REDUCING POST-PROCEDURAL INFLAMMATION

Alexander Turkevych discusses the findings from his study using combinational treatments of injectable hyaluronic and succinic acid implants provided after energy based devices and other interventional treatments

ABSTRACT

Background

Energy-based devices treatments and thread lifts cause severe traumatic inflammation in the early post-procedure period, what leads to unwanted downtime and decreasing of quality of life index within 7–14 days after treatment. Hyaluronic acid associated with succinic acid would improve (decrease) the inflammation due to its anti-inflammation properties, which may minimise downtime after mentioned above procedures.

Objective

This publication objective is to share the results of the study, which was performed to evaluate the safety and benefits of using these treatments in a combined protocol with a cannula or needle injectable administration of hyaluronic acid 9mg/ml and succinic acid 16 mg/ml mixture for faster patient recovery and improvement of the aesthetic outcomes.

Methods

Four groups of volunteers were treated with either non-surgical blepharoplasty, focused high-frequency ultrasound, CO_2 fractional laser resurfacing, or poly-lactic-acid (PLA) facial thread lift. The study was open-labeled, and the study cohort was treated split-face with hyaluronic acid 9mg/ml and succinic acid 16 mg/ml mixture within the initial treatment.

Results

All participants completed the course of treatments with significant improvement in skin recovery in both the experimental and reference sides in all groups. No adverse reactions were reported or observed.

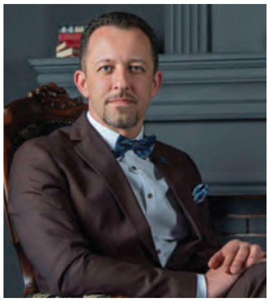
Self-evaluation

The mean score for perceived skin erythema, swelling, and aesthetic improvement evaluation improved significantly on the second visit, as well as steadily after the second visit on the experimental side of the face. Perceived skin redness and swelling decreased in comparison to the reference side within the first and the second visit (7 and 14 days). The increment was statistically significant, and the improvement in the experimental side was significantly better than the reference side.

Objective clinical evaluation using VISIA auxiliary analysis showed improvement in all groups for erythema, wrinkles, UV-spots and texture after treatments. The experimental side of the face treated (HA-SA mixture) performed better than the reference group (not treated with HA-SA mixture, according to the protocol). Erythema levels on the investigated side of the face were significantly lower than on the control side, on the 7th day after treatment in all groups. On day 14 after the treatment, levels of erythema on the treaded side in all four groups were still lower with the 5–7% rate. Wrinkle count on the investigated side of the face was 20-25% less in all four groups of volunteers on the day 14 and 5-7% less on the day 14 after the treatment. Texture levels on the control side within all groups were 12% and 6% better on day 7 and day 14 respectively. UV spots levels within treated groups for non-surgical blepharoplasty, thread lifting and HIFU were not statistically significant, for CO_2 treatment these levels showed 10% and 5% improvement respectively.

Conclusion

The subjective response from volunteers was significant as they noticed an improvement of swelling and redness in all groups on the investigated side of the face. The best results were indicated on day 7 after the treatment, which is the crucial time for the improvement of the condition and social adaptation after the energy-based or non-surgical interventional treatment. No adverse events were indicated over the treatment and follow-up period. Also, they have admitted general aesthetic improvement after the second injection of HA+SA mixture, which was followed on day 7 after the measurement of the indicators.



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TREATMENTS CARRIED OUT WITH energy-based devices and thread lifts cause severe traumatic inflammation in the early post-procedure period, which leads to unwanted downtime and a decreased quality of life 7–14 days after treatment. Hyaluronic acid injected along with succinic acid would decrease the inflammation due to their anti-inflammation properties, which may minimise downtime after the above-mentioned procedures.

The ageing process on the face is multifactorial; causing damage to the dermis and epidermis with decreased collagen, elastin, skin, fatty layers, number of cells and extracellular matrix. This, in turn, leads to changes in skin texture, firmness, radiance, volume, flexibility, and the appearance of wrinkles.

