

Histological changes of collagen types after different modalities of dermal remodeling treatment: a literature review

Alterações histológicas dos tipos de colágeno após diferentes modalidades de tratamento para remodelamento dérmico: uma revisão bibliográfica

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ABSTRACT

A range of treatment options is available to restore and increase dermal collagen. The purpose of the present review, based on articles selected on PubMed database, is to study the histological effect of four methods for skin rejuvenation: Intense Pulsed Light, non-ablative fractional laser, ablative fractional laser and percutaneous collagen induction. The therapeutic implications of each type of treatment will depend on the induced collagen type and its ability to elicit a regenerative healing response versus a fibrotic scarring response. Intense Pulsed Light and the percutaneous collagen induction produced regenerative healing response with increased collagen type I.

Palavras-chave: intense pulsed light; laser therapy; collagen; rejuvenation; skin aging

RESUMO

Uma série de alternativas de tratamento está disponível para restaurar e aumentar o colágeno dérmico. O objetivo desta revisão, apoiada em artigos selecionados na base de dados PubMed, é estudar o efeito histológico de quatro modalidades para o rejuvenescimento da pele: luz intensa pulsada, laser fracionado não ablativo, laser fracionado ablativo e indução percutânea de colágeno. As implicações terapêuticas de cada tipo de tratamento dependerão do tipo de colágeno induzido e de sua capacidade para provocar uma resposta de cura versus uma resposta regenerativa de cicatriz fibrótica. A luz intensa pulsada e a indução percutânea de colágeno produziram resposta de cura regenerativa com aumento de colágeno tipo I.

Palavras-chave: luz intensa pulsada; terapia a laser; colágeno; rejuvenescimento; envelhecimento da pele

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INTRODUCTION

Due to the rapid increase in the life expectancy of the world population, skin aging has become a field of scientific importance in recent decades, with the emergence of multiple treatment modalities.

Both intrinsic alterations (secondary to the loss of cell regeneration capacity, resulting from chronological action) and extrinsic alterations (mainly caused by the exposure to ultraviolet radiation) have influence in the skin aging process.

There are several treatment options aimed at attempting to restore and increase dermal collagen,^{1,2} though their specific histological responses are not clear and there is lack of understanding regarding the specific effects that each of them has on dermal collagen.

Characteristics of normal and pathological skin

The maintenance of the skin's tissular architecture and physiological properties are attributed to the connective tissue's extracellular matrix, which comprises a large number of components including collagen and elastic fibers, proteoglycans and glycosaminoglycans macromolecules, and several non-collagen glycoproteins. The abilities of resident cells – such as fibroblasts – to synthesize and organize the extracellular matrix are critical for the morphogenesis, angiogenesis and skin healing processes. Collagen is the main responsible for the skin's strength, elasticity and the dermal volume, corresponding to about 80% of its dry weight.

Dermal collagen synthesized by fibroblasts in normal skin contains type I collagen (80% – 85%) and type III collagen (10% – 15%). The anchoring fibrils are composed mainly of type VII collagen and contribute to the dermal-epidermal junction's stability. A reduction in the amount of non-fibrillar collagen (type I and III) seen in the chronologically aged skin and can be aggravated by photoaging.³

In chronological aging, a decrease in the dermal thickness occurs due to biochemical and structural changes in collagen and elastic fibers, and in the ground substance. There is a reduction of collagen synthesis and an increase in its degradation due to increased levels of collagenase. The total collagen amount is reduced by 1% per year throughout adult life, and the remaining collagen fibers become disorganized, more compact and grainy, with a greater number of cross-links. Elastic fibers decrease in number and diameter. The ground substance's amount of the mucopolysaccharides decreases, in special that of the hyaluronic acid (HA). These changes negatively influence the skin's turgor and also have an impact on the deposition, orientation and size of collagen fibers.^{4,5}

