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BIO-REMODELING ABNORMAL SCAR DEFORMITIES OF THE SKIN WITH HYALURONAN HYBRID COOPERATIVE COMPLEX

(2017-2022)

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CAPITOLO 1

KEY WORDS

BIO-remodelling scar deformities – changing the formed scar deformities by re-activating the physiological processes of skin healing through exogenous stimulating factor.

Scar – it is a pathological area of fibrous tissue that replaces normal skin after injury, it results from the physiological regeneration in the skin as well as in other organs and tissues of the body.

Hyaluronan hybrid cooperative complexes - it is medical device with thermally stabilized natural hyaluronic acid in the form of hybrid cooperative complexes, developed with innovative patented production process based on NAHYCO technology. The significance of NAHCO technology lies in the fact that low- and high-molecular HA complexes are first heated, then sharply cooled, which leads to the formation of hydrogen bonds between HA chains.

THE RELEVANCE OF THE PROBLEM

High incidence of cicatricial pathology of the skin, lack of protocols for highly effective conservative treatment and prevention of scarring, increased requirements for the quality of life of patients with scars make this problem relevant.

The number of patients in need of elimination of dermatogenic deformities after injuries, burns, surgical interventions, certain chronic diseases of the skin and subcutaneous fat, etc. is growing every year. According to statistics, every year, an average of 100 million people in developed countries are diagnosed with cicatricial deformities that occur for one reason or another. Among this number, on average, 15% of scars have an extremely unaesthetic appearance. Various forms of cicatricial pathology of the skin are often the cause of unpleasant subjective sensations functional, cosmetic and psychological disorders. The scientific and practical interest in this pathology is understandable. In recent decades, experimental and clinical studies have significantly expanded the understanding of the pathogenetic mechanisms of scarring and ways to influence them. Despite the achievements of medical science, which has significantly expanded the arsenal of therapeutic options for the correction of cicatricial changes, this problem has not lost its relevance to this day. This is due to such factors as the increased demands of patients and doctors for aesthetic results, expressed from negative impact on the psycho-emotional sphere, social status and social adaptation of patients. The search for new schemes for the treatment and prevention of pathological scars of the skin is expedient.

DEFINITION OF SCAR DEFORMITIES

A **scar is defined** as a pathological area of fibrous tissue that replaces normal skin after an injury. It is the result of the physiological wound repair process in the skin, as well as in other organs and tissues of the body.

The healing process involves elimination of dead cells, production of repair tissue and its re-modelling and maturation. The result is an element of a different colour and texture than the original skin, less elastic and moderately below the normal layer of the skin. When the healing process does not proceed as it should, the production phase continues longer to be followed by maturation and re-modelling and produces a hypertrophic scar. In other cases, the production phase is shorter or a deep destructive injury (inflammation) destroys the mesodermal and/or hypodermal layers of the skin, resulting in an atrophic scar.

The skin is the boundary of the human body playing a fundamental role in protection against external effects and internal homeostasis control.

Skins scars are the result of regeneration or atrophy arising from damage of skin layers: epidermis, dermis, and hypodermis.

Scars may form as a result of previous skin diseases (abscessed forms of acne, Rosacea, deep pyodermae, lupus erythematosus, scleroderma, cutaneous tuberculosis, etc.), injuries, burns, and surgical interventions.

SCAR CLASSIFICATION

The scar nature depends on the depth and area of a pathological process, on the one hand, and competence of repair mechanisms involved in healing processes, on the other hand.

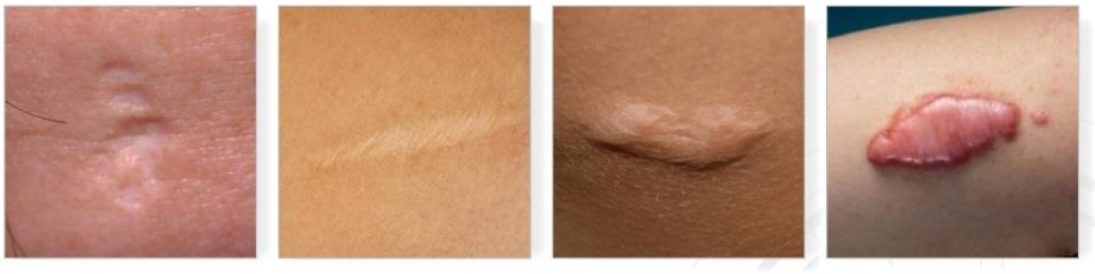
There are more than 15 different classifications of skin scars, some of which repeat one another.

The most commonly used **classification of scars** by their nature is based on the ratio of their surface height and the surrounding skin level.

This classification by V.V. Yudenich et al. (1985 y.) supplemented later by A.E. Reznikova (1999 y.) identified:

1. Normotrophic
2. Atrophic
3. Hypertrophic
4. Keloid scars

Fig.1 Examples of scars according to classification V.V. Yudenich and others



atrophic normotrophic hypertropfic keloid

Other classifications:

By form: linear, Z-shaped, stellate, fan-shaped, in the form of a cicatricial band.

By length: long, short, limited, extensive.

By width: wide, narrow.

By number: single, multiple.

By depth: deep, superficial

By colour: normal skin colour, hypo- and hyperpigmented, erythematous, mosaic.

By changes in sensitivity: with hypaesthesia, hyperaesthesia, normaesthesia, dysaesthesia.

By effect on the function of the affected areas: affecting, not affecting the function.

By aesthetic parameters: aesthetically acceptable, aesthetically unacceptable.

Currently, the clinical and morphological classification of A. B. Shechter and A. E. Guller (2008) is relevant. Its new principle is to distinguish the concepts of "clinical type of scar" (atrophic, normotrophic, hypertrophic) and "scar tissue type". For the first time, the authors identified fibrotic-altered dermis (FAD), as well as normotrophic, typical and nodular hypertrophic, keloid scar tissue. It was found that in 87% of cases, scars have a combined tissue composition, that is, they consist of different types of scar tissue.

PATHOLOGIC PHYSIOLOGY OF THE WOUND PROCESS

Understanding the pathologic physiology of the wound process enables correct determination of pathogenesis links, acting upon which may lead to optimization of the wound healing process, as well as periods and timing of this action. According to the **classification** by Ippolit Davydovsky, the following types of **wound healing** are distinguished:

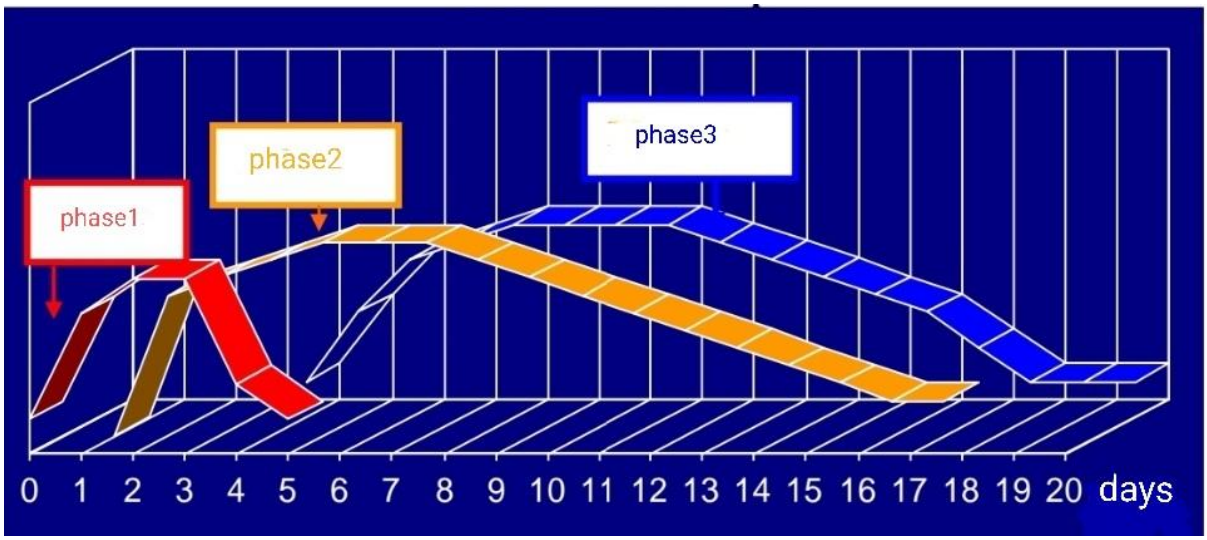
1. Direct closure of an epithelial cover defect without scar formation
2. Healing under the eschar without scar formation
3. Healing by primary intention (per primam intentionem) without pyogenesis, resulting in the formation of a neat linear scar
4. Healing by secondary intention (per secundam intentionem) terminating in formation of a rather coarse connective tissue scar.

Wound healing phases

The wound healing process can be divided into three phases:

1. Inflammatory phase (exudative) includes arrest of bleeding and wound cleansing;
2. Proliferative phase includes development of granulation tissue;
3. Differentiation phase (tissue re-modelling), includes scar maturation and formation.

Fig.2 Subsequence of wound healing phases



Inflammation, granulation, and differentiation phases often occur sequentially and not infrequently go simultaneously.

1. ***Inflammatory phase (exudative)***

It lasts for approximately three days and has two consecutive stages: vascular changes and wound cleansing due to suppression of microflora and rejection of non-viable tissues. Inflammation starts immediately after the injury, when damaged blood vessels release their content, some part of which is involved in blood clot formation, and the other part (vasoactive substances) causes vasoconstriction.

The primary objective of the first stage is to prevent a large loss of blood until the moment when platelet aggregation ensures closing of the injured vessels. This stage lasts for about 10 minutes.

At the second stage, the formation and strengthening of blood clots in the vessels occurs. The key role here is played by platelets, which activate as they leave the damaged vessels and begin to release a large number of mediators: von Willebrand factor, adenosine

diphosphate, thromboxane A2, 5-hydroxytryptophan, fibrinogen, fibronectin and thrombospondin.

Fibrinogen, which converts thrombin into fibrin, strengthens the formed blood clot in the vessel and participates in formation of a provisional matrix around it.