But the ageing process is more than this—it is a complex interaction between the facial elements, which occurs like a domino effect. And it happens in four main facial layers: skin, fat, muscles, and bones.

The amount of fat held in the face decreases with age, causing a deflated effect, which leads to volume loss and the hydrating and elastic functions of the skin no longer work as efficiently. The fibroblasts producing elastin, collagen, and hyaluronic acid work better when they are under a degree of stretch. When the fat depletes, this stretch is reduced, and the skin sags.

On the muscle front, it will be noted as part of the ageing process that some muscles get looser while others get tighter.

The facial skeleton also changes—mainly the facial apertures. The nose and the eye sockets increase by 20% in size with age and the infraorbital region physically moves backwards, so the holes are not only larger but are also pushed back. ▷

COVER STORY

KEYWORDS
Inflammaging, ROS, free radicals, hyaluronic acid, succinic acid

Inflammaging

A pervasive feature of ageing is a progressive and chronic state of systemic low-grade inflammation referred to as inflammaging[1-5]. Major age-related diseases (ARDs) all share a common inflammatory background. The role of various inflammatory molecules—mainly tumour necrosis factor-α (TNF-α), interleukin-1b (IL-1b), IL-6, and transforming growth factor-β (TGF-β)—in the promotion or exacerbation of a wide range of ARDs is increasingly emerging. A number of mechanisms, pathways, and cell types have been shown to possibly contribute to inflammaging. Initial hypothesis confirmed that inflammaging was mainly due to the long-lasting exposure to acute and chronic infections and the consequent life-long antigenic burden. However, a long list of sterile potential sources of inflammatory molecules during both the cellular and the organismal ageing process have been proposed.

The 'old' free radical theory of ageing (FRTA) states that organisms age because cells accumulate free radical-induced damage over time. One interesting view that coupled FRTA with inflammaging is the oxi-inflammaging theory. Accordingly, excessive or uncontrolled free radical production can induce an inflammatory response, and free radicals are themselves inflammation effectors. Oxidative metabolism at the mitochondrial level is probably the primary source of intracellular ROS production, which, in turn, represents long-lasting stress. Ageing-related ROS accumulation can contribute to telomere attrition and oxidative genomic damage, but can also act as signalling molecules in the development and maintenance of the senescent phenotype of the cell[2,4].

The presence of hyaluronic acid (HA) in the skin declines over the years, resulting in a loss of moisture, elasticity, firmness, and volume. Our bodies are in a continual state of rebuilding—new skin cells, for example, replace old ones approximately every 20 to 30 days. Usually, hyaluronic acid in skin tissue is broken down by hyaluronidase, and the rate of degradation can be increased by an excess intake of riboflavin (B2), ultraviolet radiation exposure or viruses. Signs of natural skin ageing become apparent with wrinkles, loss of volume in the cheeks and thinning lips.

Hyaluronic acid is a polysaccharide composed of alternating molecules of N-acetyl glucosamine and D-glucuronic acid present in almost every cell in the human body. It can be found within collagen throughout the body, but 50% of hyaluronic acid is concentrated in the skin. In the extracellular matrix, hyaluronic acid performs a range of biological functions, but it mainly regulates moisture, increases fibroblast activity, and stimulates collagen synthesis.

> "Hyaluronic acid is a polysaccharide composed of alternating molecules of N-acetyl glucosamine and D-glucuronic acid present in almost every cell in the human body."

When a patient is injected with hyaluronic acid, we re-create volume and by creating more tension, more hydration is achieved. Unfortunately, the effects are temporary, short-lasting and HA does not re-create what is continuously disappearing. The ageing phenomenon of the face is not limited to collagen and fibroblasts but also to bone loss on which HA does not have any effect.

However, while the use of HA alone may not reduce many of the effects of ageing. Sodium succinate can provide an antioxidant effect by actively blocking free radicals and stimulating metabolic processes in the skin, therefore aiming to reduce the signs of ageing[6-9].

