Yumed Text
OPEN ACCESS JOURNALS

Archives of Clinical & Experimental Dermatology

Review | Vol 4 Iss 1

Biostimulators and their Mechanisms of Action

Marisa Gonzaga da Cunha[1*], Ana Lúcia Gonzaga da Cunha[1], Marcela Engracia Garcia[1] and Maria Aparecida da Silva Pinhal[2]

[1]Discipline of Dermatology, Faculty of Medicine of ABC, Santo André, SP, Brazil

[2]Discipline of Biochemistry, Faculty of Medicine of ABC, Santo André, SP, Brazil

[*]**Corresponding author:** da Cunha MG, Discipline of Dermatology, Faculty of Medicine of ABC, Santo André, SP, Brazil, E-mail: dramarisagonzaga@yahoo.com.br

Received: March 16, 2022; **Accepted:** March 25, 2022; **Published:** March 31, 2022

Abstract

In the skin aging process, both intrinsic alterations, secondary to the loss of cell regeneration capacity resultant from chronological action and extrinsic alterations, mainly caused by the exposure to ultraviolet radiation, can be observed and change the tissue architecture and skin physiological properties. The treatments that restore the collagen production and stimulate fibroblasts to synthesize and organize the extracellular matrix is critical for morphogenesis, angiogenesis and skin healing.

Biostimulation is the ability of a polymer to provide cell benefits or tissue response through specific clinical applications. The potential use of products that stimulate the production of collagen, a component that plays a fundamental role in theproperties of the extracellular matrix, nowadays represents a promising perspective for the improvement of skin quality and its mechanical properties by introducing a new concept of therapeutic approach when treating changes caused by skin aging. Its clinical effects are due to the stimulation of a desired controlled inflammatory response, which leads to the slow degradation of the material and culminates with the deposition of collagen in the tissue.

Keywords: ***Biostimulation; Fibroblasts; Collagen; Poly-l-lactic acid; Calcium hydroxyapatite***

1. Introduction

The maintenance of tissue architecture and skin physiological properties are attributed to the extracellular matrix of the connective tissue, which includes a great number of components such as collagen and elastic fibers, proteoglycan-glycosaminoglycan macromolecules and many non-collagenous glycoproteins [1]. In the skin aging process, both intrinsic alterations, secondary to the loss of cell regeneration capacity resultant from chronological action (the dermis becomes relatively acellular and avascular), and extrinsic alterations, mainly caused by the exposure to ultraviolet radiation, can be observed [1,2].

During chronological aging, dermal thinning occurs due to biochemical and structural changes in collagen and elastic fibers and in the ground substance [3,4]. There is a reduction in collagen synthesis and an increase in its degradation because of the increase in collagenase levels. Collagen content decreases in adulthood, and the remaining fibers become more disorganized, compact and grainy, with a greater number of cross-links. The different collagen types show distinct rates, with the predominance of type I collagen in the young and type III collagen in the elderly. Elastic fibers decrease in number and diameter. The amount of mucopolysaccharides in the ground substance decreases, especially that of the hyaluronic acid (HA). These changes not only negatively influence the skin turgor, but also have an impact on the deposition, orientation and size of collagen fibers [4,5].

In extrinsic aging, the changes, especially caused by solar radiation, affect dermal cell components and the extracellular matrix with the accumulation of disorganized elastic fibers, the fragmentation of collagen fibers and the reduction in type I and type I collagen proportion [6,7]. Such alterations are generated by the direct radiation action on the collagen fibers and the increase in metaloproteinases (collagenase in particular). There is still an interruption in the synthesis of new collagen caused by the altered interaction of fibroblasts with the extracellular matrix, which exerts an inhibitory mechanism on collagenesis [6].

The ability of resident cells like 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-beta (TGF-β), a family member of growth factors released by macrophages that stimulates the expression of many genes of the extracellular matrix, including those that codify types I, III, IV and V collagen, apparently through the transformation of TGF-β into connective tissue growth factor (CTGF) in the fibroblasts. Levels of these growth factors decrease in the aging process [8]. The release of these factors by macrophages would be the proposed mechanism for collagen production stimulation in the skin healing process and after treatments with the application of biostimulators, which act through the induction of a tissue inflammatory response [5,9,10].