Blood clot formation does not occur in all vessels, but only in the ones located in the wound. This process is controlled by endothelial cells of damaged vessels. Their fibrinolytic activity is associated with the synthesis of prostacyclin by endotheliocytes, suppressing platelet aggregation, antithrombin III inhibiting thrombin activity; C-protein neutralizing coagulation factors V and VIII, plasminogen activator lyzing the blood clot due to plasminogen conversion into plasmin. In such a manner, a stage-by-stage controlled blood coagulation leads to a blood clot formation, which stops bleeding in the wound, protects it from fluid loss and contamination by pathogenic flora.

In addition to the synthesis of mediators, during the first minutes, aggregated platelets begin to release **platelet-derived growth factor (PDGF)**, being a chemotactic agent for leukocytes. This cytokine attracts leukocytes to the site of injury and maintains their functional activity. At the same time, leukocytes produce other important substances involved not only in the inflammation process, but also in the implementation of the proliferative phase: **transforming growth factor β (TGF-β)**, platelet factor 4, peptides that activate connective tissue, β-thromboglobulin and neutrophil-activating peptides-2.

After some time, previously narrowed arterioles begin to expand influenced by vasoactive substances of mast cells (histamine, bradykinin and complement components). These substances increase vascular permeability, facilitate penetration of neutrophils and monocytes into the wound space. Clinically, the process is manifested by redness and an increase in skin temperature around the wound.

Phagocytosis and protection against infection

Leukocytes, primarily, neutrophils and monocytes, are found in the wound as early as in 24 hours after the injury. The neutrophils present in the wound for several days subject the wound to primary "cleaning", phagocytize the cellular detritus, microorganisms and small foreign substances remaining in the wound. In the case of bacterial contamination,

leukocyte migration continues and phagocytosis increases, which increases the time of wound healing.

Neutrophils and monocytes are attracted to the wound surface with the help of **platelet-derived growth factor (PDGF)**, as well as with kininogenase and fibrinopeptides – chemotactic factors released from the breakdown products of fibrinogen and fibrin. The activity of neutrophils is enhanced by integrins – receptors on the cell surface, through which interaction between the cells and the extracellular matrix occurs. Integrins allow neutrophils to recognize, phagocytize, and eliminate bacteria and detritus.

As the inflammatory process continues, neutrophils are replaced by monocytes, which, under the action of PDGF, are transformed into tissue macrophages. Monocytes and tissue macrophages are the main cells in phagocytosis completion and full wound cleansing; they also synthesize cytokines which stimulate fibroblast proliferation, formation of granulation tissue and extracellular matrix, **fibroblast growth factor**, **TGF-α** and **β**, and **vascular epithelial growth factor**.

2. ***Proliferative phase***

It starts on the 4th day after the injury, but its prerequisites are created as early as during the inflammatory phase. At this stage, the forming granulation tissue and ground substance (provisional fibrin-fibronectin matrix) begin to fill the wound space. The main healing cells in this phase are epithelial cells, fibroblasts and endothelial cells. Changing phenotypically, they migrate to the wound space, where release various substances involved in forming a provisional matrix and restoring damaged epidermis and basement membrane. The remaining viable basal cells of the epidermis along the wound edges and in the follicles under the influence of cytokines: TGF-β, epidermal growth factor and provisional matrix proteins (fibronectin, as well as type I and IV collagens) undergo a number of morphological changes: they become flattened, undergo retraction, which makes it possible to move along the collagen fibers into the wound space of cells with protruding processes – lamellipodia directed into the wound space.

Laminin, as a large glycoprotein and a potent inhibitor of epithelial cell migration is the main component of the lamina lucida of the dermoepidermal junction (DEJ), and in intact skin it prevents direct contact between epithelial cells and type IV and VII collagens contained in DEJ and the dermis (type I, III and VI collagens). In case of DEJ destruction, basal cells come into direct contact with the underlying collagen and migrate. As soon as epithelial cells move into the wound space and line it, laminin reappears in the DEJ zone, i.e as early as on the 3rd day the epithelium, moving along the fibrin-saturated matrix, partially closes the wound space. On the 7th day, epithelial cells, moving towards each other from opposite sides of the wound, completely close it. However, this is only possible in superficial wounds; in deep skin wounds, migration of epithelial cells occurs only as the tissue defect is filled with granulation tissue. The process of re-epithelialization of the wound space is accompanied by **fibroplasia** and **angiogenesis**.

Fibroplasia is the formation of granulation tissue and a provisional matrix. The cells involved in the formation of granulation tissue are endotheliocytes and fibroblasts. One of the stimuli for their activation is a low level of oxygen in the wound (hypoxia) as a result of damage to blood vessels.

Fibroblasts, like epithelial cells, undergo phenotypic changes, due to which they start migrating into the wound, synthesize a large amount of collagens, proteoglycans, elastin, and other albuminous compounds of the matrix (they participate in the reduction of the wound space).

The main component of the early provisional matrix is **fibronectin** located along fibroblasts; therefore, newly formed collagen fibers have the same orientation. Fibronectin matrix appears approximately on the 5th day after the injury and, together with fibroblasts, participates in contraction of the wound space.

Hyaluronic acid, being another component of the provisional matrix**,** promotes cell mobility due to their adhesion to the matrix, while fibronectin and heparan sulfate, on the contrary, enhance the attachment of cells to the matrix. In the early healing phases, **fibroblasts** produce much more hyaluronic acid than in intact skin. Influenced by TGF-β, the most powerful stimulator of collagen synthesis, they actively synthesize type I and III collagens, elastin, glycosaminoglycans and proteoglycans. As the provisional matrix is formed, part of them under the influence of TGFβ continues to change and transform

into actin-rich **myofibroblasts**, which have the properties of both fibroblasts (producing a large number of matrix proteins) and smooth muscle cells (due to their ability to contract) and are located in the granulation tissue along the wound edges. Influenced by mediators (angiotensin, prostaglandins, bradykinins and endothelins), myofibroblasts start contracting, which leads to convergence of the wound edges. In superficial wounds, cutaneous appendages with preserved epithelial cells remain intact, so the process of wound space contraction is less pronounced and epithelialization is observed both from the wound edges and from the adnexal structures of the damaged skin.

Angiogenesis (neovascularization) is the process of formation of many capillaries that feed main regeneration cells, closely related to fibroplasia and processes occurring in the extracellular matrix.

Endotheliocytes, as the main cells of angiogenesis, like epithelial cells and fibroblasts, undergo specific changes, due to which they are able to migrate. As early as on the 2nd day after the injury, endothelial cells located along the wound edges begin to move into the perivascular space, and the cells remaining in the vessels proliferate and participate in the formation of new vessels.

The angiogenesis process is stimulated by various factors: hypoxia, cytokines, a number of components of the provisional matrix (acidic protein enriched with cysteine, tenascin, thrombospondin, heparin, fibronectin).

3. ***Differentiation phase (tissue re-modelling)***

The third and final stage of wound healing including the formation of scar tissue itself, starts from the 6th-10th day and can last for several months. During this period, the entire granulation tissue is replaced by scar tissue, due to which the barrier functions of the skin are restored to a greater extent.

The transformation of granulation tissue into the connective one is carried out by reducing the cell number and degradation of the provisional extracellular matrix. The number of capillaries and fibroblasts decreases, myofibroblasts disappear (this sharp decrease in the cell number in the transforming granulation tissue occurs due to apoptosis).

The provisional fibronectin matrix is destroyed by cells and plasma proteases. Hyaluronic acid is replaced by sulfated proteoglycans: chondroitin-4-sulfate and dermatan sulfate; they significantly increase compressive properties of scar tissue and inhibit cell movement and proliferation. Within a short period of time, fibronectin is replaced by type III collagen, and a year or later – by type I collagen.

PHASES OF SCAR FORMATION

The outcome of the physiological process of wound healing is normally the formation of a tender scar with a slight fibrosis. Thanks to this process, the integrity and functions of the organ are restored.

Scar formation

In its formation, the scar goes through four successive stages:

Stages of scar formation:

I Stage of inflammation and epithelialization (7th-10th day from the moment of injury)

II Stage of a "young" scar (10th-30th day from the moment of injury)

III Stage of an "old" scar (30th-90th day from the moment of injury)

IV Stage of final scar transformation (from the 4th month to a year from the moment of injury)

I ***Stage of inflammation and epithelialization.***

It lasts from the 7th to the 10th day from the moment of injury. It is characterized by a gradual decrease in skin swelling and inflammation. Granulation tissue is formed, bringing together the wound edges; there is no scar yet. If there is no infection or dehiscence of the wound surface, the wound heals by primary intention with formation of a barely noticeable thin scar. In order to prevent complications at this stage, it is possible to overcast atraumatic sutures; daily dressings with local antiseptics are performed. Physical activity is limited to avoid dehiscence of the wound edges.

II ***Stage of a "young" scar formation.***

It includes the period from the 10th to the 30th day from the moment of the injury. It is characterized by formation of collagen-elastin fibers in the granulation tissue. The scar is immature, loose, easily extensible, bright pink in colour (due to increased blood supply to the wound). At this stage, secondary wound injury and increased physical activity should be avoided.

III ***Stage of a "mature" scar formation.***

It lasts from the 30th to the 90th day from the injury. Elastin and collagen fibers grow into bundles and line up in a certain direction. The blood supply to the scar is reduced, causing it to thicken and turn pale. There are no restrictions on physical activity at this stage, but re-traumatization of the wound may cause formation of a hypertrophic or keloid scar.

IV ***Stage of the final scar transformation.***

It lasts from the 4th month after the injury to a year; the final maturation of the scar occurs: death of blood vessels, collagen fiber tension. The scar thickens and turns pale. It is during this period that the doctor becomes clear about the scar condition and further tactics of its correction.

It seems to be impossible to get rid of scars completely. However, with the help of modern techniques, it is possible to make the scar **aesthetically** more acceptable. Choosing the technique and treatment efficacy depends on the stage of a scar defect formation, as well as on the scar type. The following rule should be considered: the sooner medical care is provided, the better result is expected.

Scar formation: clinical and histomorphologic pattern

Wound closing with epithelium: collagen fibers are located parallel to the skin surface. Within 2 to 2.5 weeks, the scar coarsens, the pale pink epithelium becomes whitish, denser and approaching the colour of the surrounding skin.