It has been suggested in an *in vitro* trial, when combined, HA and sodium succinate act synergistically to stimulate fibroblast cells to increase (in both number and metabolic activity) with a more significant effect when compared to HA mono-component therapy[6,8]. The impact on the metabolic processes can include strengthening cellular respiration, normalising ion transport, increasing protein synthesis and increasing energy production, through the stimulation of the Krebs cycle in mitochondria. The Krebs cycle is essential for cellular respiration; taking place in the mitochondria, the cycle converts pyruvate (from glycolysis) to produce nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP) through a number of reactions and intermediate molecules—succinate is one such molecule forming the complete chain in the cycle. This should then translate to the restoration of cells, an increase in skin elasticity, firmness and tightness, improved colour and texture, as well as a reduction in the signs of ageing and fatigue.

Carbon dioxide and Er:YAG lasers

Conventional CO_2 and erbium:yttrium-aluminium-garnet (Er:YAG) lasers were considered the gold standard for skin laser resurfacing. Despite superior clinical outcomes, this particular modality has been associated with lengthy recovery time and a high risk of side-effects and complications, particularly in patients with darker skin types.

The newly developed ablative fractional photothermolysis laser (FP) was designed to minimise these shortcomings of ablative laser while maintaining its clinical efficacy. Ablative FP results in a microscopic pattern with spatial separation of normal tissue by thermally-affected epidermal and dermal columns. Immunohistochemical studies have revealed that during this peculiar process of wound healing, the induction of epidermal heat-shock protein and collagen remodelling occurs, lasting up to 3 months post-ablative FP treatment *in vivo*. This effect may be exploited to treat photoaging, skin tightening, acne scars, non-acne atrophic scars, and burn scars. Various adverse effects, ▷

Figure 1 CO_2 fractional laser treatment on the control side. Slight erythema still visible over upper lip

however, such as HSV outbreak, bacterial infection, acneiform eruption, erosion, and post-inflammatory hyperpigmentation (PIH) are being reported after ablative FP treatment. Also, earlier studies on ablative FP treatment have found that the ideal candidates for laser interventions by FP are mainly Fitzpatrick skin type I–III individuals and that the side-effects tend to develop more readily in individuals with darker skin types. PIH, in particular, is a relatively common adverse outcome after ablative FP and is more likely to occur in darker skin types. Although there are ongoing studies on the efficacy and adverse effects of ablative FP on Asian patients with darker skin types, there is no set standard for optimal treatment frequency, interval and treatment parameters.

Ablative FP can be achieved with an Er:YAG, yttrium-scandium-gallium-garnet (YSGG) or CO_2 laser, with the wavelengths exhibited by these lasers being highly absorbed by water. While various types of ablative FP on the basis of maximum power, energy, and penetration depth are currently available, a 10,600 nm CO_2 FP device, which uses pulse energy from 5 to 70 mJ covering 10% to 70% of the treated area in increments of 5%, was used in this study.

Non-surgical low-temperature plasma blepharoplasty

Plasma is the fourth state of matter in which electrons are separated from atoms to form an ionised gas. The flow of plasma stimulates the formation of new collagen and elastin fibres, improves sun damaged skin, reduces wrinkles and smoothes the skin surface. Each cell has its membrane potential (the electric potential difference between the two sides of the membrane). On the inner side of the cell membrane is the negative charge, on the outer side is a positive charge. Skin ageing leads to an uneven distribution of electric charge (potential) along the cell membrane and thus changes the membrane voltage. By passing direct current (DC) through the cells, this changes its membrane potential and returns it to equilibrium. When this process occurs in the majority of skin cells in a specific area, retightening of the skin is then visible to the naked eye. By contrast, equipment that works with alternating current (AC) cannot depolarise the membrane, because the alternating current does not pass through the cell membrane. Using higher intensities, Jett Plasma Lift can, in a controlled manner, destroy cells of the epidermis, because at such intensities the electric arc produced by the ionisation of gases can sublimate the cells with which it comes in contact, without affecting surrounding tissue. The electrical channel created by the direct current is very accurate (ø1 mm); therefore, the treatment and destruction of cells is very accurate.