Biostimulation is the ability of a polymer to provide cell benefits or tissue response through specific clinical applications. Its clinical effects are due to the stimulation of a desired controlled inflammatory response, which leads to the slow degradation of the material and culminates with the deposition of collagen in the tissue. The process is conditioned by the biomaterial properties, the characteristics of the patient and the technique through which the polymer was injected in the tissue [11]. The materials used as biostimulators will have different biocompatibility properties according to a variety of physicochemical factors, like the chemical composition, the size of the particle, molecular conformation, contact angles, structure, surface tension and surface loads. For instance, particles with irregular pores or surface are potentially more reactive and can trigger an inflammatory response, whereas those with a smooth surface are encapsulated by fibrous tissue in the induction of foreign body response regulated by protease-activated receptor 2 (PAR 2), a protein involved in cell proliferation and in the regulation of acute inflammatory response [12]. Microspheres with diameters between 0.5 μm and 20 μm are phagocytosed by a variety of cells, which results in a cascade of cytokines characterized by the production of tumor necrosis factor-α (TNF-α) and interleukins IL-1 and IL. On the other hand, particles with greater diameters are not phagocytosed and do not induce the production of TNF-α [13,14]. The degradation process of the polymer that constitutes the implant must also be taken into account since molar mass, composition, thermal history, crystal structure and the applied amount vary from polymer to polymer. The corresponding monomers, or the products generated from them, also undergo metabolic action in living organisms in an

aqueous environment, thus inducing a biological response [12,15]. The biomaterial degradation should result in non-reactive molecules given the fact the degradation products cannot bring about the stimulation of inflammatory cells, especially macrophages and giant cells, or interfere with material biocompatibility [12]. Studies involving soft tissue filler substances that do not induce a relevant inflammatory response have revealed a great number of possibilities of biomaterials in different procedures.

Capsule formation and inflammatory cell infiltrate are characteristics of foreign body reaction to the biomaterial. Depending on its surface properties, distinct extracellular proteins can be attached [16,17], and the combination of these proteins and their concentrations determine cellular behavior [18]. Proteins from the host that are absorbed by the biomaterial surface include fibrinogen, complement fragments, vitronectin, fibronectin, immunoglobulin G and albumin [19,20]; the first three proteins are recognized by macrophage receptors and neutrophils [21]. In order to stimulate inflammatory cell migration, mastocytes release histamine [20,22]. Furthermore, monocytes and Th2-helper cells infiltrate the tissue. Monocytes mature to macrophages and release chemoattractants, guiding even more macrophages to the biomaterial. Platelets and subsequently macrophages produce platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), which promote the migration of fibroblasts [22]. TGF-β seems to act as a mediator not only for collagen synthesis but also for the differentiation of fibroblasts to α-smooth muscle actin-rich (α-SMA-rich) myofibroblasts. PGDF promotes the proliferation of myofibroblasts [23]. Macrophages fuse together under the influence of IL-4 and IL-13, forming giant cells of foreign body in case the material cannot be phagocytosed. In an alternative condition, the macrophages produce pro-fibrotic factors like TGF-β1 and PDGF, which stimulate fibroblasts to produce collagen, leading to the formation of a capsule that engulfs the material [23,24].

Initially, there is a deposition of type III collagen fibers around the microspheres of the biostimulator, with a subsequent fibroblastic tissue response and the deposition of type I collagen on the periphery. As months pass by, a remodeling process of type III collagen occurs, which results in the prevalence of type I collagen in the newly formed tissue [25,26]. Maturation phase begins with collagen reticulation, which brings about its contraction and network organization with a subsequent increase in tissue tension [26].

Cell fusion and the formation of giant cells are an adaptation to the difficulties found in the elimination of the foreign body. The biomaterial is recognized by the host in the physiological reaction of the foreign body, with the activation of the circulating monocytes. Once activated, they firmly adhere to the substrate, releasing proteins that initiate a specific recognition on cell-surface receptors, which determine an expected inflammatory response. However, some factors may modify this physiological response, attracting Langerhans cells and lymphocytes and triggering a pathological foreign body reaction: chemical composition; size and volume of the particles; morphology of the implant (irregular-shaped particles activate more prostaglandin E2 and tumor necrosis factor); superficial area; electric discharge and implantation site; and the host's individual response [16].