In photoaging – a term that refers to skin changes associated with chronic exposure to ultraviolet light – the epidermal and dermal changes affect cellular components and the extracellular matrix with the accumulation of disorganized elastic fibers, loss of collagen fibers, and a reduced proportion of type I : type III collagen^{6,7} (Figure 1). Clinically, it manifests as roughness, loss of elasticity, appearance of fine wrinkles, dyschromias and melanoses. Varani et al.⁶ showed that the reduction of collagen in the photodamaged skin is caused by both the increased collagen degra-

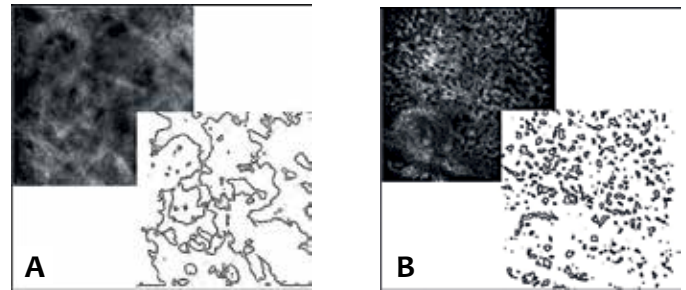


Figure 1: Confocal microscopy shows increased collagen fragmentation with advancing age. A. Image of a 25-year old person. B. Image of a 68-year old person. (<http://www.orion-concept.com>)

ation by the action of metalloproteinases (especially collagenase) and the decrease in the production of collagen by fibroblasts. This interruption in the synthesis of new collagen is caused by the interaction with an altered extracellular matrix, which exerts an inhibitory mechanism on fibroblasts. When isolated, fibroblasts regain their ability to grow and produce collagen.

Facial wrinkles have histologic differences in the photodamaged skin. A recent study compared the static wrinkles of the forehead with the adjacent skin and noticed that the wrinkles show significant reduction of type VII collagen, elastin and tropoelastin. The levels of collagen type I and III are similar to that of the adjacent photoaged skin.⁷ The reduction in type VII collagen in the wrinkles' bottom seems to contribute to the appearance of a thin, flattened dermal-epidermal junction, weakening the connection between the epidermis and dermis, leading to their proliferation.

There are currently many procedures designed to stimulate neocollagenesis in the treatment of the aging skin, aiming at remodeling the dermis and consequently improving the sagging skin and wrinkles. The main issues regarding the collagenesis are linked to its control and stimulus, especially in rejuvenation treatments. Controlling the formation of collagen is critical to maintaining a proper dermal structure and the lack of control contributes for the formation of hypertrophic scars and keloids, for instance.

In the wound repairing process, healing results in a fibrous inflammation with predominance of type III collagen, which is stronger and more resistant, and scars are classified into three types: normotrophic, hypertrophic and keloid. Verhaegen et al.⁸ found differences in the collagen's morphology among the scar types and the normal skin. When compared to the normal skin, hypertrophic scars and keloids have collagen fibers arranged in more parallel pattern. In keloid scars, the collagen bundles are significantly thicker and the distance between them is increased.

In hypertrophic scars and keloids, fibroblasts produce collagen excessively as compared to normal skin fibroblasts. Oliveira et al.⁹ found that this increase is caused by an increase

in type III collagen. They compared hypertrophic with normotrophic scars and showed that hypertrophic scars have greater amounts of type III collagen accumulated in the deep dermis, and that both scars have equal amount of collagen type I. These findings are consistent with those of Syed et al.¹⁰ who have ratified the fact that the ratio collagen type I : collagen type III is altered in keloids and showed that fibroblasts of the growing perilesional skin have a greater collagen production than that of other regions in the same keloid. Moreover, differences are observed in the production of collagen in different locations of the same keloid, with a decrease in the ratio collagen type I : collagen type III. The perilesional region has an increase of collagen type III and a slight decrease in collagen type I as compared to the intralesional area.