> “Interestingly, whether it be wound healing, liver injury or lung injury, there is a potent mechanism for clearing HA following tissue injury.”

High-frequency ultrasound

Microfocused ultrasound (MFUS), also commonly referred to as intense focused ultrasound, is a novel technology introduced in 2009 for the correction of mild to moderate skin and soft tissue laxity. A commercially available device for the lifting of eyebrows and lax skin of the neck and submental areas, as well as for the treatment of the chest to improve wrinkles of the décolleté. Short duration (25–50 ms) pulses of transcutaneous ultrasound energy with frequencies in the megahertz (MHz) range create precise 1.0–1.5 mm^3 areas of spatially focused, chromophore independent thermal (60–70°C) coagulative damage, increasing dermal thickness and reticular dermal collagen while sparing intervening tissues or overlying skin.

Currently available transducers produce a set number of thermal injury zones (TIZs) at 4.5 mm (4 MHz or 7 MHz), 3.0 mm (7 MHz), and 1.5 mm (10 MHz) fixed focal depths. Treatment lines are pulsed adjacent to each other (<3 mm apart) in a parallel fashion, with a full face and upper neck typically requiring 650–900 lines per the most recent protocols. The amount of lines and number of treatments required may vary between patients based on the extent of pretreatment skin laxity. While the 4.5 mm transducer yields a maximum of 17 TIZs spaced 1.5 mm apart, 3.0 mm, and 1.5 mm transducers produce a maximum of 23 TIZs spaced 1.1 mm apart, each within a straight 25 mm line.

The 4.5 mm transducers can selectively, predictably, and reproducibly target the superficial musculoaponeurotic system (SMAS), a continuous fibroelastic network that mediates the interaction between facial skin and underlying muscles of facial expression. The mechanical properties of the SMAS generate greater holding forces and diminished relaxation following skin tightening procedures than other cutaneous or subcutaneous tissues. Diagonal vectors with 4.5 mm transducers are often utilised on the cheeks to enhance lifting capacity via the SMAS. The 3.0 mm/7 MHz and 1.5 mm/10 MHz transducers, on the other hand, target reticular dermal tissue at respectively decreasing depths.

Few complications have been reported in previously published clinical trials and retrospective studies with MFUS, with no prior reports of ulceration or cutaneous necrosis. A recent retrospective review of 39 patients treated with MFUS demonstrated a complication rate of 23%, including localised oedema, mandibular ecchymosis, and transient lip and eyebrow paralysis. One patient developed an area of localised oedema that eventuated in cutaneous atrophy (dermal scarring),

Figure 2 Non-surgical low-temperature plasma blepharoplasty. (A) Day 0 and (B) 1 month after treatment. Erythema is significantly higher on the patient's left-hand side, which is the control (not treated with HA+SA mixture) side.

▷ while another patient with hyaluronic acid filler placed previously in her cheeks developed facial subcutaneous pseudoatrophy following administration of local anaesthesia containing hyaluronidase[10].

Non-surgical thread lift

The first threads for thread lifting were mainly considered for plastic surgeons. They were composed of silicone and polypropylene and were inserted through an incision and general anaesthesia. An extended downtime and other specific features did not make them popular.

The crucial step for future success was the appearance of absorbable threads on the market. Being used for more than 30 years, it proved its safety and reserved a prominent position in surgery. The threads consist of L-polylactic acid in combination with Σ-caprolactone (P(LA/CL)) in a 3:1 ratio. In addition, the main component L-polylactic acid is responsible for the rejuvenation effect. It stimulates the natural revitalisation of tissues and prolongs the ageing process. During thread biodegradation, L-lactic acid is released into surrounding tissues, stimulating neocollagenesis and their rehydration. Σ-caprolactone, the second component of the thread, slows down its biodegradation, increasing the duration of the tightening effect, and serves as a lactic acid delivery system to surrounding tissues. The overall percentage of degradation of the material from day one to 72 weeks was 41.2%. The full reabsorbtion period for the PLA threads is between one and a half and two years. The tissues react to the implantation of the P(LA/CL) threads by the formation of collagen type 1 and type 3, and by the development of a fibrous capsule. Even after full biodegradation, the fibrous capsule continues to hold soft tissue for 3 to 5 months.

