicity and holding a certain amount of water and ions, provide tissue turgor (mechanical tensions). With age (after 25 years), the amount of collagen and hydrophilicity (water content) of the sol changes downwards. This causes the skin to gradually lose its mechanical properties and manifestation of sagging and wrinkles[8, 9].

The study of changes in the mechanical properties of living tissues in health and pathology has allowed the development Ultrasound Strain Elastography (SE) [1]. However, the first attempts to use SE in dermaoncology did not lead to significant progress in the mind of the operator- and apparatus-dependence method [1, 10, 11].

New perspectives have emerged in the development of the Share Wave Elastography (SWE) for an objective and quantitative assessment of stiffness / elasticity by of Young's modulus in kPa. The great interest are works on the use of the one-dimensional transient elastometry of the skin (1D TE) [12].

It was shown experimentally and in the clinic the possibility of studying thin layers and biomembranes (dermis, penile tunica albuginea) by 2D-SWE [13, 14].
There was a possibility of accurate navigation in real time of the colorized region of interest (ROI) of elastography and sample volume (SV) of elastometry within the thickness of the dermis of 1.2-2.2 mm by B-mode [14].

It is of interest to evaluate with the influence on the mechanical properties of the skin of the combinational treatments of injectable hyaluronic and succinic acid (HA+SA) intradermal implants by the 2D-SWE [9].

**Aim** was to evaluation of the skin redermalization (RD) effects by Hyalual booster injection (combination HA 22 mg/ml and SA 16 mg/ml) by Real-time 2D-SWE.
Methods and materials

The skin thickness and stiffness at the rear wrists and cheeks were evaluated in 7 subjects (1 male and 6 women, age 28-55 years) on both sides. Real-time 2D-SWE of the skin performed by US machine Soneus P7 (Ultrasign, Ukraine) with conventional broadband linear 5-12 MHz probe.

The unique property of equipment is the absence of the elastography "dead zone" in the dermis under the contact surface of the probe aperture.

All subjects underwent three procedures of the skin RD by Hyalual booster intradermally injection with an interval of 2 weeks [9].

Real-time 2D-SWE performed before and two weeks after the last injection.

First, we performed real-time B-mode of the echostructure of the skin and subcutaneous fatty tissue, as well as underlying tissues (fascia, muscles, bones, tendons, etc.).

The thickness of the dermis was measured at 3 points along the sensor aperture of 38 mm and the average value was calculated.

The choice of the SV 2D-SWE position and diameter was determined in accordance with the thickness of the dermis in B-mode.

In order to eliminate the precompression artifact of the dermis by the probe, we applied the principle of a "gel pillow". Standardization of the gel layer thickness of the 1-2 mm and prevention of precompression was achieved by using the original nozzle rubber band. Fig.1.

It is also known that the skin mechanically exhibits anisotropic properties and its ability to stretch and shrink in different directions varies significantly [4, 15].

In order to avoid the influence of anisotropy of the orientation connective tissue fibers of the skin on the results of elastometry, we located the US prober in the same standard direction. On the rear wrists the probe was oriented along the long axis of the bones above the interosseous gaps. On the cheeks the probe was standardly oriented in a direction parallel to the edge of the mandibula.

The quality control of elasto-visualization and elastometry was always carried out in real time by the standard deviation (SD) value and the mapping of errors of the 2D-SWE in the ROI on a pink-green scale (#, %). Fig.2.

Statistical data processing of the research results was carried out using IBM SPSS Statistics Base v.22. The statistical significance of the data was set at $p < 0.05$. Results are presented in $M \pm SD$. 
Fig. 1: The rubber nozzle around the probe. Protective hygiene film covers the nozzle [16]. The screen displays of the skin 2D-SWE color mapping on ROI (square green frame) and SV (white circumference).

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**Fig. 2:** Quality control of Real-time 2D-SWE imaging of the cheek dermis by SD and Real-time mapping of the error (#,%) in the ROI on a pink-green scale.

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Results

The skin thickness of the rear wrists was initially: on right 1.4±0.5 mm, on left 1.1±0.3 mm. After the RD it was on right 1.1±0.3 mm, on left 0.9±0.3 mm (p>0.05).