OBJECTIVES

The objectives of the present study are to evaluate the evidence published in the literature about different types of collagen in four skin rejuvenation methods (Intense Pulsed Light – IPL), Non-Ablative Fractional Laser – NAFL), Ablative Fractional Laser – AFL) and Collagen Induction Therapy – CIT); and evaluate the therapeutic implications of each procedure and infer which of them can most closely achieve outcomes with characteristics of normal skin.

The hypothesis of the present study is that IPL and ICT, acting through a process of regeneration rather than healing, are the procedures that can most closely achieve outcomes with characteristics similar to those of healthy skin.

METHODOLOGY

The methodology consists of reviewing the scientific literature, based on selected articles on PubMed database, using the keywords *intense pulsed light, ablative fractionated laser, nonablative laser, percutaneous collagen induction, rejuvenation, dermal collagen, scar, fibrosis, photoaging* and *complications*. The English, Spanish and Portuguese languages, as well as publication dates between 1990 and 2015, related to dermatology, were the parameters used in the search filter.

RESULTS

One of the most important modulators of the connective tissue's gene expression is the transforming growth factor - type (TGF-) belonging in the family of the growth factors released by macrophages that stimulates the expression of various extracellular matrix genes, including those encoding collagen I, III, IV and V, apparently by the transformation of TGF- into connective tissue growth factor (CTGF) in the fibroblasts. With the aging process, these factors have their levels reduced.⁸ This would be the proposed mechanism for stimulating collagenesis during the healing process and after treatments that act through the induction of an inflammatory response.^{5,11,12}

Histological effects of IPL

IPL devices emit non-coherent and not collimated diffuse light – or polychromatic light, whose characteristics are

different from those of the laser light. The latter has collimated and coherent rays, and always with a single wavelength.³ In this manner, due to the fact that IPL has various wavelengths (ranging from 500nm to 1,200nm) is capable of treating melanocytic and vascular lesions,¹³ as well as stimulating neocollagenesis.¹⁴

The effectiveness of IPL in the remodeling of the extracellular matrix of skin aging has been proven in several clinical studies.¹⁵ The stimulation of fibroblasts, resulting in neocollagenesis, in addition to the dermal remodeling and decreased in elastosis can be seen histologically for up to six months after the treatment.¹⁵⁻¹⁷ However, its precise mechanism of action in photorejuvenation is not fully elucidated.

Research studies have shown that irradiation with IPL has a stimulating effect on cutaneous fibroblasts *in vitro*, promotes cell viability and increases the expression of collagen types I and III.¹⁸⁻²⁰

Feng et al.²¹ studied the effect of IPL on 58 patients. Cut-off filters of 560nm, 590nm and 640nm, with fluences from 14 to 22 J/cm² and pulse durations of 2 to 4 ms were employed. After 3 sessions, 62% of patients had improvement in their wrinkles and skin texture, 85% had reduction in pigmentary lesions, and 81% had a decrease in telangiectasia. Histological results of four patients showed an increase in collagen fibers types I and III.

Another study performed in six female patients showed increased collagen after six treatment sessions with IPL. The increase in type I collagen was higher than that of type III. These results, however, were not statistically significant.²²

A histological analysis in 14 patients with poikiloderma of Civatte (PC) evidenced an increased number of fibroblasts associated with increased compression, thickness and density of collagen. Three IPL sessions were carried out at monthly intervals with 570 nm and 540 nm cut filters, fluence of 18 J/cm², and pulse duration of 15 ms. In 86% of cases there was more homogeneous redistribution of the melanin pigment in the basal layer of the epidermis, consistent with the improvement in the PC's pigmentary component.²³

Regarding the IPL's beneficial effect on hypertrophic scars and keloids in a series of 109 patients, Erol et al.²⁴ reported an improvement of over 75% in the pigmentation and a 50% reduction in size and thickness of hypertrophic scars. The parameters used were energy at 30 to 40 J/cm², cut filters of 550 nm to 590 nm and pulse duration of 2.1 ms to 10ms. The number of sessions ranged from 1 to 24, depending on the severity of the scar.