The potential use of products that stimulate the production of collagen, a component that plays a fundamental role in the properties of the extracellular matrix, nowadays represents a promising perspective for the improvement of skin quality and its mechanical properties by introducing a new concept of therapeutic approach when treating changes caused by skin aging.

Among the biomaterials used, poly-L-lactic acid and hydroxyapatite stand out due to their biocompatibility and bioreabsorption characteristics; besides, since their mechanisms of action are vastly known, they are products most often used in treatments.

Generally speaking, when it comes to implants, the characteristics of the host also contribute to variable responses regarding the interaction between the biomaterial and the organism response [13]. Such characteristics will determine the amount of collagen, which varies according to age, sex, general health condition, concomitant diseases, lifestyle and the pharmacological status of the patient.

2. Poly-L-Lactic Acid (PLLA)

Injectable PLLA has been administered as a cosmetic filler since 1999 for the correction of facial and skin volume loss caused by the gradual and prolonged aging process. Results are natural and harmonious, with low risks of adverse effects [15,27]. It is a synthetic polymer derived from the alpha-hydroxy-acid family of heavy molecular weight (140 kD) with the property of self-organization and the formation of colloidal micelles in aqueous environment. It is supplied in sterile vials as a lyophilized powder containing PLLA spherically-shaped, smooth-surfaced microparticles, 4.45% of sodium carmelose and 2.67% of non-pyrogenic mannitol. The vial content should be diluted in 8ml of distilled water 24 to 72 hours prior to implantation. The aqueous vehicle is absorbed within 24 to 48 hours [23,26]. PLLA microspheres have a more regular size, with diameters between 40 μm and 63 μm. They act as a substrate that will promote suitable cellular activity, inducing or favoring molecular and/or mechanical signaling so that tissue regeneration can be optimized without causing any local harmful or systemic response to the host.

PLLA is considered to be of superior biocompatibility. Despite the fact it can be affected by tissue enzymes and other chemical species, like superoxides and free radicals, its degradation route is through non-enzymatic hydrolysis. Initially, water-soluble monomers and dimers are formed and phagocytosed by macrophages, metabolized in CO_2 (eliminated through the respiratory system), water, or incorporated to glucose. A 31-day half-life is estimated, and it is totally cleared from the body after 18 months [15,28]. Besides, it is a bioabsorbable material given the fact its degradation occurs through the decrease in molecule size, resulting in the in vivo absorption of metabolites and their complete elimination through metabolic pathways.

After the implantation of PLLA in the deep reticular dermis or in the superficial hypodermis, the normal reaction starts with a minimal wound caused by the injection. The release of platelets in the extracellular matrix triggers homeostatic and chemotactic factors that attract fibroblasts, neutrophils and monocytes from circulation. Two hours after the application, the inflammatory phase begins: activated neutrophils start to phagocytose the foreign body and to secrete cytokines and proteolytic enzymes; edema shows up to make cell migration easier; monocytes turn into macrophages to eliminate apoptotic neutrophils and particles that are too big to be phagocytosed. Between 7 and 10 days after the implantation, there is an increase in the level of macrophage fusion, with the associated reduction in number of apoptotic cells as well as a slight initial inflammatory response as a foreign body reaction. Macrophages then fuse into giant cells in order to try to phagocytose the particles. Additionally, macrophages also secrete growth factors to initiate the proliferative phase of repair [23,28].

Fibroblasts produce components of the extracellular matrix, type I collagen at first, the major structural protein of dermal extracellular matrix that plays a paramount role in skin tension and resilience, followed by the production of lower levels of

type III collagen (FIG. 2). After this neocollagenesis, a marked fibroblastic activity and proliferation can be observed, with a gradual deposition of more collagen fibers and the formation of mature vascularized fibrous tissue followed by PLLA degradation without any indication of acute inflammatory response [28].

