Biostimulators and their mechanisms of action

Bioestimuladores e seus mecanismos de ação

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ABSTRACT

In the skin aging process, both intrinsic alterations, secondary to cell regeneration capacity loss resulting from chronological action, and extrinsic alterations, caused by to ultraviolet radiation exposure, can be observed. Treatments that restore collagen production and stimulate fibroblasts to synthesize and organize extracellular matrix are critical for morphogenesis, angiogenesis, and skin healing. Potential uses of products that stimulate collagen production, a component that plays a fundamental role in the extracellular matrix, represents a promising perspective for improving skin quality and its mechanical properties by introducing a new concept of therapeutic approach when treating changes caused by skin aging.

Keywords: Collagen; Hydroxyapatite; Skin Aging

RESUMO

No envelhecimento da pele, as alterações intrínsecas, secundárias à perda da regeneração celular, e extrínsecas, causadas pela exposição à radiação ultravioleta, podem ser observadas e alteram a arquitetura tecidual e as propriedades fisiológicas da pele. Tratamentos que restauram a produção de colágeno e estimulam os fibroblastos a sintetizar e organizar a matriz extracelular são críticos para a morfogênese, angiogênese e cicatrização. Potencial utilização de produtos que estimulam a produção de colágeno, que desempenha papel fundamental na matriz extracelular, representa perspectiva promissora para a melhoria da qualidade da pele e das propriedades mecânicas, introduzindo um novo conceito de abordagem terapêutica no tratamento de alterações causadas pelo envelhecimento da pele.

Palavras-chave: *Colágeno; Hidroxiapatita; Rejuvenescimento*

Review

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INTRODUCTION

The maintenance of the tissue architecture and skin physiological properties is attributed to the extracellular matrix of connective tissue, which comprises a large number of components including collagen and elastic fibers, proteoglycans and glycosaminoglycans macromolecules, as well as several non-collagen glycoproteins.[1]

In the skin aging process, both intrinsic and extrinsic changes occur. The intrinsic changes are secondary to cell regeneration capacity loss due to chronological action, with the dermis becoming relatively more acellular and avascular in senescence. Moreover, the extrinsic changes are caused mainly by chronic exposure to ultraviolet radiation.[1,2]

In chronological aging, there is a thinning of the dermal thickness, which occurs due to biochemical and structural changes in collagen, elastic fibers, and the ground substance.[3,4] The collagen synthesis decreases and its degradation increases due to higher levels of collagenase. The collagen content reduces throughout adulthood, and the remaining fibers appear disorganized, more compact, and granular, with a higher number of crosslinks. The rate of collagen types also changes, with a predominance of type I collagen in young individuals and type III collagen in the elderly. The elastic fibers decrease in number and diameter. The number of mucopolysaccharides in the ground substance decreases, especially that of hyaluronic acid. These changes negatively influence the skin's turgor and also impact on deposition, orientation, and size of collagen fibers.[4,5]

In extrinsic aging, the alterations caused mainly by solar radiation affect the dermal cellular components and the extracellular matrix, with the accumulation of disorganized elastic fibers, fragmentation of collagen fibers, and reduction in the proportion between type I and type III collagens,[6,7] which occur both by the direct action of radiation on collagen fibers and by the increase in metalloproteinases (mainly collagenase). The altered interaction of the fibroblast with the extracellular matrix also causes an interruption in the synthesis of new collagen, exerting an inhibitory mechanism on collagenesis.[6,7]

The ability of resident cells, such as fibroblasts, to synthesize and organize the extracellular matrix is critical for morphogenesis, angiogenesis, and skin healing. One of the most important modulators of connective tissue gene expression is the transforming growth factor type β (TGF-β), a member of the family of growth factors released by macrophages. It stimulates the expression of several genes in the extracellular matrix, including those encoding collagens I, III, IV, and V, apparently transforming TGF-β into connective tissue growth factor (CTGF) in the fibroblast. These growth factors have their levels reduced in the aging process.[8] The release of these factors by macrophages would be the proposed mechanism for stimulating collagen production, both in the healing process and after treatments with the biostimulators application, which act by inducing a tissue inflammatory response.[5,9,10]