Swelling of scar tissue: loosely located collagen fibers penetrated by vessels and an undeveloped horny layer of the epidermis are revealed in the intercellular substance. Visually, the scar rises above the skin level, reddens and becomes sensitive to touch. After 3-4 weeks, the erythema turns cyanotic, the pain decreases, the scar becomes evenly dense, it sharply protrudes above the skin surface in some places, which makes it look like a keloid scar. Subsequently, the scar softening may occur or its transformation into a keloid or hypertrophic scar.

With a keloid or hypertrophic scar: fibroblasts dominate and are activated, collagen fibers become denser, more compact and form spirals and nodules; during neovascularization, expanding vessels give the scar an intense red colour.

OVERVIEW OF SCAR CORRECTION METHODS AND SIGNIFICANCE OF HYALURONIC ACID IN THE PROCESS OF SCAR FORMATION

Scar correction methods

Existing methods of skin scar correction are divided into two groups: **surgical and conservative** (non-surgical).

The latter is divided into: **invasive and non-invasive.**

General list of the **conservative and mini-invasive methods** of scar treatment:

1**. *Pharmacological:***

- proteolytic enzymes (e.g., Hyaluronidase)
- intralesional corticosteroid injections
- silicone gels (e.g., Dermatix)

2. ***Physical (compression therapy):***

- compressing bandages

- compression underwear

- silicone patches

3. ***Physiotherapy:***

- Grenz ray therapy

- phonophoresis

- electrophoresis

- electrostatic massage (e.g., Hivamat – therapy)

4. ***Cosmetology:***

- chemoexfoliation (chemical peels)

- dermabrasion

- laser correction

- cryodestruction

- contour plastic (filling with stabilized hyaluronic acid preparations)

- mesotherapy

- botulinum therapy

- cosmeceuticals (topical preparations)

5. ***Cosmetic (camouflage).***

- tattoo

- cosmetics

6. ***Psychotherapy.***

Lipofilling, a mini-invasive plastic surgery, should be noted as an effective method of scar correction. This method is related to conservative methods of treatment of scar deformities, because it is implemented without violating the integrity of the scar. Lipofilling consists in introducing autologous adipose tissue into areas that need correction.

In my practical work with patients, I use many of the above-mentioned methods of scar correction.

However, I would like to find, metaphorically speaking, "**the ideal method"** for scar correction. In my opinion, it should meet the following **conditions**:

1. Effective

2. Atraumatic

3. Fast-acting

4. Cost-efficient

The surgical excision or lipofilling of the scar from the point of view of the above criteria cannot be satisfactory, since it may lead to the formation of new scars and require additional rather long rehabilitation. Among the cosmetic methods of correction of hypotrophic scar the method of volumetric correction with fillers is usually used . Fillers or injectable implants are gel -injectable products in Aesthetic Medicine that allow to perform contouring without surgery. Most fillers are cross- linking agent (BDDE= butandiol diglycidyl ether or DVS=divinylsulfone) stabilized hyaluronic acid products. The period of their volumetric action usually does not exceed 6-9 mounths, which makes it necessary to repeat the correction of the scar on a regular basis. Laser ablation and cryodestruction are quite effective methods of correction of cicatricial changes in the skin. However, they provide for a fairly long period of rehabilitation and can lead to a number of complications such as hypopigmentation, hyperpigmentation and the formation of new cicatricial deformities. The remaining of the above methods of scar correction require an earlier start of treatment or are significantly less effective.

Scientific research in recent years devoted to the study of the effect of a modern injection drug from hyaluronic acid (HA) on the biological processes occurring in the skin has created prerequisites for its use in order to correct scar deformities.

CAPITOLO 2

HYALURONIC ACID: DEFINITION, STRUCTURE, PHYSIOLOGICAL FUNCTION, MEDICAL USES, STABILIZATION OF HYALURONIC ACID, CLINICAL STUDIES.

Definition

Hyaluronic acid (HA), also called hyaluronan, is an anionic, nonsulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues. It is unique among glycosaminoglycans as it is non-sulfated, forms in the plasma membrane instead of the Golgi apparatus, and can be very large: human synovial HA averages about 7 million Da per molecule, or about 20,000 disaccharide monomers, while other sources mention 3–4 million Da.

The average 70 kg person has roughly 15 grams of hyaluronan in the body, one third of which is turned over (i.e., degraded and synthesized) per day.

As one of the chief components of the extracellular matrix, it contributes significantly to cell proliferation and migration.

Hyaluronic acid is also a component of the group A Streptococcal extracellular capsule, making it a major source of biosynthetic HA.

Structure

Hyaluronic acid (HA) is a linear glycosaminoglycan (GAG), an anionic, gel-like, polymer It is part of a family of structurally complex, linear, anionic polysaccharides. The carboxylate groups present in the molecule make it negatively charged, therefore allowing for successful binding to water, and making it valuable to cosmetic and pharmaceutical products.[46]

HA consists of repeating β4-glucuronic acid (GlcUA)-β3-*N*-acetylglucosamine (GlcNAc) disaccharides, and is synthesized by hyaluronan synthases (HAS), a class of integral membrane proteins that produce the well-defined, uniform chain lengths characteristic to HA

Physiological function

Hyaluronic acid is part of the extracellular matrix. Hyaluronic acid is a major component of the synovial fluid and was found to increase the viscosity of the fluid. Along with lubricin, it is one of the fluid's main lubricating components. Also hyaluronic acid is an important component of articular cartilage. As a result of its physico-chemical and rheological properties such as viscoelasticity and capacity to retain water, Hyaluronic Acid (HA) plays a pivotal role in the control of tissue hydration and permeability to small or large molecules, and these properties contribute to its excellent biocompatibility and immune barrier function. In the human dermis, the high percentage of HA allows hydration, maintaining at the same time a proper tissue volume which buffers skin cells from mechanical damage. Alone or in combination with other molecules, HA accelerates in vitro processes related to wound healing and in vivo tissue regeneration (e.g., burns, ulcers).

Furthermore, HA can provide both anti-inflammatory and bio- stimulating effect, and also activate other signaling pathways through the interaction with cell membrane receptors such as CD44, TLR-4, and RHAMM. All the above mentioned properties make HA an excellent dermal agent, able to correct soft tissue defects including scar and prompt biosynthetic processes. The skin matrix is responsible for structural integrity, mechanical elasticity, stability and many other functions.

Medical uses

Hyaluronic acid has been FDA-approved to treat osteoarthritis of the knee via intra-articular injection. Intra-articular injection of high molecular weight HA improved both pain and function in people with knee osteoarthritis.

Hyaluronic acid has been used to treat dry eye. Hyaluronic acid is a common ingredient in skin care products. Hyaluronic acid is used as a dermal filler in cosmetic surgery. It is typically injected using either a classic sharp hypodermic needle or a micro-cannula. Currently, hyaluronic acid is used as a soft tissue filler due to its bio-compatibility and possible reversibility using hyaluronidase.

Stabilization of hyaluronic acid

For the prolonged stay of the HA-based gel in the tissue, the structure of the polysaccharide is stabilized by the formation of intermolecular cross-links. This type of connections leads to the formation of a spatial 3D HA structure, which ensures the slow degradation of the hyaluronic gel for several months. The following chemical compounds are used as a "crosslinking agent", a cross - linker: most often **1,4 - butanediol diglycidyl ether** (BDDE); diepoxyoctane; divinylsulfone; formaldehyde.

Italian pharmaceutical company IBSA Farmaceutici has created for the first time a product with **thermally stabilized natural hyaluronic acid in the form of hyaluronan hybrid cooperative complexes**, developed with unique and innovative patented production process based on NAHYCO technology. The significance of NAHYCO technology lies in the fact that low- and high-molecular HA complexes are first heated, then sharply cooled, which leads to the formation of hydrogen bonds between HA chains. This allows not to use a chemical cross-linking agent. This medical device is called PROFHILO. Each syringe of this product contains 3.2% - 32 mg (H-NA) + 32 mg (L-HA) in 2 ml hyaluronic acid sodium salt for intradermal injection.

Clinical studies

There are some clinical studies demonstrating the influence of hyaluronan hybrid cooperative complexes of HA on cellular bioprocesses reactivation.

The **first study** to be mentioned demonstrates an **increase in the expression levels of type I and type III collagen in fibroblasts and keratinocytes, and type IV and type VII collagen in, as well as increase in elastin expression with the hybrid cooperative complexes**. Antonella Stellavato and others carried out the study, which evaluated the multi-faceted interaction between keratinocytes and dermal fibroblasts in presence of the novel hybrid cooperative complexes HA formulation has been evaluated. The in vitro model employed has made possible the functional interaction between the two cell types, involving the synthesis and assembly of the skin extracellular matrix (ECM) proteins. Also a full thickness skin model was used toward better resembling in vivo effect. The results showed a notably different biological response, regarding collagen and elastin expression and synthesis, of HA hybrid cooperative complexes respect to native HA formulations, with a potential for better global bio-remodelling performance. These findings could overall corroborate the in vivo clinical data obtained on the HA hybrid cooperative complex.