The main peculiarity of threads is the disposition of their multidirectional barbs. This configuration gives the opportunity to evenly distribute the tension of the tissues along the entire length of the thread, and the three-dimensional arrangement of the barbs allows for the most reliable fixation to be achieved.

One of the most important characteristics is the thread is preinstalled in a special atraumatic cannula with the rounded tip and a hole aside.

> "The mechanisms by which HA accumulates in tissue injury have been studied. Elegant work from Swedish investigators has demonstrated that growth factors such as PDGF and TGF-β that accumulate following bleomycin lung injury, stimulate lung fibroblasts to produce HA."

The rounded tip of the cannula does not give the possibility to go beyond the right plane and pushes the tissue without any breaks. Thus, the superficial muscular-aponeuroses system (SMAS) perforation and damage in deeper structures are practically impossible. The risk of the skin retraction is also minimal.

However, implantation of the PLA thread is provided with the trauma, and a reaction to the material induces an inflammation reaction and causes swelling and tenderness over the face.

Interventional inflammation and effects of hyaluronic and succinic acid

Hyaluronan is a normal constituent of the basement membrane, and, in its native form, HA exists as a high molecular weight polymer, typically in excess of 106 Da. Hyaluronan plays a role in maintaining the structural integrity of tissues, such as the joints where it is responsible for maintaining the viscosity of joint fluid. In addition to existing as a soluble polymer, HA is bound to large proteoglycans, such as aggrecan and versican. Hyaluronan side chains of aggrecan are vital in cartilage organisation, and recent data have suggested that HA is essential for maintaining cartilage integrity. At sites of inflammation, such as during inflammatory arthritis or in wound healing, HA becomes depolymerised into lower molecular weight forms[7-9].

There appear to be two mechanisms for depolymerising HA, enzymatic and non-enzymatic. The enzymes that degrade HA are hyaluronidases, chondroitinases, and hexosaminidases. Most hyaluronidases are lysosomal enzymes and require an acid pH for maximal activity. However, the hyaluronidase PH-20, found in sperm, is active at PH7. Recently, a soluble form of PH-20 has been identified.

In addition, a novel hyaluronidase (Hyal-2) that generates HA fragments of 10–20×103 Da has been described. Hyal-2 is expressed in fibrotic lung injury.

Hyaluronan can also be degraded into smaller fragments by exposure to ROI. This is an important mechanism for generating HA fragments at sites of inflammation. Interestingly, hyaluronidases are endo-glucosaminidases whereas ROIs fragment HA randomly at internal glycoside linkages[7-9].

Hyaluronan degradation products appear to have biological functions distinct from the native high molecular weight polymer. Oligosaccharides of less than 20 disaccharides have been shown to be angiogenic. Low and intermediate molecular weight HA (2×104–4.5×105 Da) stimulate gene expression in macrophages, endothelial cells, eosinophils and specific epithelial cells. Hyaluronan degradation products are purported to contribute to scar formation. Fetal wounds heal without scar formation and wound fluid HA is high molecular weight. When hyaluronidase is added to generate HA fragments, there is increased scar formation. Collectively, these data support the concept that high-molecular-weight HA promotes cell quiescence and supports tissue integrity, whereas generation of HA breakdown products

is a signal that injury has occurred and initiates an inflammatory response. Interestingly, whether it be wound healing, liver injury or lung injury, there is a potent mechanism for clearing HA following tissue injury. This suggests that while the generation of HA breakdown products may be necessary to initiate the inflammatory response, removal of these fragments may be critical for the resolution of the repair process.