Biostimulation is the polymer's ability to generate cellular benefit or tissue response in a particular clinical application through a desired controlled inflammatory response, leading to slow degradation of the material. It culminates in collagen deposition in the tissue, conditioned by biomaterial properties and by the technique that injected the polymer into the tissue.[11] The materials used as biostimulators will have different biocompatibility according to various physicochemical factors such as their chemical composition, particle size, physical shape, contact angles, structure, surface tension, and surface charges. For example, particles with pores or an uneven surface are potentially more reactive and can initiate an inflammatory response, while the smooth ones are encapsulated by fibrous tissue and induce the foreign body response regulated by the Protease Activated Receptor 2 (PAR 2), a protein involved in cell proliferation and regulation of the acute inflammatory response.[12] Microspheres with diameters between 0.5 μm and 20 μm are phagocytized by a variety of cells, resulting in a cascade of cytokines characterized by the production of tumor necrosis factor α (TNF-α) and interleukins IL-1 and IL, while the particles with larger diameters are not phagocytized and do not induce TNF-α production.[13,14]

The process of polymer degradation that constitutes the implant must also be considered, as it varies with its molar mass, composition, thermal story, crys-

talline structure, and amount of polymer applied. The corresponding monomers or the products generated from them, in an aqueous environment, also undergo metabolic action in living organisms and can generate a biological response.[12-15] The degradation of the biomaterial should result in non-reactive molecules, as their degradation products cannot cause stimulation of inflammatory cells, especially macrophages and giant cells, or interfere with their biocompatibility.[12] The search for substances for soft tissue fillers that do not evoke an important inflammatory response has led to the use of a variety of biomaterials.

The formation of a capsule and inflammatory cell infiltration are characteristic of the foreign body reaction to the biomaterial and, depending on their surface properties, distinct extracellular proteins can be attached.[16,17] The combination of these proteins and their concentrations determines cell behavior.[18] Host proteins that are absorbed by the biomaterial surface include albumin, complement fragments, fibrinogen, fibronectin, immunoglobulin G, and vitronectin.[19,20] Fibrinogen, complement, and vitronectin are recognized by macrophage and neutrophil receptors.[21] To stimulate inflammatory cell migration, mast cells release histamine.[20,22] Monocytes and Th2 helper cells infiltrate the tissue. Monocytes turn into macrophages releasing chemoattractants that attract more macrophages around the biomaterial. Platelets and macrophages produce platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β), which promote the fibroblasts migration.[22] TGF–β seems to be the mediator for collagen synthesis, as well as for the differentiation of fibroblasts from myofibroblasts, the alpha-smooth muscle actin (aSMA)-rich, its contractile form. PDGF promotes myofibroblast proliferation.[23] Macrophages fuse under the influence of IL-4 and IL-13 to form foreign body giant cells, in case the material cannot be phagocyted. In an alternative condition, macrophages produce pro-fibrotic factors, such as TGF-β1 and PDGF, which stimulate fibroblasts to produce collagen, leading to the formation of a capsule that surrounds the material.[23,24]

There is initially the deposition of collagen type III fibers around the biostimulator microspheres, with a fibroblastic tissue response and type I collagen deposition in the periphery. Over the months, there is a remodeling process of type III collagen, resulting in the predominance of type I collagen in the newly formed tissue.[25,26] The maturation phase begins with the collagen crosslinking, which will cause its contraction and adjustment of the network, with the return of the tensile force to the tissue.[26]