The results of this study are presented below in the form of diagrams and photographs

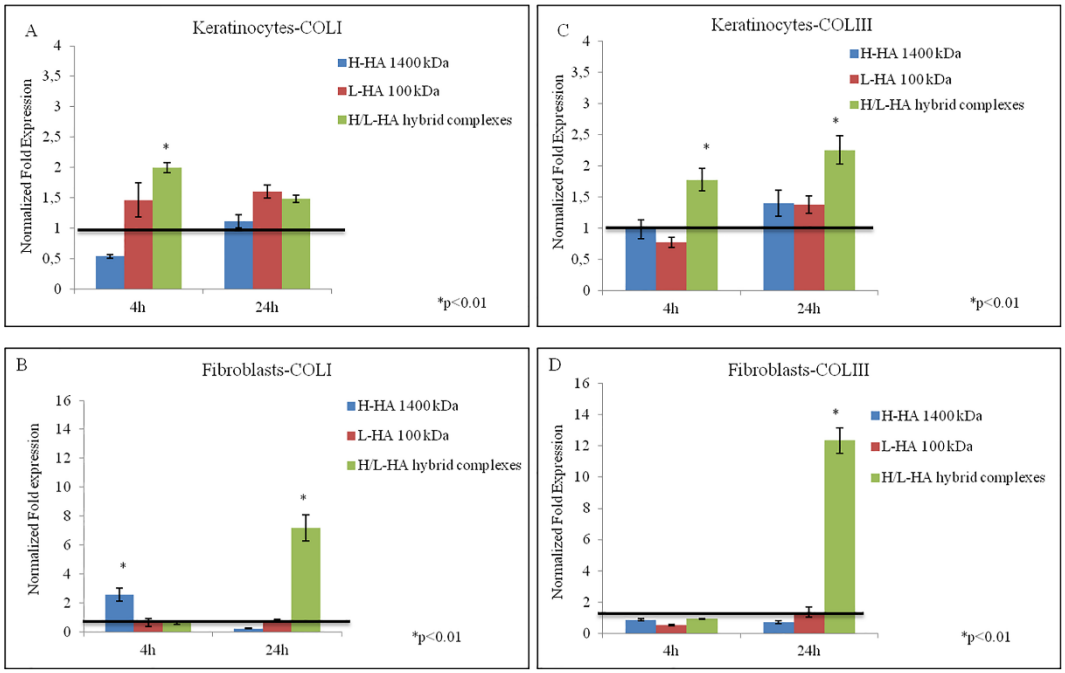


Fig.3 Increased the expression levels of type I and type III collagen in keratinocytes and fibroblasts in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecular weight HA (H-HA) and low molecular weight HA (L-HA) after 4 and 24 hours incubation

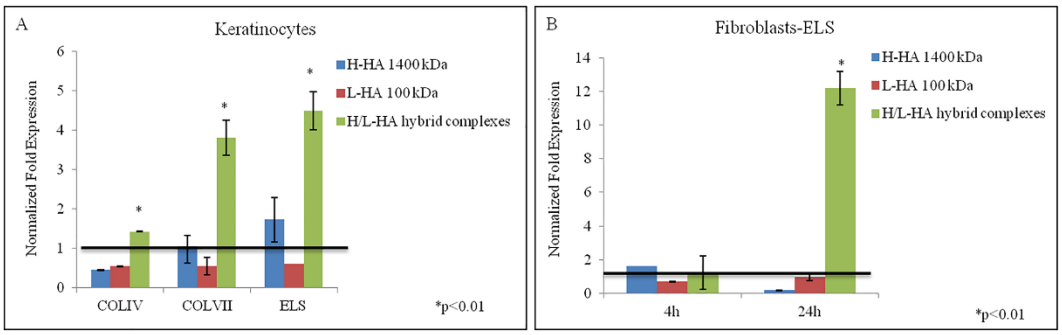


Fig. 4 Increased the expression levels of type IV , type VII collagen and elastin (ELS) in keratinocytes and fibroblasts in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecular weight HA (H-HA) and low molecular weight HA (L-HA) after 4 hours incubation

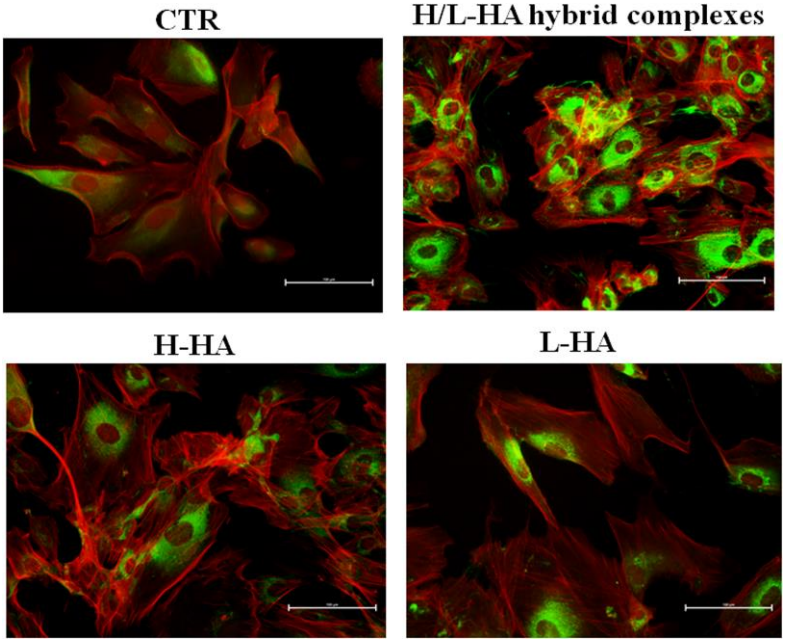


Fig.5 Increased the expression levels of type I collagen in keratinocytes and fibroblasts in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecular weight HA (H-HA) and low molecular weight HA (L-HA)) immunofluorescence picture

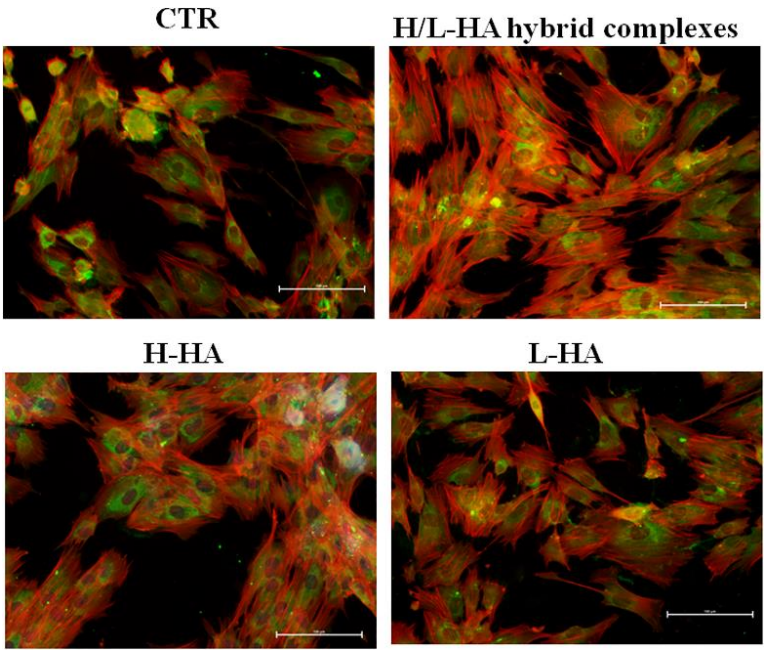


Fig.6 Increased the expression levels of type III collagen in keratinocytes and fibroblasts in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecula weight HA (H-HA) and low molecular weight HA (L-HA)) immunofluorescence picture

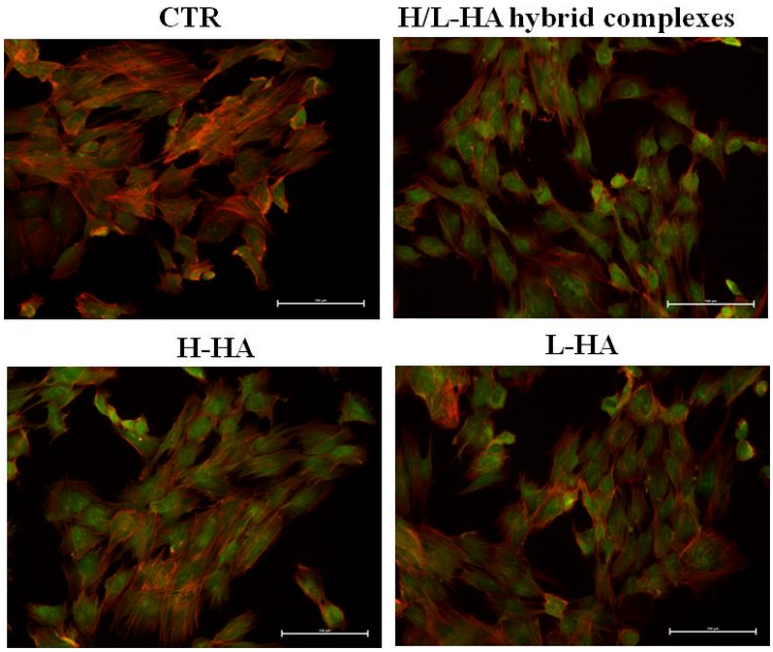


Fig.7 Increased the expression levels of elastin (ELS) in keratinocytes and fibroblasts in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecular weight HA (H-HA) and low molecular weight HA (L-HA)) immunofluorescence picture

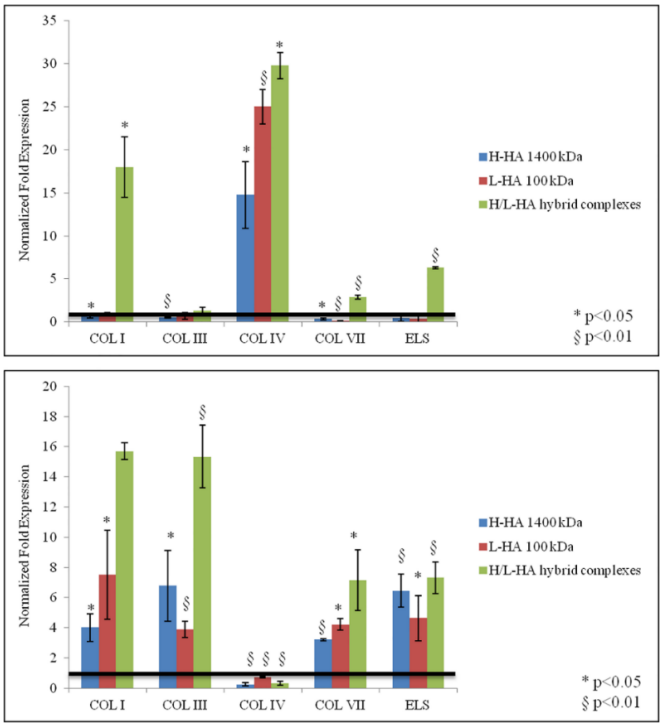


Fig.8 Increased the expression of type I collagen, type III collagen, type IV collagen, type VII collagen and elastin for the full thickness skin model samples in the presence of high/low HA hybrid cooperative complexes (H/L-HA) compared to high molecular weight HA (H-HA) and low molecular weight HA (L-HA) at 24 h. (a) and 7 days (b)

The **second study** to be mentioned demonstrates identification of **ability of Hyaluronan hybrid cooperative complexes (HCC) to recruit and differentiate stem cells in adipocytes, and considerably improving fat tissue renewal**. Antonella Stellavato and others carried out the study which demonstrates for the first time that hybride cooperative complexes potentiate Adipose-derived Stem Cells (ASCs) differentiation, preserving both morphology and viability. The quality and the efficiency of the differentiation are greater than that obtained with the other HA formulations, both in terms of gene, protein and morphological expression, and with the formation of large and numerous lipid vacuoles. This is of major importance in clinical use. Possible to assume that this substance can affect the differentiation of resident fat cells that are present in both the dermis and hypodermis, which in turn may lead to replenishment of the local deficiency of subcutaneous adipose tissue.The results of this study are presented below in the form of diagrams and photographs.