Therefore, fibroblasts isolate the implant in fibrous collagen capsules that will gradually be replaced by fibrocytes, and each foreign particle will finally be encapsulated independently from the others. As PLLA is degraded, the response of the connective tissue around the implant results in a gradual filling with the formation of new collagen fibers at the site where PLLA was originally implanted. This fibroplasia brings the desired cosmetic result with an increase in the dermis thickness [23,28]. The new collagen starts to form one month after the implantation, and this formation continues to increase over a period of nine months to one year. PLLA-induced augmentation is most likely based on capsule formation orchestrating macrophages, (myo) fibroblasts, and a substantial deposition of type III collagen close to the particles and type I collagen at the periphery of PLLA encapsulation. There is still the expression of genes related to collagen metabolism, with the presence of CD68(+) macrophages close to the PLLA particles, CD90(+) and α-SMA- positive fibroblasts, which indicate the presence of myofibroblasts and neovascularization. RNAm expressions for the transcription of types I and III collagen and growth factors TGF-β1 e TIMP1 are significantly high [28].

After six months, many particles become porous due to the enzymatic degradation, and they are surrounded by macrophages. At the end of this period, because of the remodeling process, there is a prevalence of type I collagen, and α-SMA-positive fibroblasts as well PLLA particles disappear [15,23]. Statistically speaking, there is a significant increase in type I collagen and a non-significant increase in type III collagen after treatment. The inflammatory response is absent or attenuated at month 3 and 6 and absent at month 12 (FIG. 3) [20]. The neocollagenesis effect lingers on for many months after the product is injected [18]. Maturation phase begins with collagen reticulation, which brings about its contraction and network organization with a subsequent increase in tissue tension [28].

Histological exam of a tissue sample removed six months after PLLA injection, stained with HE and analyzed using polarized light microscopy.

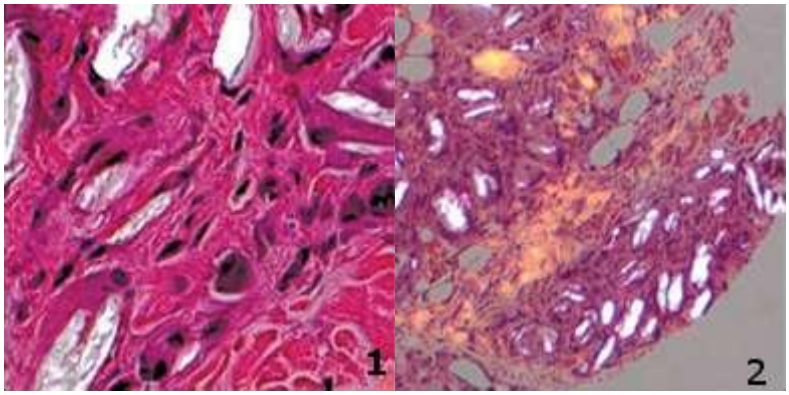

FIG. 1. **Orange-stained collagen fibers in the right lower corner (magnification of ×40).** FIG. 2. **PLLA crystals are shown in white; they can be seen surrounded by multinucleated cells (magnification of ×10) [28].**
(Courtesy of Goldberg D.et al from *Dermatol Surg* 2013)