Cell fusion and the formation of giant cells is an adaptation to the difficulties in eliminating the foreign body. In the expected and physiological foreign body reaction, the host, with activation of circulating monocytes, recognizes the biomaterial. Once activated, they adhere firmly to the substrate, releasing proteins that initiate specific recognition at cell surface receptors, determining an expected inflammatory response. However, some factors can modify this physiological response, attracting Langerhans cells and lymphocytes, causing a pathological foreign body reaction: chemical composition; particle size and volume; implant morphology (irregularly shaped particles activate more prostaglandins E2 and tumor necrosis factor); surface area; electrical load, and implantation site, including the individual response of the host.[16]

The potential use of products that stimulate the collagen production, a fundamental component for the properties of the extracellular matrix, currently represents a vital treatment perspective for improving the skin quality and its mechanical properties, opening a new concept for the therapeutic approach to changes caused by skin aging. Among biomaterials, poly-L-lactic acid and hydroxyapatite stand out due to their biocompatibility and bioreabsorption characteristics. They also have the most studied and well-known mechanisms of action and are, therefore, the most widely used products.

For implants, in general, the characteristics of the host also contribute to the variable responses in the interaction between biomaterial and organism response,[13] which will determine the amount of collagen, variable according to age, sex, general health, concomitant diseases, lifestyle, and pharmacological status of the patient.

OBJECTIVE

Review the articles on poly-L-lactic acid (PLLA) and calcium hydroxyapatite (CaHA),

highlighting their different mechanisms of action and their therapeutic indications.

MATERIAL AND METHODS

We searched for articles published in English on the PUBMED, with the keywords: poly-l-lactic acid, calcium hydroxyapatite, biostimulator, neocollagenesis, and collagen.

RESULTS

Twenty-nine articles were selected, specifically on biostimulators. Of these, ten were related to the clinical use of poly-L-lactic acid and nine to the clinical use of calcium hydroxyapatite. Only one article cited the clinical indications of the two products together, but not in a comparative way. Regarding the mechanisms of action, three articles on PLLA and five articles on CaHA were published. In the introduction to this article, we discussed the biological response to biostimulators, in a review of 10 articles relevant to the topic.

DISCUSSION

Poly-L-lactic acid

Injectable PLLA has been applied as a cosmetic filler since 1999 to correct facial and cutaneous volume losses caused by aging in a gradual, progressive, and prolonged manner, promoting natural and harmonious results, with low risks of adverse events.[15,27]

It is a high molecular weight organic polymer (140 kD), of the family of α-hydroxide acids, derived from lactic acid. It presents self-organization property and formation of colloidal micelles in aqueous solution, in the form of spherical particles with a smooth surface, dispersed as lyophilized powder in sterile flask, added to 4.45% of carmellose sodium and 2.67% of non-pyrogenic mannitol. It must be diluted in 8 ml of distilled water for 24 to 72 hours before implantation. The aqueous vehicle will be absorbed in 24 to 48 hours.[23,26]

PLLA microspheres have more uniform sizes, between 40 μm to 63 μm in diameter. They act as a substrate that promotes appropriate cell activity, inducing or facilitating molecular and mechanical signaling to optimize tissue regeneration without causing any local or systemic harmful response to the host.

PLLA is considered to have superior biocompatibility. Although tissue enzymes and other chemical species, such as superoxides and free radicals, can affect it, its degradation pathway occurs through non-enzymatic hydrolysis. They initially form water-soluble monomers and dimers, which are phagocytized by macrophages, metabolized in CO2 (eliminated by the respiratory route), H2O, or incorporated into glucose. Its estimated half-life is 31 days and is eliminated from the body after 18 months.[15,28] It is considered a bioresorbable material, as its degradation occurs by decreasing the size of the molecule, and its metabolites are absorbed in vivo and completely eliminated by metabolic routes.