The mechanisms by which HA accumulates in tissue injury have been studied. Elegant work from Swedish investigators has demonstrated that growth factors such as PDGF and TGF-β that accumulate following bleomycin lung injury, stimulate lung fibroblasts to produce HA. However, whether increases in HA are due to increased production or decreased degradation were challenging to sort out at that time because the genes encoding HA synthases were unknown.

However, as stated above, three isoforms of HA synthase have recently been cloned. The three isoforms exhibit different patterns of expression in developing embryos. HAS1 is expressed early from day 1–5, HAS2 is expressed throughout development, and HAS3 is expressed in late development. The three isoforms map three distinct chromosomes, suggesting that they arose from ancient gene duplication.

Targeted deletion of HAS2 has recently been reported[11]. HAS2 deletion results in an embryonic lethal condition, whereas HAS1 and HAS3 mice develop normally. The HAS2 deletion appears to present a similar phenotype to that described for a naturally occurring versican knockout. There are major abnormalities in heart and blood vessel development. Interestingly, *in vitro* data have suggested that HAS1 and HAS2 produce high molecular weight HA, whereas HAS3 produces lower molecular weight HA[12].

Materials and methods

Sixteen female and one male volunteers, aged 25 to 65, not pregnant, not breastfeeding and not having any other treatment for skin pigmentation or improvement during the time of the study, as well as 3 months before and 3 months after the treatment. Systemic autoimmune diseases and other chronic disorders were excluded from this study.

The first group of 5 volunteers was recruited and received a pseudo-blepharoplasty procedure for upper eyelids, followed by the injection of 0.75ml of HA+SA mixture with a 25G cannula in the left periorbital area and evaluated before treatment, 30 minutes after treatment, 2 weeks, and 4 weeks later. For ethical compliance, all volunteers received the injection of HA+SA mixture with a 25G cannula for full face during the second visit. Volunteers were not randomised and not blinded.

Patient consent forms were recorded, as well as:

- Pictures under regular, UV and polarised light, always in the same position and exposure (objective)
- GAIS Scale: global aesthetic improvement questionnaires for volunteers
- Skin analysis: Visia Complexion Analysis device

> **“Red areas: represent a variety of conditions, such as acne, inflammation, rosacea or spider veins. Blood vessels and haemoglobin contained in the papillary dermis, a sub-layer of skin, give these structures their red colour, which is detected by the RBX Technology in VISIA.”**

(Canfield, USA), which allows measuring and evaluating of different parameters for the skin.

- » Wrinkles: are furrows, folds or creases in the skin, which increase in occurrence as a result of sun exposure, and are associated with decreasing skin elasticity. This skin feature has the greatest variability from image to image as it is highly dependent upon the facial expression of the client. Wrinkles are identified by their characteristic long, narrow shape
- » Texture: is primarily an analysis of skin smoothness. Texture measures skin colour and smoothness by identifying gradations in colour from the surrounding skin tone, as well as peaks (shown in yellow) and valleys (shown in blue) on the skin surface that indicate variations in the surface texture
- » UV spots: occur when melanin coagulates below the skin surface as a result of sun damage. UV spots are generally invisible under normal lighting conditions. The selective absorption of the UV light by the epidermal melanin enhances its display and detection by VISIA
- » Red areas: represent a variety of conditions, such as acne, inflammation, rosacea or spider veins. Blood vessels and haemoglobin contained in the papillary dermis, a sub-layer of skin, give these structures their red colour, which is detected by the RBX Technology in VISIA. Acne spots and inflammation vary in size but are generally round in shape. Rosacea is usually larger and diffuse compared to acne, and spider veins are typically short, thin, and can be interconnected in a dense network.

Non-invasive blepharoplasty or pseudo-blepharoplasty is a medical treatment provided by a sequence of spark discharges generated by DC voltage. Spark discharges generate heat that warms the skin. Mechanism of direct ▷

Figure 3 PLA threads. Investigated side on the seventh day after implantation. Little to no inflammation is visible

▷ current fulguration, in contrast to discharge caused by AC, affects a much smaller spot of the skin, is very precise, and the DC discharge does not damage surrounding tissues. The discharge (at the edge of which is a corona) is formed between the tip of the device and the conductively interconnected skin of the patient (to the device) at a distance of a tip over the skin of 2mm. Air that contains free electrons at the point of discharge takes up a lot of energy that leads to air breakthrough, which stops being an insulator and starts to drive an electric current (a shock occurs).