More recently, Hultman et al.²⁵ described the efficacy of IPL in the treatment of dyschromias in burn scars in 20 patients using cut filters of 560 nm to 650 nm, and fluence of 10 J/cm² to 22 J/cm². Sarkar et al.²⁶ reported decreased vascularity, flattening and prevention of hypertrophy in recent scars after burns. Four sessions were performed with 590 nm cut filter and fluence at 25 J/cm².

According to the authors,²³⁻²⁵ the beneficial effect of IPL on hypertrophic scars could be explained both by the inhibitory action of IPL on the blood vessels and the inhibition of type III collagen synthesis. So far there is still absence of studies on the

histological effect of IPL in scars, since those found in the literature describe only clinical parameters.

Histological effects of non-ablative laser

Non-ablative lasers (NAL) have arisen in order to enhance the undesirable effects of ablative lasers. Non-ablative resurfacing, performed with 1,064 nm Nd:YAG for instance, has a deeper penetration in the dermis that does not cause dermal ablation.²

In 1997, studies by Golberg²⁷ proved the positive effects with few adverse effects using Q-switched Nd:YAG laser in skin rejuvenation, observing that only 4 of the 6 patients showed a slight increase of collagen in the papillary dermis and concluded that the clinical effects did not correlate to histological effects.²⁸ Studies in animals²⁹⁻³¹ showed increased collagen type I and type III associated with a decrease of metalloproteinases after irradiation with QS Nd:YAG. In the three studies,³⁰⁻³² the expression of type III procollagen protein was greater than that of type I procollagen. Moreover, Nd:YAG leads to increased expression of types I and III procollagen when compared to IPL.³²

The concept of fractional photothermolysis introduced by Manstein et al.³³ was first used for non-ablative fractional lasers (NAFL). It consists of thermal damage inflicted in the shape of dermoepidermal coagulation columns, without ablation of the epidermis, leaving areas of untreated skin between them. Tw2

Orringer, et al. 34 studied molecular mechanisms of the treatment with 1,550 nm Erbium NAFL in 20 patients with cutaneous photoaging. Biopsies were performed once a week, for one month. It was possible to observe early inflammatory response with a significant increase in pro-inflammatory cytokines (interleukin-1 β and tumor necrosis factor α) followed by increasing metalloproteinase. After 24 hours, there was a decrease in the expression of types I and III collagens, which was soon reversed to progressively increase during the course of two weeks. Increased levels of collagen types I and III were proportional to the energy used.

In order to evaluate the result of NAFL on burn scars in their chronic phase, Taurdorf et al.³⁵ performed a randomized controlled study with 20 patients. The treatment was carried out with 1,540 nm Erbium:Glass laser, firstly using a deep, and then a superficial tip. Clinical and histological analyzes were performed at one, three and six months after the treatment. The 15 patients who completed the study showed overall improvement in the appearance of the scars, nonetheless 11 patients had one or more prolonged adverse effects, such as erythema, hyperchromia and hypochromia. It was possible to histologically observe an improvement of the flatness of the dermal-epidermal junction and a reorganization of elastic fibers and collagen.³⁶ Both studies conclude that the flat and atrophic scars respond better to treatment with NAFL; however hypertrophic scars have limited clinical response.^{35,36}

When compared to AFL, NAFL led to similar clinical response in the treatment of post-surgical scars, with a lower rate of adverse effects.³⁷

Histological effects of AFL

Carbon oxide 10,600nm and 2,940nm Erbium:YAG ablative lasers were first used for skin rejuvenation. Results were encouraging, however, due to the fact that they cause complete ablation of the epidermis, both showed all possible complications stemming from the total exposure of the dermis in the postoperative period.