After PLLA implantation in the deep reticular dermis or superficial hypodermis, the normal reaction begins with the injection wound, although minimal. The release of platelets in the extracellular matrix releases homeostatic and chemotactic factors that attract fibroblasts, in addition to neutrophils and monocytes from the circulation. Two hours after the injection, the inflammatory phase begins. Activated neutrophils begin to phagocytize the foreign body and secrete cytokines and proteolytic enzymes. Edema appears to facilitate cell migration. Monocytes are transformed into macrophages to eliminate apoptotic neutrophils and particles too large to be phagocyted. Between seven and ten days after the implant introduction, the level of macrophage fusion increases with the associated reduction in the number of apoptotic cells. There is a slight initial inflammatory response with foreign body reaction, in which the macrophages fuse into giant cells to try to phagocytize the particles. Macrophages also secrete growth factors to initiate the proliferative phase of reconstruction.[23,28]

Fibroblasts secrete components of the extracellular matrix, initially type I collagen, the most abundant structural protein in the dermal extracellular matrix, which plays a significant role in skin tension and resilience, accompanied by a smaller production of type III collagen. This neocollagenesis is followed by marked fibroblast activity and proliferation, with gradual deposition of more collagen fibers and the formation of mature vascularized fibrous tissue, accompanied by PLLA degradation, with no indication of acute inflammatory response.[28]

Thus, fibroblasts isolate the implant with a fibrous collagen capsule, which will gradually be replaced by fibrocytes, and each foreign particle will finally be encapsulated independently of the others. As the PLLA is degraded, the connective tissue's response around the implant results in a gradual filling with new collagen fibers at the site that it formerly occupied. This fibroplasia produces the desired cosmetic result, with increased dermis thickness.[23,28]

The new collagen begins to form after one month and continues to increase for nine months to a year. PLLA-induced tissue augmentation was based on capsule formation, orchestrating macrophages, myoblasts and fibroblasts, and substantial deposition of type III collagen close to particles and type I collagen in the periphery of the encapsulated PLLA. There is an expression of genes related to collagen metabolism, with the presence of CD68(+) macrophages next to PLLA particles, as well as CD 90(+) and α-SMA-positive fibroblasts, indicating the presence of myofibroblasts and neovascularization. MRNA expression for types I and III collagen transcription and growth factors TGF-β1 and TIMP1 are significantly elevated.[23]

In the sixth month, many particles become porous, due to enzymatic degradation, and surrounded by macrophages. At the end of this period, due to the remodeling process, there is a predominance of type I collagen, and α-SMA-positive fibroblasts, as well as PLLA particles, disappears.[15,23] Quantitatively, there is a statistically significant increase in type I collagen, without a significant increase in type III collagen after treatment. The inflammatory response after treatment is absent or with low intensity after three and six months and absent at 12 months.[20] The effect of neocollagenesis continues many months after the injection of the product.[18] The maturation phase begins with the collagen crosslinking, which will cause its contraction and adjustment of the network, with the return of the tensile force to the tissue.[28]

Calcium hydroxyapatite

The use of calcium hydroxyapatite (CaHA) as a biostimulator was approved by the US Food and Drug Administration (FDA) in 2006 to correct facial wrinkles and folds and to replace volume in patients with facial lipodystrophy associated with the HIV.[29] In 2009, the FDA approved a protocol that included lidocaine to the compound with CaHa for better comfort during application. Since 2016, the CaHA implant already added to lidocaine has become a formulation available for use in Europe.[30]

CaHA is a synthetic substance composed of calcium and phosphate ions, biodegradable, biocompatible, non-mutagenic, with no evidence of local and systemic toxicity. Its chemical composition is similar to that of inorganic constituents of bones and teeth. It decomposes in the same way as bone debris after fractures, which guarantees its biocompatibility and safety.[30,31]

CaHA corresponds to a group of compounds with the chemical formula Ca10(PO4)6(OH)2, which vary significantly in their three-dimensional structure and their biological behavior in tissues. Biologically active CaHA particles are generally subdivided into macroporous and microporous. The synthetic macroporous CaHA molecules have a highly organized structure with pore sizes ranging between 10 μm and 500 μm. Larger pores can be osteoconductive and allow fibrovascular growth within the particles. Microporous CaHA particles, on the other hand, have smaller pores that vary between 2 μm and 5 μm, which do not allow this fibrovascular growth.[25]