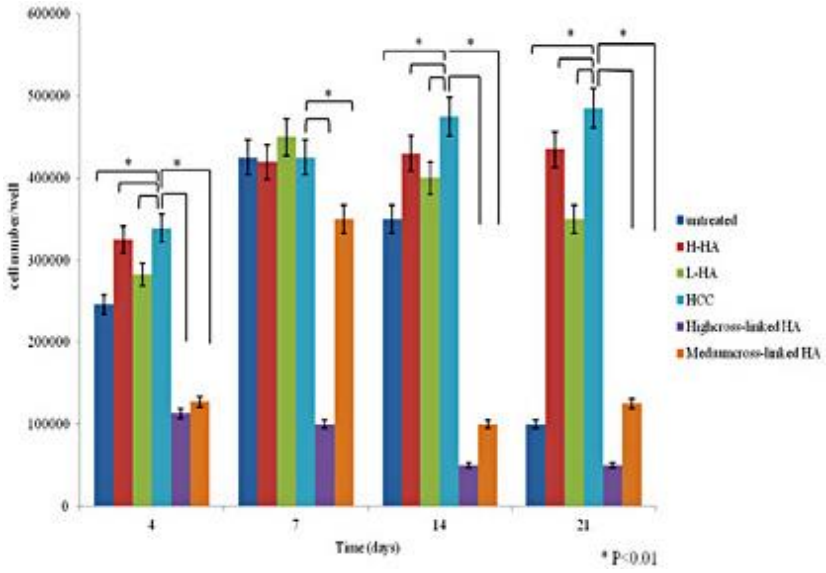


Fig.9 At long times of culture and starting from 14 days, hybrid cooperative complexes (HCC) showed a higher proliferation rate than other HA gels (L-HA, H-HA, High and Medium cross-linked HA formulations) and control. Evaluation of cell proliferation at 4, 7, 14 and 21 days in presence of different HA gels

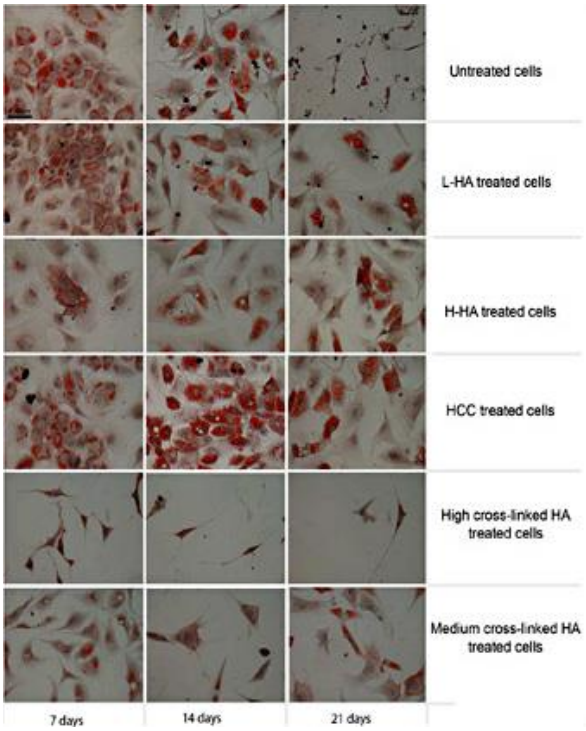


Fig.10 The Oil Red O staining showed that untreated and treated ASCs, differentiated into adipose cells. HCCs was found to be the better complex of HA to induce the adipogenic differentiation. At 14 days, cells showed typical multi-vacuolated adip The cells become senescent at 21 days and showed few and small droplets ocytes with rich lipid droplets cytoplasm and strong positivity for Oil red O staining.

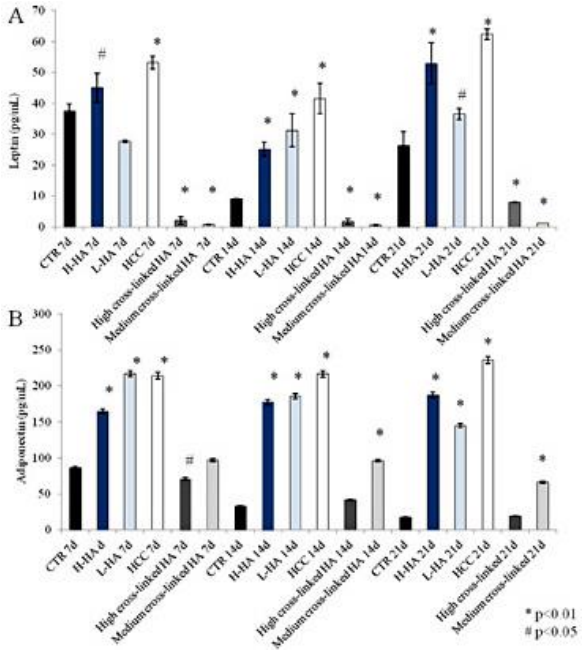


Fig.11 Increased the adipogenic cytokine secretion, including leptin and adiponectin with HCC compared with other HA gels (L-HA, H-HA, High and Medium cross-linked HA formulations) and control (CTR) Evaluation of adipogenic cytokine secretion at 7, 14 and 21 days were evaluated.

Presented results of scientific research demonstrate biological activity of a commercially available hyaluronic acid, PROFHILO, which is a **thermally stabilized hyaluronan hybrid cooperative complexes** based on NAHYCO technology. HCC act as an activator of biological cascades, stimulat cell migration and proliferation, thus, it creates prerequisites for its **use in formulations for injectable use as a dermal inductor for wound healing and bio-remodeling of scar deformities of the skin**.

CAPITOLO 3

EXPERIENCE OF CLINICAL APPLICATION OF HYALURONAN HYBRID COOPERATIVE COMPLEXES FOR THE PURPOSE OF CORRECTION OF SCAR DEFORMITIES OF THE SKIN.

INTRODUCTION

Hyaluronan hybrid cooperative complexes promotes multi-level dynamic bio remodeling of skin. In vitro studies have shown that hyaluronan hybrid cooperative complexes, on the example of the use of a commercially available PROPHILO, improves the extracellular environment: maintaining suitable conditions for the viability of - fibroblasts, keratinocytes and adipocytes. Stimulating effect of PROFHILO leads to a reconstruction of the extracellular matrix and creates prerequisites for optimizing skin healing processes, including bio remodeling of the finally formed scars.

OBJECTIVE

Define the role of stabilized hyaluronic acid injectable gel in the form of hybrid cooperative complexes for early and late treatment of scar deformities concerning both on skin and subcutes.

PATIENTS AND METHODS

Medical device gel

PROFHILO is the first product with thermally stabilized natural hyaluronic acid in the form of hyaluronan hybrid cooperative complexes, with concentrations of HA 64mg /2ml. Each syringe of this product contains 3.2% - 32 mg (H-NA) + 32 mg (L-HA) in 2 ml hyaluronic acid sodium salt for intradermal injection.

For scar correction, PROFHILO were administered to the patients in 3 or 4 (media 3,5) treatment sessions with 4 weeks interval. Bolus injections of 0,1 ml were carried out in the deep dermis of the scar area and in the intact skin around the edge of the scar using needle 30 G in a volume of 0,5 to 2,0 ml (media 1,25 ml). In addition to treatment, the recommendations were made to not use topical therapy, not apply irritating agents and avoid ultraviolet exposure were given. Lidocaine 2,5% and Prilocaine 2,5% creams were applied as local anaesthetic.

Patients

The Patients were a group of 4 people aged 18 to 50 years (media 34 years), 3 women and 1 man, with 5 different scar deformities (atrophic scar - 2, hypertrophic scar - 2, retraction scar - 1). The causes of scarring were: surgery, injury, burn.

Table 1. Inclusion and Exclusion criteria for the patients

Inclusion criteria	Exclusion criteria
age patient ≥ 16 years,	chronic skin diseases in the acute stage
age scar ≥ 6 weeks	corticosteroid therapy
length scar ≥ 3cm	inability to carry out course treatment
cause of scar is known	no visible normal skin on similar anatomical area as a controlavailable
caucasian	

Assessment

For the assessment of the scar status, physical methods of examination (visual inspection, palpation) , measurements with a caliber, a scale “Scala dell’osservatore POSAS” were performed. The results of the treatment were documented photographically befor and after therapy.

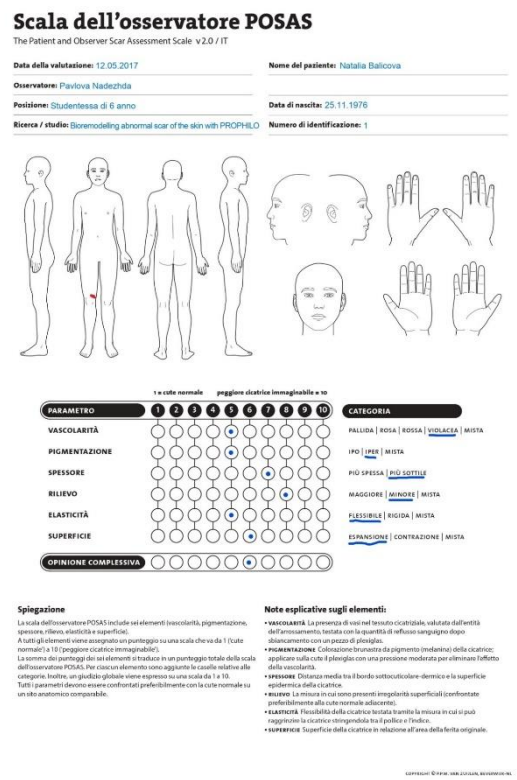
Patient and Observer Scar Assessment Scale (POSAS)

The POSAS is composed of two numerical scales that evaluate signs and symptoms of healing. It consists of the two following parts: a scale for patients and a scale for observers. Both contain six items punctuated numerically from 1 to 10, which comprise the “total score” of the scale for both the patient and observer. Each item evaluates a specific parameter. In addition, the patient and observer also mark their “general opinion” regardless of the “total score,” also scored from 1 to 10.