Given that the Erbium:YAG laser emits a wavelength that approximates that of the water absorption's peak (3,000nm), almost all energy is absorbed in the epidermis and the papillary dermis, producing a more superficial ablation with a lower underlying thermal damage than that of the CO₂ laser.³⁸

Ablative fractional laser (AFL) employs an innovative technology that combines the principles of classic ablative techniques with fractional photothermolysis. The beam applied on a fraction of the skin surface produces a microscopic area of treated skin, comprising a central ablative focus encircled by thin necrotic area, which in turn is surrounded by a coagulation band. The areas of untouched skin among the treated areas enable faster tissue proliferation aimed at repairing the damaged zones.³⁹

In the literature, there is a discussion on whether the lifting type tension effect of AFL is caused by the ablation of the abnormal tissue – resulting in the regeneration and contraction of the collagen – or by the stretching resulting from dermal heating.⁴⁰

In order to determine whether there is a difference between the cutaneous stretching caused by collagen contraction mediated by heat, and that occurring secondarily to a regenerative process, Fitzpatrick et al. 41 treated the upper eyelids of 9 patients with two types of AFL: CO₂ and Erbium:YAG. Ultrasonographic measurements and skin biopsies were performed monthly for 6 months. The authors demonstrated that the Erbium:YAG laser acts through a purely ablative mechanism. The stretching effect starts to be noticed after 1 week after the damage has been inflicted and being a result of the transformation of fibroblasts into myofibroblasts. This mechanism of wound contraction leaves a microcicatrical aspect. With the CO₂ laser, the ablative effect is smaller and there is also immediate stretching of the skin through dermal heating. This reaction is the result of a dissociation of intermolecular peptide bonds of the collagen's triple helix, leading to a longer lasting tension effect. The authors concluded that the tissular stretching and thickening produced by the ablative effect results from the stretching of the collagen, which is caused by the wound contraction mechanism, leading in turn to a healing response. The more intense is the laser ablation, the greater the risk of scarring. In this study, 41.43% of patients showed permanent scars, which were treated with blepharoplasty.

In order to objectively evaluate the histological and immunohistochemical effects of the Erbium:YAG laser, El-Do-myati et al. 42 carried out a comparative study of 12 patients treated with the ablative and the fractional modalities of Erbium:YAG laser. With serial biopsies, they demonstrated a quantitative increase of collagens type I, III and VII in both treatment

modalities after 4 sessions. This increase in collagen was maintained for up to 6 months after the last treatment. The same authors described similar findings in 10 patients after 5 sessions with Erbium:YAG AFL in the skin rejuvenating treatment of the upper third of the face.⁴³

Although CO₂ AFL has proven effective in the treatment of atrophic acne scars,⁴⁴ a recent randomized controlled study could not confirm its clinical effectiveness in different types of scars.⁴⁵

Ozog et al.⁴⁶ evidenced a significant increase of collagen type III along with a significant decrease of collagen type I in scars of burns after treatment with CO₂ AFL. Ten patients were included in the study, with 8 dropping out after the first session of treatment due to adverse effects such as pain, infection and ulceration. Two patients reported improvement in thickness and pigmentation of the scar. This finding coincides with what Laubach et al.⁴⁷ described: that ALF produces epidermal microinjuries and damage to the dermal collagen, which, through a regeneration process, is replaced by collagen type III.

Histologic effects of collagen induction therapy

In 1995, Orentreich and Orentreich⁴⁸ described the term “subcision” as a means to stimulate the connective tissue located beneath scars and retracted wrinkles. Based on these ideas, the collagen induction therapy (CIT) was developed. This is a method that uses a device with a variable number of microneedles of different lengths, which cause microtraumas to the skin and formation of microchannels by performing multiple punctures in the skin. These microchannels function as conduits for active principles, facilitating the absorption of substances, and promoting collagen induction.