The CaHA particles with micropores, in the compound used commercially as a biostimulator, have diameters between 25 μm and 45 μm and correspond to 30% of the formulation. They are suspended in a sterile, non-pyrogenic carrier gel composed of highly purified water, glycerin, and sodium carboxymethyl cellulose, equivalent to 70% of the final volume.[26,31]

The carrier gel is cohesive and has high viscosity and elasticity, properties that allow a high integration into the tissues and guarantee easy manipulation. The final product made up of the gel and the particles of CaHA has demonstrated efficacy, safety and good tolerability.[25,26]

After implanting the product, its immediate action is to produce a filling effect for soft tissue volumizing, with a defect correction rate of 1:1, which avoids overcorrections. Over a few months after application (about two to four months), the carboxymethyl cellulose particles gradually collapse until phagocytosis promotes their complete resorption.[26,30,31] The im-

mediate volumizing effect is not necessary to induce neocollagenesis.

The newly formed collagen will gradually replace the initial volume of the gel. [31-32] The small deposited CaHA microspheres act as a foundation, which activates the fibroblasts by stretching and supporting the new tissue in with subsequent new collagen formation.[26,30,31] This process starts in up to four weeks and lasts for about 12 months. However, the clinical effects of CaHA can last from one to three years. [29,30]

The mechanism of stimulating the initial macrophage activity, associated with the sodium carboxymethyl cellulose gel, determines the formation of the fibrous capsule around the individual microspheres and appears to be of minimal intensity, with no significant inflammatory response.[34] In addition to this mechanism, others are described in response to the implantation of CaHA microspheres, such as fibroblast stretching, local tissue destruction, and increased cytokine production, such as TGF-β.[29] The microspheres would stabilize the extracellular matrix's three-dimensional structure, facilitating the adhesion of fibroblasts to dermal fibers, making it similar to that of young skin. Thus, the original collagen architecture and layout would be restored, which supports the growth of fibroblasts and the formation of new collagen without calcifications, physiologically inducing neocollagenesis by a process in which type I would gradually replace the type III collagen.[31]

Elastin deposition was also demonstrated four and nine months after implantation. There was a significant and progressive increase in Ki-67 (a marker of cell proliferation of collagen-producing cells with the consequent remodeling of the extracellular matrix).[26,28,29]

There was an increased CD34 density (a marker of angiogenesis), which suggests that increased blood flow and better delivery of nutrients to the skin accompany the formation of new tissue - which are vital for the dermis supply in repairing and remodeling without accentuating an inflammatory response.[28,30]

We gradually visualize a more uniform dermal structure, with a more dense and linear arrangement of fibers in the superficial and deep layers, which produces an improvement in the skin quality, which becomes more elastic and firm, in addition to the increase in the skin dermis thickness. As a result, we have greater effectiveness in the treatment of folds and wrinkles with more extended durability of the aesthetic clinical effects.[26,29,30]

At this stage, there is a small amount of type III collagen and a predominance of type I collagen due to tissue remodeling, which, associated with the increase in elastic fibers, results in higher tissue tensile strength and greater elasticity.[26,34]

Also, during natural skin aging, collagen fibers become irregular and disorganized. The accumulated collagen fragments combined with the lack of three-dimensional structure of these fibers interfere negatively in their adherence, affecting the function of the fibroblast.31 Clinically, this can be seen by the accentuation of facial folds and skin atrophy.[29] After the application of CaHA, the microspheres stabilize the adhesion of the fibroblast, making it similar to that of young skin. Thus, the original collagen architecture and layout is restored.

Regarding CaHA application plan, n comparative histological studies conducted on animals and analyzing intradermal and subdermal injection as to the resulting collagen production, it was found that intradermal applications produce a greater amount of collagen, it was found that intradermal applications produce a more significant amount of collagen. However, there is also a higher nodulation index than in the subdermal plane.[31] Nevertheless, there is still no evidence that this leads to better clinical efficacy.