The skin thickness of the cheeks was initially: on right 1.6±0.2 mm, on left 1.7±0.5 mm. After the RD it was: on right 1.6±0.3 mm, on left 1.8±0.4 mm (p>0.05).

The skin thickness of the rear wrists was initially: on right 13.9±5.3 kPa, on left 13.9±3.3 kPa. After the RD it was: on right: 10.8±2.5 kPa (p>0.05), on left 9.7±2.3 kPa (p<0.05).

The skin thickness of the cheek was: on right 10.8±0.9 kPa, on left 10.4±1.2 kPa. After the RD it was: on right 6.7±0.8 kPa, on left 8.0±1.3 kPa (p <0.001), respectively.

Consequently, the initial values of thickness and elasticity did not have a statistically significant difference for the symmetric zones of the rear wrists bilaterally (p > 0.05). Similar data without bilateral asymmetry were obtained for the cheeks dermis. The stiffness of the dermis of the healthy volunteers was consistent with data from other researchers [12, 13].

The thickness of the dermis of the rear wrists and cheeks did not significantly differ after the course of RD from the initial values. The stiffness of the dermis of the rear wrists and cheeks after a course of RD decreased in comparison with the initial value according to 2D-SWE (p>0,05). The stiffness of the dermis of the rear wrists and cheeks without bilateral asymmetry were obtained (p>0,05). Fig. 3-10.

The results of the Real-time 2D-SWE of the skin revealed a significant effect of the course of intradermal injection of the HA+SA combination on dermis. The RD reduced the stiffness of the dermis of the rear wrists and cheeks bilaterally. This means an increase in the elasticity of the dermis.
Fig. 3: Three measurements of the thickness of the rear wrist dermis before RD.

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Fig. 4: Real-time 2D-SWE of the stiffness of the rear wrist dermis on kPa before RD.

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**Fig. 5:** Three measurements of the thickness of the rear wrist dermis after RD.

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Fig. 6: Real-time 2D-SWE of the stiffness of the rear wrist dermis on kPa after RD.

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**Fig. 7:** Three measurements of the thickness of the cheek dermis before RD.

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Fig. 8: Real-time 2D-SWE of the stiffness of the cheek dermis on kPa before RD.

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Fig. 9: Three measurements of the thickness of the cheek dermis after RD.

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Fig. 10: Real-time 2D-SWE of the stiffness of the cheek dermis on kPa after RD.

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Conclusion

The results of the RD can find an explanation in the properties of the HA+SA combination to influence on the skin metabolic (plastic and energy) processes and the hydrophilicity of the ECM. One of the SA key point is the ability to block reactive oxygen species (ROS). SA shows antioxidant activity. As is known, ROS are the basis of the theory of skin aging (Inflamejding). The HA+SA mixture has a positive effect on the control of the clinical inflammation, which is proved by the subjective and objective measurement of swelling and erythema levels. [9, 17, 18, 19].

Thus, today it is possible to consider the SWE as an instrument for an objective quantitative assessment of the mechanical properties of the dermis itself and the subcutaneous fat layer. The use of SWE is described in normal/pathological conditions and in case of skin damage to various etiologies, as well as during plastic and surgical operations, cosmetic procedures [9, 20, 21, 22, 23].

However, it is necessary to take into account a number of restrictions when using skin 2D-SWE [1, 14, 15]. The surface location of the skin leads to its additional compression (precompression) with an US probe. Precompression can lead to an overestimation of the results of measuring the stiffness of the dermis. Another limitation of skin elastography is the presence of a "dead zone" in skin layers under the probe.

It is important to avoid reverberation artifact from strong reflectors under the skin, such as bones, tendons, and cartilage.

Since elastography appeals to the information contained in the connective tissue of the skin (ECM, collagen fibers and HA), it is logical to use Real-time 2D-SWE as a tool for objectifying the effect of Hyalual booster intradermally injection (combination HA 22 mg/ml and SA 16 mg/ml) on the mechanical properties of the dermis during RD.

1. Real-time 2D-SWE of the skin is available as a routine procedure with the ability to control quality elasto-visualization and eliminate elasto-artifacts: precompression, reverberation and the "dead zone".

2. The course of redermalization with a HA+SA combination did not change of the thickness but increase of the dermis elasticity of the rear wrists and cheeks.
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