Second group

The second group of 3 volunteers received a treatment with poly-l-lactic acid threads for face lifting. The investigated side of the face was treated with injection of 0.75ml of HA+SA mixture with a 25G cannula over the middle and lower third of the face, using linear (vector) retrograde injection, and prior to thread implantation. Entry point for the injection was the same as the entry point of thread implantation. After HA+SA mixture injection, volunteers were treated with five threads per side (10 threads for the middle and lower third). Volunteers were evaluated before treatment, 30 minutes after treatment, 2 weeks, and 4 weeks later. For ethical compliance, all volunteers received the injection of HA+SA mixture with 30G needle injection for full face during the second visit. Volunteers were not randomised and not blinded.

Patient consent forms were recorded, as well as the points covered for group one.

Third group

The third group of 5 volunteers was treated with high-frequency ultrasound for the lower face with the aim of lifting, followed by the injection of 0.75ml of HA+SA mixture with a 25G cannula in the left middle and lower third of the face. Subjects were evaluated before treatment, 30 minutes after treatment, 2 weeks, and 4 weeks later. For ethical compliance, all volunteers received the injection of HA+SA mixture with a 25G cannula for full face during the second visit. Volunteers were not randomised and not blinded.

Patient's consent forms were recorded, as well as the previous points covered for group one and two.

Fourth group

The fourth group of 4 volunteers was treated with CO_2 fractional laser for the face with the aim of rejuvenation and post-acne treatment. Volunteers were treated with an injectable administration of 0.9% hyaluronic acid and 1.6% succinic acid mixture via cannula 30 minutes after laser resurfacing, 2 weeks, and 4 weeks later. For ethical compliance, all volunteers received the injection of HA+SA mixture with a 25G cannula for full face during the second visit. Volunteers were not randomised and not blinded.

Patient consent forms were recorded, as well as:

- Pictures under regular, UV, and polarised light, always in the same position and exposure (objective)

Table 1 Average percentile change within treatment and control groups

	Wrinkles, percentile*		Texture, percentile *		UV Spots, percentile*		Redness, percentile*	
Thread lift measurements (average)	control	investigated	control	investigated	control	investigated	control	investigated
7 days after treatment (T2)	52	56	76	81	26	27	37	43
14 days after treatment (T3)	47	54	81	87	26	27	36	40
Non-surgical low temperature plasma measurements (average)	control	investigated	control	investigated	control	investigated	control	investigated
7 days after treatment (T2)	73	76	96	97	5	6	18	52
14 days after treatment (T3)	83	87	97	97	10	10	20	27
CO2 fractional laser measurements (average)	control	investigated	control	investigated	control	investigated	control	investigated
7 days after treatment (T2)	61	60	83	88	21	18	91	92
14 days after treatment (T3)	32	45	80	77	17	17	92	94
High-frequency ultrasound (average)	control	investigated	control	investigated	control	investigated	control	investigated
7 days after treatment (T2)	66	83	90	84	75	71	89	93
14 days after treatment (T3)	70	84	85	83	71	70	81	87

*Percentile scores compare to those of a similar sex, age and skin type (which can include ethnic background.)
For example, 'Your Pores score is better than 78% of women your age and skin type', according to Canfield Visia internal database.

- Skin analysis: Visia Complexion Analysis device, Canfield, USA, which allows measuring and evaluating of different parameters for the skin
- GAIS Scale: Global aesthetic improvement questionnaires for volunteers.

Results

All participants completed the course of treatments with significant improvement in skin recovery in both the experimental and reference sides in all groups. No adverse reactions were reported or observed.

Self-evaluation

The mean score for perceived skin erythema, swelling, and aesthetic improvement evaluation improved significantly on the second visit, as well as steadily after the second visit in the experimental side of the face.