In a study conducted to assess the quantitative production of collagen on weeks 4, 16, 32, 52, and 78 after application of CaHA, an immediate increase was observed at week four, higher than at week 16, explained by scar formation initial or tissue edema. Then there is a progressive increase until week 78.[31]

Immunohistochemical and histomorphological analyzes of skin biopsies treated with CaHA with two applications (at baseline and at four months) demonstrated a significant increase in the collagen type I expression in the analysis of four and seven months after the first application compared to the baseline. As for type III collagen, an increase was observed in four months with a subsequent decrease in its concentration at seven months, but still above the baseline.[29]

These findings were associated with improved skin elasticity and flexibility measured through cuto-

metry, a technique that uses a non-invasive suction instrument that measures the vertical deformity of the skin surface and quantifies its extensibility, delayed distention, deformity, and final retraction.[29]

Ultrasound images showed a statistically relevant increase in the dermis thickness, from 1462.3 mm at the baseline to 1642.8 mm after four months ($p<0.01$), with progressive growth after the second treatment, reaching values of 1865.9 mm at seven months.[29]

About six months after biomaterial injection, next to the deposition of the new collagen around and eventually inside the microspheres, the surface of the particles becomes slightly uneven. Over time, after the carrier gel is fully metabolized, the microspheres become particulate and distributed in the intra and extracellular space. CaHA is metabolized through a standard homeostatic mechanism that naturally occurs in the organism via macrophage phagocytosis, similar to the breakdown of small bone fragments. This results in calcium and phosphate ions, which are eliminated by regular metabolic routes, leading to the total disappearance of the particles after about 18 months.

CONCLUSION

Clinical implications of the mechanism of action of biostimulators

The mechanism of action of biostimulators has important practical implications, including the application form, the results optimization, and the adverse events minimization.[35] Its application on the skin allows the correction of sagging skin and wrinkles by the gradual increase of tissue volume.[36,37] Each treatment will lead to collagen formation, and the magnitude will depend on the concentration and volume used, which must be individualized. Subsequent injections promote continuous stimulation of tissue response, with deposition of more extracellular matrix and the resulting improvement in skin flaccidity and facial contour.

Unlike poly-L-lactic acid, CaHA, when applied, has immediate effects due to the carrier gel.[35] The glycerin present in the gel can cause a pronounced, but temporary, edema from 24 to 72 hours.[36] As the carrier gel presents high viscosity, density, and cohesiveness, it becomes an adequate product for tissue elevation and immediate improvement of the facial contour. It is also considered an ideal agent for supraperiosteal application, and can be used to restore volume in areas of bone resorption.[36,37]

As the biomaterial implantation results may not be evident for weeks, it is essential to wait for the biological response between applications to happen. The use of additional treatments should be conduct at intervals of at least four weeks so that there is no overcorrection.[35] Response time and degree of correction depend on each patient's characteristics, which vary according to age, sex, skin quality, phototype, and diet.

Regarding the application plan of both products, histological studies conducted in animals, comparing the resulting collagen production after intradermal and subdermal injections of the biostimulators, demonstrated that intradermal applications produce a more significant amount of collagen; 2 however, they also determine a higher rate of undulations and nodule formation due to product accumulation, generally palpable and not visible, which respond well to conservative treatment with digital massage or infiltration of saline or lidocaine.[37,38] When comparing the two products, PLLA must be hydrated hours in advance, while CaHA can be applied directly or with the addition of lidocaine at the time of use. CaHA has an immediate and sustained volumizing effect. However, it can present significant edema in the first 24 to 48 hours due to reaction to glycerin present in the carrier gel, while in PLLA, the effect presented immediately after application is due to the diluent volume and disappears with its absorption in 24 to 48 hours. Its effect is late and gradual, only reappearing when the dermal thickening resulting from neocollagenesis starts. Both products have good clinical results proven and maintained for long periods, with the formation of type I collagen and, in a smaller amount, type III collagen. ●

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