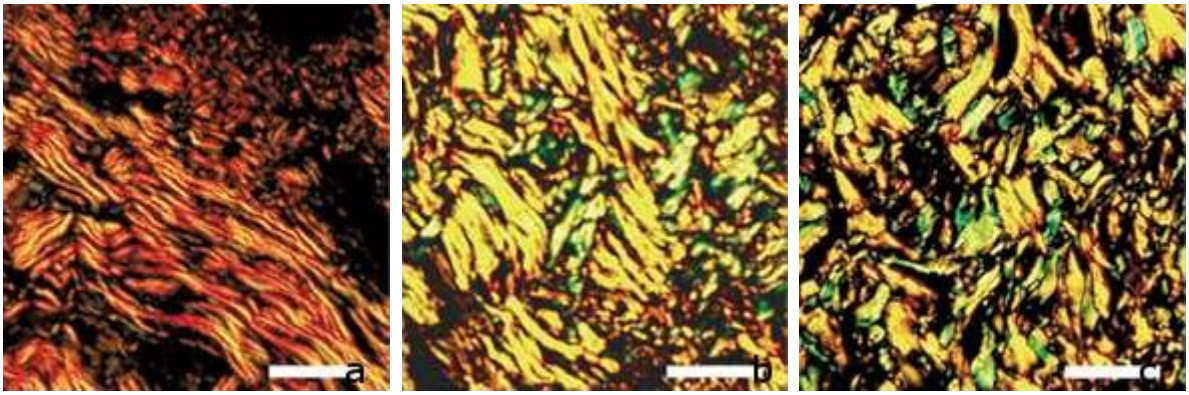

FIG. 3. **Skin samples of one of the patients stained with picro-sirius red. Analysis performed at baseline (a), three (b) and six months (c) after treatment with PLLA. The intensity corresponding to type I and type III collagen formations (larger yellow fibers and smaller green fibers respectively) is shown. Scale bar of 50 μm [28]. (Courtesy of Goldberg D. etal from *Dermatol Surg* 2013)**

3. Calcium Hydroxyapatite

In 2006, the US Food and Drug Administration (FDA) approved the use of calcium hydroxyapatite (CaHA) implants as a biostimulator for the treatment of wrinkles and facial furrows and for HIV lipodystrophy [29]. In 2009, the FDA approved the addition of lidocaine to CaHA for better therapy comfort. In Europe, this practice has been in effect since 2016. CaHA is a non-toxic synthetic biodegradable, biocompatible and non-mutagenic substance composed of calcium and phosphate ions. Its chemical composition is similar to that of inorganic constituents of bones and teeth, and it decomposes in the same way as bone debris after fractures, assuring its biocompatibility and safety [30,31].

CaHA corresponds to a group of compounds with chemical formula Ca10(PO4)6(OH)2. There is a significant variation in its tridimensional structure and its biological behavior in the tissue. Biologically active CaHA particles are usually subdivided into macroporous and microporous. The macroporous molecules of synthetic CaHA have an extremely organized structure, with pore sizes that vary in range from 10 μm to 500 μm. Bigger pores can be osteoconductive, and they allow for the fibrovascular growth within the particles. Microporous particles of CaHA, on the other hand, have smaller pores that range in size between 2 μm and 5 μm, which do not allow for fibrovascular growth [25]. The microporous particles of CaHA in the compound commercially used as a biostimulator have diameters between 25 μm and 45 μm and correspond to 30% of the formulation. They are suspended in a gel carrier, composed of highly purified water, glycerin and sodium carboxymethylcellulose, equivalent to 70% of the final volume [26,31]. The cohesive gel carrier is highly viscous and elastic, properties that allow for its high integration to tissues and make manipulation easier. The final product, composed of gel and CaHA particles, has demonstrated to be effective, safe and well tolerable [25,26].

After the product is implanted, its immediate action is to provide a filling effect for the volumization of soft parts with a defect correction rate of 1:1, preventing overcorrections. Around two to four months after the application, the carboxymethylcellulose particles gradually collapse until phagocytosis promotes their complete reabsorption [26,30,31]. The immediate volumizing

effect is not necessary to induce the neocollagenesis. The initial gel volume will gradually be replaced by the newly formed collagen since the small deposited CaHA microspheres act as a scaffold (FIG. 4 and 5) that supports the new forming tissue and activates fibroblasts with a subsequent collagen neoformation. This process is triggered in up to four weeks and lasts for about twelve months. However, the clinical effects of CaHA can last from one to three years [26,29-32].

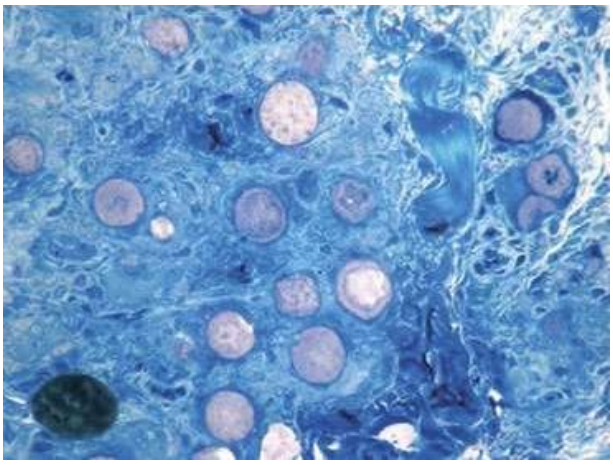

FIG. 4. **Optical microscopy showing microspheres forming the scaffold from where the formation of the new tissue occurs [32]. (From Marmur ES, Phelps R, Goldberg DJ; with permission)**