Items and total scale scores for the patient and observer: Each item on both scales has a score of 1 to 10. The lowest score is 1 and corresponds to the normal skin situation. The total score of both scales can be calculated simply by adding the scores of each of the six items. The total score will range from 6 to 60.

Categories: Category boxes are provided to mark items not only quantitatively but also qualitatively. In this way, not only gravity but also the direction of the disorder is addressed. The items in the categories are not included in the total POSAS score, even though they are considered clinically relevant for complete documentation.

General scale for the patient and scale for the observer: Both the patient and observer provide their general opinion on the appearance of the scar. The 10-point scale is again used, where 10 is the worst scar imaginable.



Scala dell'osservatore POSAS

The Patient and Observer Scar Assessment Scale v 2.0 / IT

Data della valutazione: 12.05.2017 **Nome del paziente:** Natalia Balicova

Osservatore: Pavlova Nadezhda

Posizione: Studentessa di 6 anno **Data di nascita:** 25.11.1976

Ricerca / studio: Bioremodelling abnormal scar of the skin with PROPHILO **Numero di identificazione:** 1

1 = cute normale peggiore cicatrice immaginabile = 10

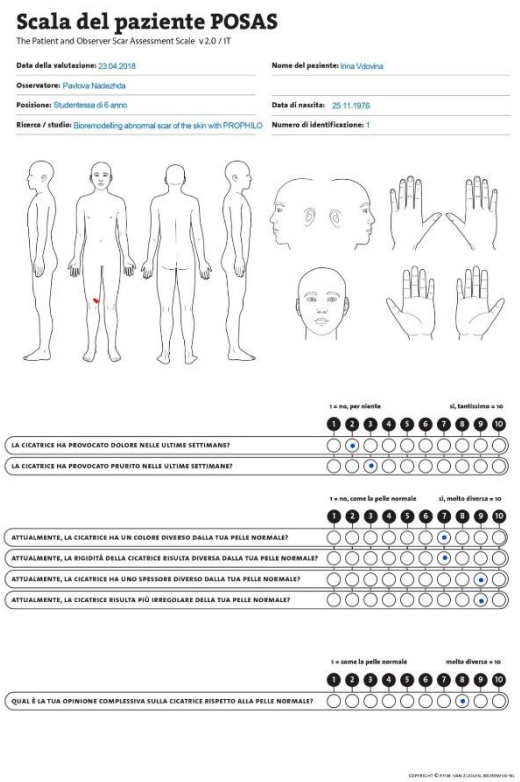
PARAMETRO	1	2	3	4	5	6	7	8	9	10	CATEGORIA
VASCOLARITÀ					●						PALLIDA | ROSA | ROSSA | VIOLACEA | MISTA
PIGMENTAZIONE					●						IPO | IPER | MISTA
SPESSORE							●				PIÙ SPESSA | PIÙ SOTTILE
RILIEVO								●			MAGGIORE | MINORE | MISTA
ELASTICITÀ					●						FLESSIBILE | RIGIDA | MISTA
SUPERFICIE						●					ESPANSIONE | CONTRAZIONE | MISTA
OPINIONE COMPLESSIVA						●					

Spiegazione

La scala dell'osservatore POSAS include sei elementi (vascolarità, pigmentazione, spessore, rilievo, elasticità e superficie).
A tutti gli elementi viene assegnato un punteggio su una scala che va da 1 ('cute normale') a 10 ('peggiore cicatrice immaginabile').
La somma dei punteggi dei sei elementi si traduce in un punteggio totale della scala dell'osservatore POSAS. Per ciascun elemento sono aggiunte le caselle relative alla categorie. Inoltre, un giudizio globale viene espresso su una scala da 1 a 10.
Tutti i parametri devono essere confrontati preferibilmente con la cute normale su un sito anatomico comparabile.

Note esplicative sugli elementi:

- **VASCOLARITÀ** La presenza di vasi nel tessuto cicatriziale, valutata dall'entità dell'arrossamento, testata con la quantità di reflusso sanguigno dopo sbiancamento con un pezzo di plexiglas.
- **PIGMENTAZIONE** Colorazione brunastra da pigmento (melanina) della cicatrice; applicare sulla cute il plexiglas con una pressione moderata per eliminare l'effetto della vascolarità.
- **SPESSORE** Distanza media tra il bordo sottocuticolare-dermico e la superficie epidermica della cicatrice.
- **RILIEVO** La misura in cui sono presenti irregolarità superficiali (confrontate preferibilmente alla cute normale adiacente).
- **ELASTICITÀ** Flessibilità della cicatrice testata tramite la misura in cui si può raggrinzire la cicatrice stringendola tra il pollice e l'indice.
- **SUPERFICIE** Superficie della cicatrice in relazione all'area della ferita originale.



Scala del paziente POSAS

The Patient and Observer Scar Assessment Scale v 2.0 / IT

Data della valutazione: 23.04.2018 **Nome del paziente:** Irina Vdovina

Osservatore: Pavlova Nadezhda

Posizione: Studentessa di 6 anno **Data di nascita:** 25.11.1976

Ricerca / studio: Bioremodelling abnormal scar of the skin with PROPHILO **Numero di identificazione:** 1

	1 = no, per niente									sì, tantissimo = 10
LA CICATRICE HA PROVOCATO DOLORE NELLE ULTIME SETTIMANE?		●								
LA CICATRICE HA PROVOCATO PRURITO NELLE ULTIME SETTIMANE?			●							

	1 = no, come la pelle normale									sì, molto diversa = 10
ATTUALMENTE, LA CICATRICE HA UN COLORE DIVERSO DALLA TUA PELLE NORMALE?							●			
ATTUALMENTE, LA RIGIDITÀ DELLA CICATRICE RISULTA DIVERSA DALLA TUA PELLE NORMALE?							●			
ATTUALMENTE, LA CICATRICE HA UNO SPESSORE DIVERSO DALLA TUA PELLE NORMALE?								●		
ATTUALMENTE, LA CICATRICE RISULTA PIÙ IRREGOLARE DELLA TUA PELLE NORMALE?								●		

	1 = come la pelle normale									molto diversa = 10
QUAL È LA TUA OPINIONE COMPLESSIVA SULLA CICATRICE RISPETTO ALLA PELLE NORMALE?								●		

Fig.12 An example of a filled POSAS scale.

Scale “Scala dell’osservatore POSAS” were filled by the doctor and each of the patients before and after treatment, respectively. The evaluation results were subjected to statistical analysis.

STATISTICAL ANALYSIS AND RESULTS

A group of 4 people aged 18 to 50 years (media 34 years), 3 women and 1 man, with 5 different scar deformities (atrophic scar - 2, hypertrophic scar - 2, retraction scar - 1). The causes of scarring were: surgery, injury, burn.

For scar correction, each Patient on an outpatient basis (in regime ambulatoriale) received PROFHILO were administered to the patients in media 3,5 (range of 3 or 4) treatment sessions with 4 weeks interval. Bolus injections of 0,1 ml were carried out in the deep dermis of the scar area and in the intact skin around the edge of the scar using needle 30 G in a volume of 0,5 to 2,0 ml (media 1,25 ml) . Lidocaine 2,5% and Prilocaine 2,5% creams were applied as local anaesthetic.

Each individual procedure and the full course of treatment did not cause any complications in any patient. The patients' well-being was satisfactory during the procedure and after. After the procedure, there was a slight erythema at the injection site, which completely passed within 20-30 minutes after the procedure.

Table 1. Assessment of scar criteria POSAS before and after treatment carried out by a **doctor** in 5 patients

criterii / paz	1 pre	1 post	2 pre	2 post	3 pre	3 post	4 pre	4 post	5 pre	5 post
Vascolarità	5	1	4	1	5	2	3	2	7	5
Pigmentazione	5	1	3	2	7	3	3	2	9	7
Spessore	7	1	4	2	8	4	3	2	6	1
Rilievo	8	1	4	2	8	4	4	2	4	1
Plasticità	5	1	5	3	10	5	1	2	9	1
Superfice	6	1	3	3	8	4	3	2	7	4
Opinione complessiva	6	1	4	2	8	4	3	2	7	3
MEDIA	6	1	3,9	2,1	7,7	3,7	2,9	2	7	3,1
DEV STANDARD	0,7	0,0	0,0	0,7	2,1	1,4	0,0	0,0	0,0	1,4

Table 2. Assessment of scar criteria POSAS before and after treatment carried out by a **patients** in 5 patients

criterii / paz	1 pre	1 post	2 pre	2 post	3 pre	3 post	4 pre	4 post	5 pre	5 post
Dolore	2	1	1	1	3	2	2	1	2	1
Prurito	3	1	3	1	5	2	4	1	4	2
Colore	7	1	4	3	7	4	7	3	10	8
Rigidità	7	1	4	3	10	5	8	3	10	1
Spessore	9	1	5	3	10	5	8	4	10	1
Irregolarità	9	1	4	3	10	5	9	3	10	5
Opinione complessiva	8	1	4	3	9	5	8	3	10	5
MEDIA	6,42	1	3,6	2,4	7,7	4,0	6,6	2,57	8	3,0
DEV STANDARD	4,2	0,0	2,1	1,4	4,2	2,1	4,2	1,4	5,7	1,4

The comprehensive opinion of the doctor regarding the initial condition of the scar evaluated in the range of 3-8 (media 5,6) and after treatment in the range of 1-4 (media 2,4). At the same time, the comprehensive assessment of the initial condition of the scar by patients differed from the medical opinion and evaluated in the range of 4-10 (media 7,8), and after treatment in the range of 1-5 (media 3,4). Patient ratings were higher. This fact demonstrates the presence of an emotional component of the perception and evaluation of the scar, which in some cases aggravates the situation. A significant difference in the indicators of a comprehensive assessment of the scar before and after treatment indicates a significant improvement in the condition of the scar. That is, the scar deformity after the treatment became less different from the healthy surrounding tissues, both according to the doctor and according to the patients.