Perceived skin redness decreased from an average score of 8.33 to 5.21 in the experimental side, and from 8.33 to 7.31 in the control side within the first and the second visit. The improvement was statistically notable and was significantly better in the treated side than the control side.

Perceived skin swelling decreased from an average score of 8.33 to 5.21 on the treated side, and from 8.33 to 7.31 on the control side within the first and the second visit. The increment was statistically significant, and the improvement in the experimental side was significantly better than the control side.

Objective clinical evaluation

VISIA auxiliary analysis showed improvement in all groups for erythema, wrinkles, UV-spots, and texture after treatments. The experimental side of the face treated (HA-SA mixture) performed better than the reference group (not treated with HA-SA mixture, according to the protocol). Erythema levels on the investigated side of the face were significantly lower than on the control side, on the 7th day after treatment in all groups. On day 14 after the treatment, levels of erythema on the treaded side in all 4 groups were still lower with the 5–7% rate. Wrinkle count on the treated side of the face was 20–25% less in all four groups of volunteers on day 7 and 5–7% less on day 14 after the treatment. Texture levels on the control side within all groups were 12% and 6% better on day 7 and day 14, respectively. UV spots levels within treated groups for non-surgical blepharoplasty, threadlifting, and HIFU were not statistically significant, for CO_2 treatment these levels showed 10% and 5% improvement, respectively.

Discussion

The subjective response from volunteers was significant as they noticed an improvement of swelling and redness in all groups on the investigated side of the face. The best results were indicated on day 7 after the treatment, which is the crucial time for the improvement of the condition and social adaptation after the energy-based or non-surgical interventional treatment. No adverse events were indicated over the treatment and follow-up period. Also, they have admitted general aesthetic improvement after the second injection of HA+SA mixture, which was followed on day 7 after the measurement of the indicators.

The objective response by controlled photos and skin parameters measurements is similar to the subjective response. As shown in *Table 1*, erythema was improved for all volunteers within the first week and kept on improving on day 14 for all investigated groups. Wrinkle count and skin texture levels improved steadily during two weeks of follow-up and were better on the investigated side with the statistical significance. UV-pigmentations level improvement was significantly better within the CO_2 group, what might be combined with the needle injection in this group (as a targeted treatment for melanin) as well as a time for melanin improvement which is usually 24-29 days. Pigmentation measurements were not statistically significant within the three other groups.

The first conclusion of this trial is that 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. This was the primary objective of the study.

The second conclusion is that the second treatment with the HA+SA mixture within a week after the combined protocol improves not only the inflammation but also the expected aesthetic results after energy-based techniques and non-surgical invasive procedures.

The addition of hyaluronic and succinic acid in the used concentration with the needle or cannula delivery injection methods should deliver a new useful treatment against the early inflammation stage after energy-based devices or PLA threads. Patients in clinical practice are usually treated with a range of products and procedures to provide synergistic effects. This combination will improve patients' satisfaction, minimise the possible adverse events, and improve aesthetic outcomes.

▶ ***Declaration of interest*** *None*

▶ ***Figures 1-3*** *© Alexander Turkevych*

▶ ***Table 1*** *© Alexander Turkevych*

[redacted]

Key points

- Energy-based devices treatments and thread lifts cause severe traumatic inflammation in the early post-procedure period
- It was believed that hyaluronic acid associated with succinic acid would improve (decrease) the inflammation due to its anti-inflammation properties
- This study looked to evaluate the safety and benefits of using in a combined protocol with a cannula or needle injectable administration of hyaluronic acid 9mg/ml and succinic acid 16 mg/ml mixture for faster patient recovery and improvement of the aesthetic outcomes
- The mean score for perceived skin erythema, swelling, and aesthetic improvement evaluation improved significantly on the second visit, as well as steadily after the second visit on the experimental side of the face
- All participants completed the course of treatments with significant improvement in skin recovery in both the experimental and reference sides in all groups. No adverse reactions were reported or observed

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