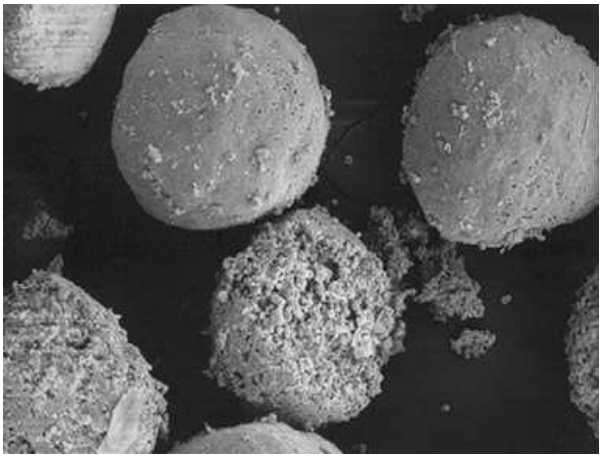

FIG. 5. **Electronic microscopy. CaHA thirty months after implantation the newly formed tissue [30]. (Courtesy of David J Goldberg, MD, JD, New York, NY).**

Besides the stimulus mechanism responsible for the initial macrophage activity (apparently of minimum intensity and associated to the carboxymethylcellulose gel to which is attributed the formation of the fibrous capsules around the individual microspheres) [34], additional mechanisms in response to the implantation of CaHA microspheres are described as follows: fibroblast stretching; local tissue destruction; and increase in the production of cytokines, such as TGF-β [29]. The microspheres would establish the tridimensional structure of the extracellular matrix, making the adherence of the fibroblasts to dermal fibers easier in such a way that the treated matrix will be similar to that of a young skin. Therefore, the architecture and original disposition of collagen, which support fibroblast growth and the formation of new non- calcified collagen, would be reestablished and would physiologically induce neocollagenesis through a process in which type I collagen would gradually replace type III collagen [31]. Elastin deposition is shown four and nine months after the application of the implant, and a

significant and progressive increase in Ki-67 (a cell proliferation marker of collagen- producing cells with a resultant extracellular matrix remodeling) can be observed [26,28,29].

An increase in the density of CD34 (an angiogenesis marker) can also be seen, suggesting that the formation of the new tissue is accompanied by an increase in blood flow and a better delivery of nutrients to the skin, two vital factors for dermal supply in repair and remodeling processes without accentuating an inflammatory response [28,30].

Gradually, a more uniform dermal structure can be visualized, with a more linear and dense arrangement of superficial and deep-layer fibers, leading not only to the improvement in the quality of the skin, firmer and more elastic, but also to an increase in dermal thickness. The result is a greater efficiency in the treatment of wrinkles and furrows with greater durability of the clinical aesthetic effects [26,29,30]. In this phase, there is a small quantity of type III collagen and the predominance of type I collagen due to tissue remodeling, which, along with the increase in elastic fibers, promotes greater tissue tension and elasticity [26,34].

Besides, during the natural skin aging process, collagen fibers become irregular and disorganized. Accumulated collagen fragments, combined with the lack of tridimensional structure of these fibers, negatively interfere with collagen adherence, thus affecting the fibroblastic function [31]. Clinically, it can be seen as the deepening of facial furrows and skin atrophy [29]. After the application of CaHA, the microspheres stabilize the fibroblast adherence, giving the skin a smoother appearance, similar to that of a young skin. As a result, collagen architecture and original disposition is reestablished.

Regarding the regimen of application of CaHA, comparative histological studies performed on animals, analyzing intradermal and subdermal injections and the resultant collagen production, reveal that intradermal applications lead to the production of a higher amount of collagen with higher rates of nodule formation when compared with the subdermal regimen. There is, however, no evidence that this brings about a better clinical efficacy. A study was carried out to evaluate the quantitative production of collagen at weeks 4, 16, 32, 52 and 78 after the application of CaHA. It was observed that there was an immediate increase at week 4, higher than at week 16, which was explained by the formation of the initial scar tissue or tissue edema. Subsequently, there was a progressive increase until week 78 (FIG. 6) [31].