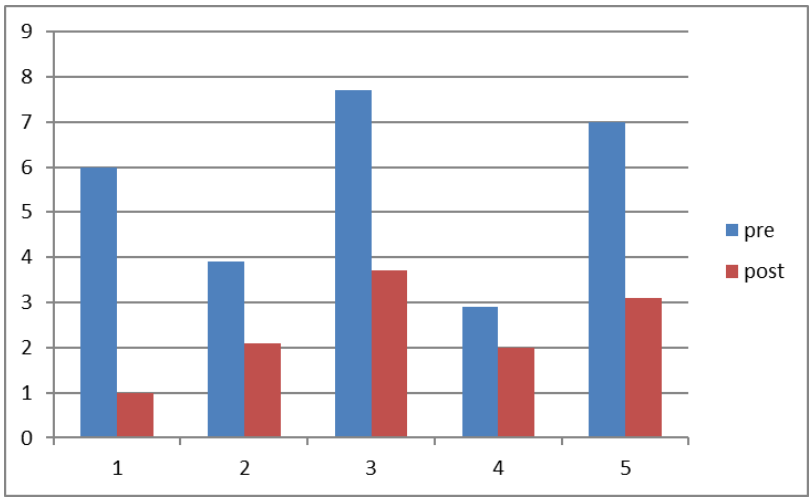


Fig.13 The results of a comprehensive assessment of the scar before and after treatment according to the doctor.

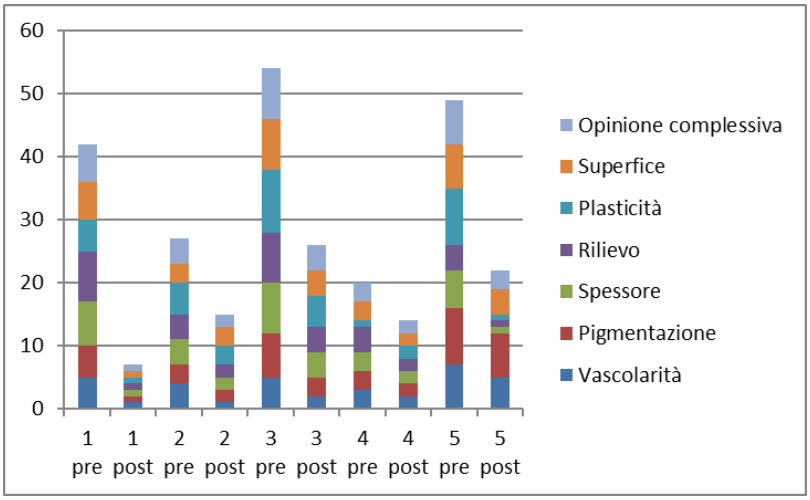


Fig.14 Evaluation by the doctor of the criteria for the condition of the scar before and after treatment for each patient.

Photographic demonstration of the results of treatment with hybrid cooperative complexes

1. The first patient

41-years-old female patient who complains about an hypotrophic scar on the skin in the medial anterior region of the knee on the right.The scar was a result of a liposuction procedure.

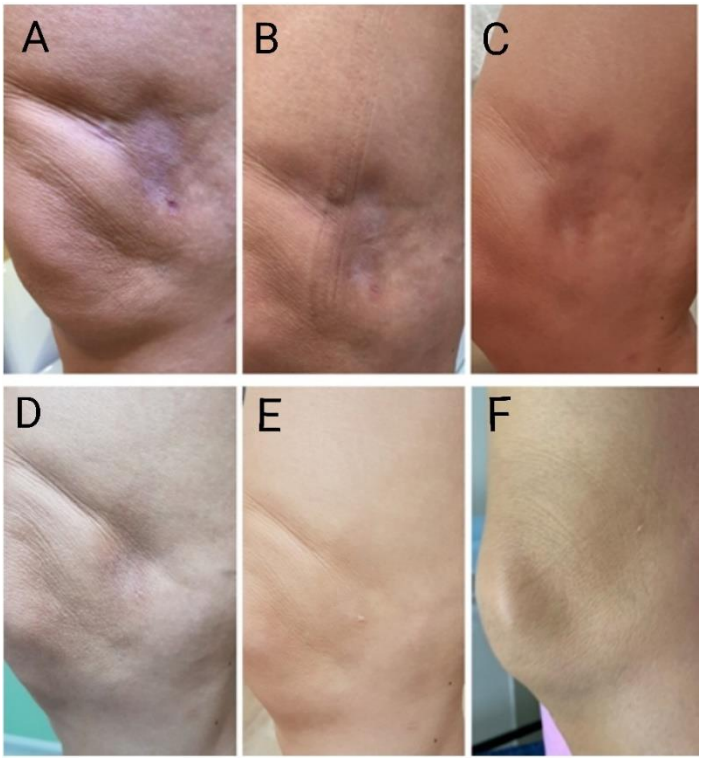


Fig. 15 Pre-treatment presentation of the atrophic scar V1 (A) and subsequent follow-up photographic documentation at V2 (B), V3 (C) and at follow-up visits: 3 months (D), 8 months (E) and 40 months (F) after the last treatment.

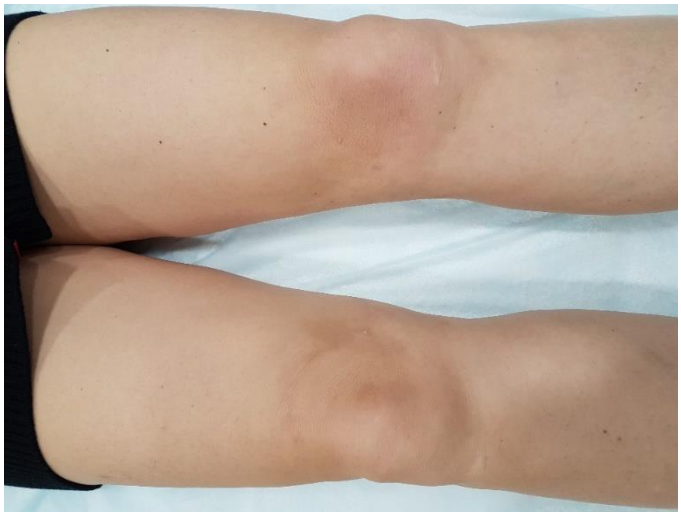


Fig.16 Post-treatment presentation of the atrophic scar 10 months after the treatment

Table 4. Hypotrophic scar on the skin in the medial anterior region of the knee

Date	Type of visit	Report of visual inspection
Jun 2017	1st treatment session (V1)	Form of scar is irregular, like a form of a star with one outstretched ray. Dimension: max in height – 60mm, width-wise – from 20mm till 85mm. Relief: congenerous, smooth, apparent depression of the abnormal focus with maximum recess in the center dimension: 50mm (height) x 40mm (width). Dotty region of deeper depression in the lower part. Color: paly cyanochroic with parts of hyperpigmentation peripherally. Peripheral outlines: uneven, obscure, ascending from depression till the level of normotermia, partly hyperpigmented. Displaceability: displaceable. Compliance: low elastic.

		Firmness: lowered. Skinfold thickness: lowered with the comparison of surrounding normal skin. Skin markings: normal anatomy, apart from dotty depression in the lower part of the scar. Painfulness: unpainful. Humidity: highly lowered (there was fine scaling of skin). Imagining of underlying anatomical organizations: while stretching subcutaneous veins can be seen well. (Fig.15A)
Jul 2017	2nd treatment session and follow-up (V2)	Significant decrease of scar's dimensions and intensity of skin depression; decrease of cyanochroic component; increase of strength, compliance and thickness of skin fold; stretching subcutaneous veins cannot be seen (Fig. 15B).
Aug 2017	3rd treatment session and follow-up (V3)	Significant decrease of scar's dimensions and intensity of skin depression, further decrease of cyanochroic component; hyperpigmentation on the whole area of the pre-existing depression; almost full smoothing of abnormal focus boarders; increase of strength, compliance and thickness of skin fold (Fig. 15C).
Sep 2017	Follow-up	Decrease of scar's dimensions with the preservation of a small depression area; decrease of hyperpigmentation intensity on the pre-existing scar and increase of hyperpigmentation around residual part of the depression; increase of strength, compliance and thickness of skin fold. A small

		hypertrophic scar appeared in the area of the previous initial defect.
Nov 2017	Follow-up	Almost full recovery of skin; presence of a small depression in the center of preexisting scar; significant decrease of hyperpigmentation area. A small hypertrophic scar in the area of the previous initial defect is still present (Figure 15D).
Apr 2018	Follow-up	Full recovery of skin in the area of pre-existing scar with low-intensity hyperpigmentation in the center. A small hypertrophic scar in the area of the previous initial defect is still present (Figure 15E).
Sep 2020	Follow-up	Normal skin (Figure 15F)

2. The second patient

50-years-old female patient who complains about a hypotrophic scar on the skin in the right cheek. The scar was a result of injury (firecracker explosion).

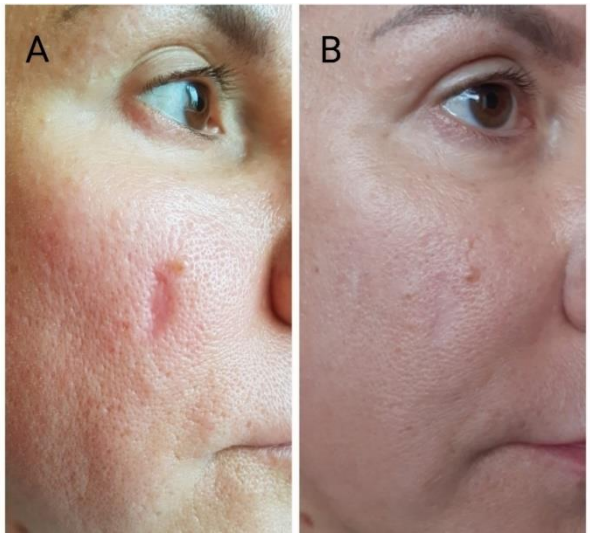


Fig. 17 Befor and after treatment

Table 5. Hypotrophic scar on the skin in the right cheek		
Date	**Type of visit**	**Report of visual inspection**
Apr 2018	1st treatment session	Htpotrophic scar 12 mm (Fig.17A)
May 2018	2nd treatment session and follow-up	Significant decrease in scar intensity of skin depression; an increase of strength, compliance and thickness of skin fold.
Jun 2018	3rd treatment session and follow-up	Significant decrease of scar's intensity of skin depression; almost full smoothing of abnormal focus borders; an increase of strength, compliance, and thickness of skin fold (Fig.17B).