The CIT was proven effective and promising for the safe treatment of scars and other dermatologic conditions, safely and without the risk of hypopigmentation.⁴⁹ It was also demonstrated to be safe and effective in the treatment of periorbital wrinkles.⁵⁰

The repair process produced by CIT comprises 3 phases.⁵¹ In the first phase (the injury phase), there is release of platelets and neutrophils responsible for the release of growth factors acting on keratinocytes and fibroblasts. In the second phase (the healing phase), there are angiogenesis, epithelialization and fibroblast proliferation, followed by the production of type III collagen, elastin, glycosaminoglycans and proteoglycans.

Concomitantly, fibroblast growth factors (TGF- α and TGF- β) are secreted by monocytes. The TGF plays a crucial role in the formation of fibrotic scars. Research on the TGF molecules' family found that TGF- β 3 induces regenerative response without producing scars, while TGF- β 1 and TGF- β 2 induce fibrotic scarring.⁵² A repair with the presence of scars is histologically characterized by abnormal dermal organization composed of small parallel bands of type III collagen and fibronectin. A regenerative response without scarring has characteristics similar to those of the normal skin.

Studies in animals show that the CIT induces the expression of TGF- β 3, which remains for 2 weeks after the procedure.⁵³ Finally, in the third stage (the maturation phase), type III colla-

gen is replaced by collagen type I.⁵⁴

Aust et al.⁵⁵ demonstrated a significant improvement in wrinkles, flaccidity and appearance of scars in 480 patients. The same authors showed that the device CIT 3mm (Environ[®] Medical Roll-CITTM) leads to an increase of collagen type I and that the joint application of retinol and vitamin C maximizes these results. Biopsies taken after 6 months and 1 year showed that the histological changes, such as thickening of the stratum spinosum, normalization of the dermal epidermal junction, and increase in collagen have remained during that period.⁵⁵

More recently, Zeitter et al.⁵⁶ demonstrated that the same effects can be obtained using 1mm long microneedles. The study was carried out in rats and found increased epidermal thickness and expression of collagen type I, and decreased expression of collagen type III. These findings were more evident in the group that underwent 4 treatment sessions, and even better in the group that underwent 4 CIT sessions with the topical application of 1% retinol and 10% vitamin C.

DISCUSSION

The healing and regeneration processes take place in different ways. While the regeneration process culminates in the production of collagen type I (stronger and more resistant), the healing process results in a fibrous inflammation with predominance of type III collagen.^{6, 44, 57}

Hypertrophic scars and keloids have increased production and amount of type III collagen.¹³⁻¹⁴ In cutaneous photoaging, there is increased collagen type III along with a reduction in collagen type I.⁷

All methods studied in this paper lead to an improvement in the texture of the skin, wrinkles and surface irregularities through collagenesis; however, the type of collagen produced can be different.

Intense pulsed light leads to increased expression of collagen type I and reduced expression of collagen type III.^{24, 34, 36} It also proved effective in the treatment of various types of scars,²⁷⁻³⁰ however the resulting histologic effect has not yet been sufficiently investigated.

ICT also generates increased expression of collagen type I and a decrease in collagen type III.^{53, 57-59}

Delayed healing and fibrosis are more frequent with the AFL and NAFL.⁵⁸ This can be explained by the type of collagen whose production is triggered by these lasers. Several articles show that NAFL produces a greater increase in type III collagen than in type I collagen.³³⁻³⁶ Regarding the genesis of collagen triggered by different types of lasers, the one that leads to the greatest increase collagen type III is the QS Nd:YAG NAFL.⁵⁹

The treatment of hypertrophic scars with AFL leads to increased collagen type III and decreased collagen type I.⁵⁰⁻⁵¹

CONCLUSION

It can be concluded that regeneration is an integral part of the clinical improvement observed in the IPL and ICT, which is associated, at the histological level, to an increase of type I collagen, which is stronger and more durable. The clinical improvement

observed with AFL and NAFL depends on the triggered healing process and fibrosis, which produces more collagen type III.

Notwithstanding, it is necessary to expand the knowl-

edge with further studies in order to arrive at credible and reliable conclusions. Many of the studies presented in the present paper have a small number of cases, and the methodologies used were limited. ●

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