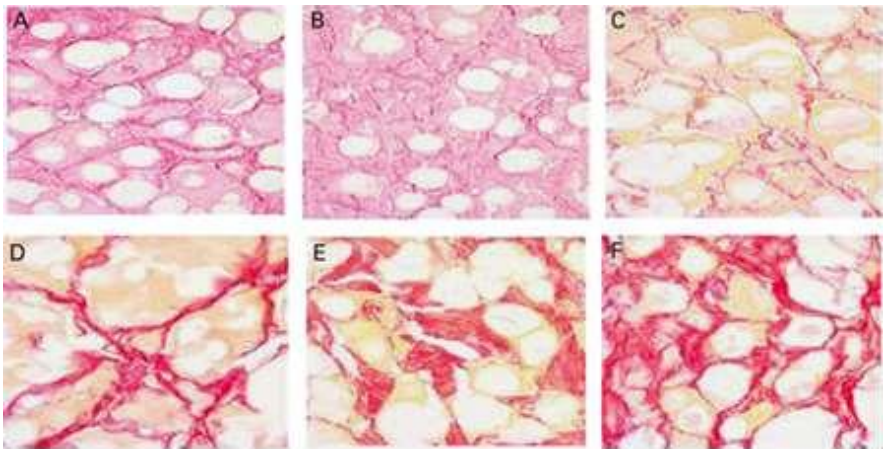

FIG. 6. **Histological findings of the increase in collagen density throughout weeks 4-78 after CaHA application. (A) 4 weeks; (B) 16 weeks; (C) 24 weeks; (D) 32 weeks; (E) 52 weeks; (F) 78 weeks [29].**

Immunohistochemical and histomorphological analyses of biopsy material from skins treated with two applications of CaHA (the first at baseline and the second at month 4) showed a significant increase in type I collagen expression at the 4-and-7-month evaluation after the first application when compared with the baseline. As to type III collagen, an increase in its concentration can be observed at the 4-month analysis, with a subsequent decrease at month 7, which, however, was still above the baseline level. These findings have been associated with the improvement in skin elasticity and flexibility measured by cutometry, a technique that uses a non-invasive suction device that measures the vertical skin surface deformity and quantifies its extensibility, delayed distension, deformity and final retraction. Ultrasound images show a statistically relevant increase in dermal thickness, from 1462.3 mm at baseline to 1642.8 mm at month 4 ($p<0.01$), progressively increasing after the second application, with values reaching up to 1865.9 mm at month 7 [29].

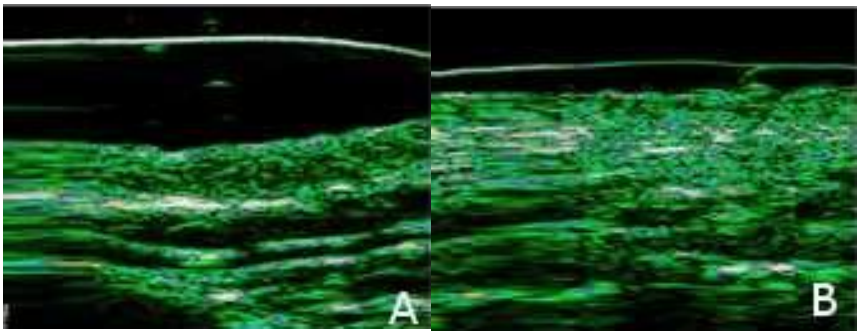

FIG. 7. **Ultrasound images of dermal thickness increase from baseline (A) to month 7 (B) after treatment with CaHA [29]. (Image courtesy of Yutskovskaya YA and Kogan EA)**

About six months after the application of the biomaterial, besides the new collagen deposition around and eventually in the microspheres, the surface of the particles becomes slightly irregular. As time passes by, after the gel carrier is totally metabolized, the microspheres become particulate, and they are distributed in both inter- and extracellular spaces. CaHA is metabolized through a normal homeostatic mechanism, which naturally occurs in the body via phagocytosis by macrophages. It is similar to the degradation process of small bone fragments, resulting in calcium and phosphate ions that are eliminated via normal metabolic pathways, which leads to the total elimination of the particles around 18 months later [29].