3. The third patien

18-years-old male patient who complains about an hypertrophic scar on the skin of the abdomen. The scar was a result of burn from hot water 12 years ago.

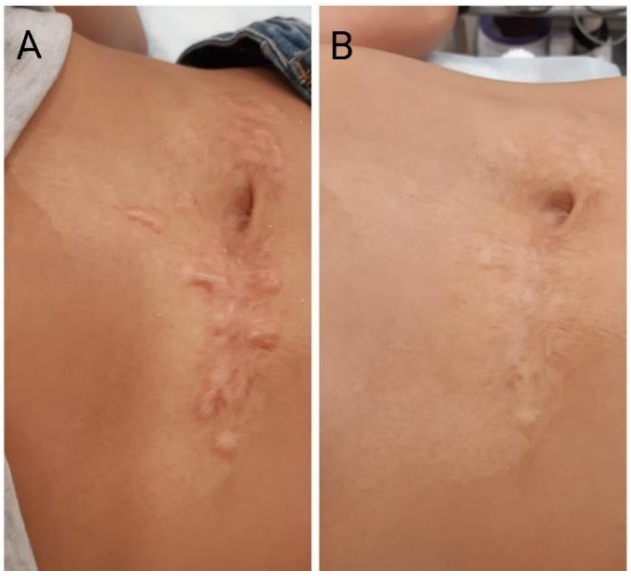


Fig. 18 Befor and after treatment

Table 6. Hypertrophic scar on the skin of the abdomen

Date	Type of visit	Report of visual inspection
Apr 2018	1st treatment session	Hypertrophic scar multiple (Fig.18A)
May 2018	2nd treatment session and follow-up	Significant decrease of scar's intensity of skin thickening; increase of elasticity, decrease in intensity of erythematous coloration
Jun 2018	3rd treatment session and follow-up	Moderate decrease of scar's intensity of skin thickening; moderate increase of elasticity, decrease in intensity of erythematous coloration; smoothing of abnormal focus boarders; decrease of skin fold (Fig.18B).

4. The fourth patient

32-years-old female patient who complaints about an retraction scar on the skin in the lateral surface of the thigh on the left and right and hypertrophic scar on the skin on the inner surface of the thigh. The scars were a result of a liposuction procedure.

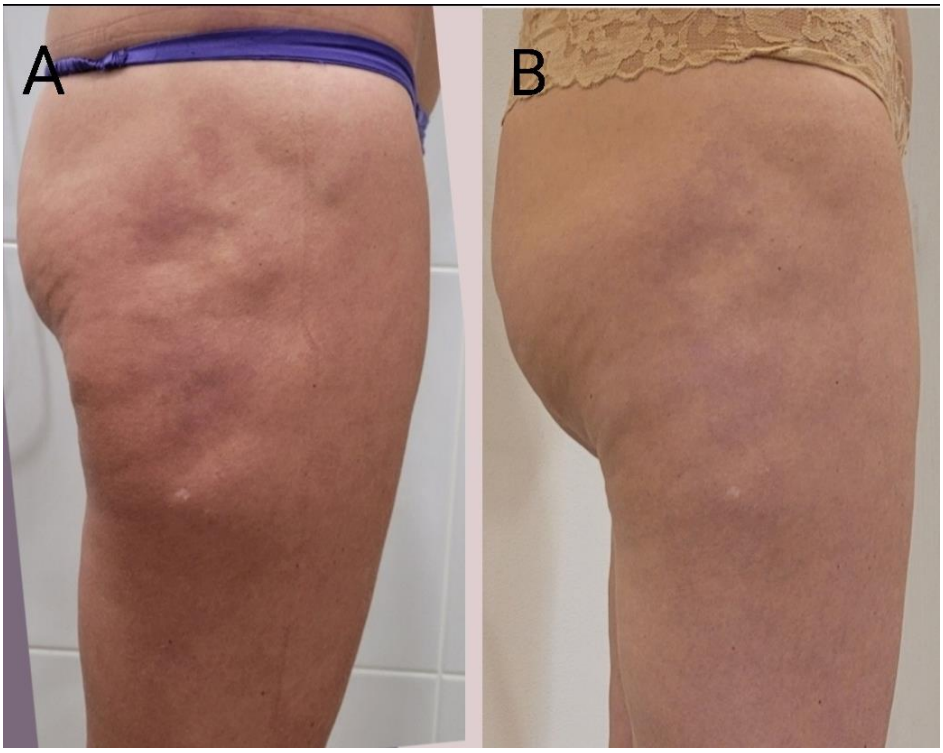


Fig. 19 Befor and after treatment retraction scar

Table 7. Retraction scar on the skin in the lateral surface of the thigh		
Date	**Type of visit**	**Report of visual inspection**
Mar 2022	1st treatment session	Uneven skin surface in the form of tuberosity with large areas of skin retraction; cyanotic skin coloration; pronounced decrease in elasticity in abnormal focus, reaching complete rigidity in some areas; the diameter of the retractions is from 2 to 7 cm (Fig.19A)
Apr 2022	2nd treatment session and follow-up	Significant decrease of intensity of skin depression; decrease of cyanochroic component; increase of compliance and elasticity of skin.
May 2022	3rd treatment session and follow-up	Significant decrease of intensity of skin depression, further decrease of cyanochroic component; smoothing of abnormal focus

		boarders; increase of compliance and elasticity of skin.
Jun 2022	4th treatment session and follow-up	Almost full recovery of skin; presence of a small depression in the center of pre-existing wide retraction; almost full smoothing of abnormal focus boarders; further increase of compliance and elasticity of skin; a small cyanochroic component is still present (Fig.19B).

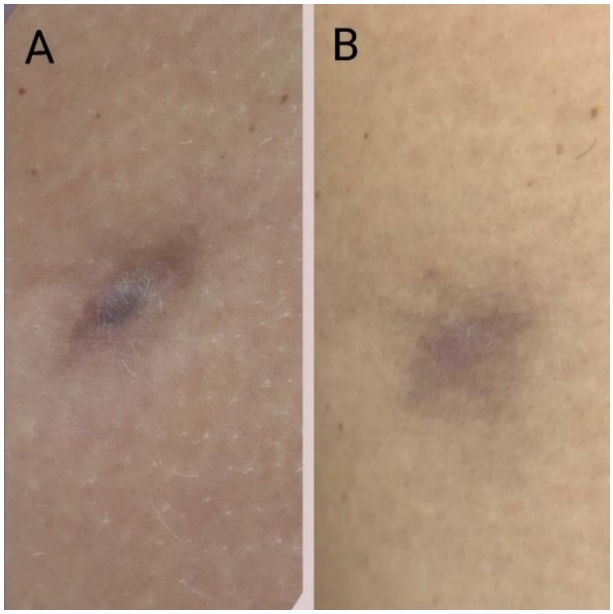


Fig. 20 Befor and after treatment hypertrophic scar

Table 8. Hypertrophic scar on the skin on the inner surface of the thigh

Date	Type of visit	Report of visual inspection
Mar 2022	1st treatment session	Significant skin thickening with cyanotic skin coloration; pronounced decrease in elasticity in

		abnormal focus, reaching complete rigidity ; the diameter of skin thickening is 7 mm (Fig. 20A)
Apr 2022	2nd treatment session and follow-up	Significant decrease of intensity of skin thickening; decrease of cyanochroic component; increase of compliance and elasticity of skin.
May 2022	3rd treatment session and follow-up	Significant decrease of intensity of skin thickening, smoothing of abnormal focus boarders; increase of compliance and elasticity of skin; full recovery of skin microrelief; full smoothing of abnormal focus boarders; a small cyanochroic component is still present (Fig.20B).

Visual assessment of scar based on photographs taken before and after treatment reveals the presence of changes in the relief, density, size, color of scar deformities in the case of each patient.

DISCUSSION

Scarring, regardless of etiology, is a challenging but treatable condition that may sensibly affect the life of patient. Countless therapeutic and surgical options are available for scar management with varying levels of evidence and success rates. The search for the ideal method of treating abnormal scars continues. Given the high biological significance of hyaluronic acid (HA), its ability to maintain a sufficient volume of tissues, accelerate the processes associated with tissue healing and regeneration, anti-inflammatory and bio-stimulating effect, it is advisable to consider this substance as a potentially effective means for correcting scar deformities of the skin and subcutes. Since 2006, HA injection have been the most accepted mini-invasive medical procedure to improve the skin. Since HA has a short half-life, cross-linking derivatives with more chemical and physical stability were developed. However, undesired side effects with characteristic of products with high cross-linking degree stimulated the scientific community to design novel HA-based formulations.

Italian pharmaceutical company IBSA Farmaceutici has created for the first time a product with **thermally stabilized natural hyaluronic acid in the form of hyaluronan hybrid cooperative complexes**, which in clinical studies has shown the ability to increase in the expression levels of type I and type III collagen in fibroblasts and type IV and VII collagen in keratinocytes, as well as increase in elastin expression. Another property of this medical device is the ability to recruit and differentiate stem cells in adipocytes, considerably improving fat tissue renewal. Taking into account the above-described properties of the hyaluronan hybrid cooperative complexes, their use for the treatment of abnormal scars is justified.

CONCLUSIONS

The experience of using injectable medical device PROFHILO, which is the product with thermally stabilized natural hyaluronic acid in the form of hyaluronan hybrid cooperative complexes, with concentrations of HA 64mg /2ml has proven to be effective in the bio-remodelling process of patological scars of skin and subcutis. Therefore its use results interesting in the clinical practice of many specialists from different areas (such as general surgery, plastic surgery, orthopedics, dermatology) and in all cases when it is necessary to create conditions that optimize the outcome of the healing process of wounds or bio-remodeling of scars.

In addition to the effectiveness, the undeniable advantages of this method of scar treatment are atraumatic, ease of use, and the possibility of using it as monotherapy. Currently, there is only a commercial use of this product for the treatment. The possibility of using the product with thermally stabilized natural hyaluronic acid in the form of hyaluronan hybrid cooperative complexes at the hospital level could significantly increase the availability of this type of correction of scar deformities and thereby make a positive contribution to solving the problem of prevention and correction of scars.

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