4. Clinical Implications of the Mechanism of Action of Biostimulators

The mechanism of action of biostimulators has important practical implications, including the way applications are performed, the optimization of results and the minimization of adverse effects of the product [35]. The application of biostimulators to the skin allows for the correction of sagging skin and wrinkles due to the gradual tissue volume augmentation [36,37]. Each treatment session will lead to the formation of collagen, and the magnitude will depend on the concentration and volume used, which should be individualized. Subsequent injections promote continuous stimulation to the tissue response, with the deposition of more extracellular matrix and a resultant improvement in skin sagging and facial contouring.

Differently from poly-L-Lactic, when CaHA is applied, the effects are immediate due to the gel carrier. The glycerin present in the gel may cause a pronounced but temporary edema that will last 24-72 hours [36]. As the gel carrier is highly viscous, dense and cohesive, it has become a suitable product for tissue elevation and immediate improvement in facial contouring. It

is also considered an ideal agent for supraperiosteal application, with the possibilityof being used in volume restoration is areas of bone reabsorption [36,37].

As the results from biomaterial implantations are not evident for weeks, the biological response is likely to happen between applications. The use of additional therapies should only be considered at intervals of at least 4 weeks so that overcorrection does not occur. The response time and correction degree depend primarily on each patient'scharacteristics, varying according to age, sex, skin quality, phototype and eating habits.

As to the regimen of application of both products, animal histological studies comparing the resultant collagen production after intradermal and subdermal injections of biostimulators indicate that the former produces a higher amount of collagen [2]. Despite the mentioned benefit, a higher rate of rippling and nodules of product accumulation, usually palpable but non-visible, can be observed. Nonetheless, these adverse effects respond well to conservative treatment, with digital massage or the infiltration of saline or lidocaine [37,38-43].

5. Conclusion

When both products are compared, PLLA must be hydrated hours before its use, whereas CaHA can be applied directly, or with the addition of lidocaine. CaHA provides an immediate volumizing and sustained effect; however, it may cause important edema in the first 24-48 hours due to a reaction to the glycerin present in the carrier. On the other hand, the effect presented right after the application of PLLA is owing to the volume of the diluent, which disappears with its absorption in 24-48 hours. Its final effect is gradual and delayed, which can only be noted as dermal thickening occurs as a result of neocollagenesis. Both products provide proven good clinical results, which are maintained over a long period of time, with the formation of type I collagen, and, in smaller quantities, of type III collagen. In conclusion, the choice of the ideal product depends on the personal experience of the applicator and the needs of each patient.

REFERENCES

1. L Baumann. Skin ageing and its treatment. J Pathol. 2007; 211(2):241-51.
2. Fenske NA, Lober CW. Structural and functional changes of normal aging skin. J Am Acad Dermatol. 1986;15(4, pt 1):571-85.
3. Varani J, Dame MK, Rittie L, et al. Decreased collagen production in chronologically aged skin. Roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol. 2006;168(6):1861-8.
4. El-Domyati M, Attia S, Saleh F, et al. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. Exp Dermatol. 2002;11(5):398-405.
5. Quan T, Shao Y, He T, et al. Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. J Invest Dermatol. 2010;130(2):415-24.
6. Varani J, Spearman D, Perone P, et al. Inhibition of type I pro collagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am J Pathol. 2001;158(3):931-42.
7. El-Domyati M, Medhat W, Abdel-Wahab HM, et al. Forehead wrinkles: a histological and immunohistochemical evaluation. J Cosmet Dermatol. 2014;13(3):188-94.

8. Verhaegen PD, van Zuijlen PP, Pennings NM, et al. Differences in collagen architecture between keloid, hypertrophic scar, normotrophic scar, and normal skin: An objective histopathological analysis. Wound Repair Regen. 2009;17(5):649-56.
9. Fitzgerald R, Vleggaar D. Facial volume restoration of the aging face with poly-l-lactic acid. Dermatol Ther. 2011;24(1):2-27.
10. Schierle CF, Casas LA. Non-surgical rejuvenation of the aging face with injectable poly-l-latic acid for restoration of soft tissue volume. Aes Surg J. 2011;31(1):95-109.
11. Griffith LG. Polymeric biomaterials. Acta Materialia. 2000;48(1):263-77
12. Morhenn VB, Lemperle G, Gallo RL. Phagocytosis of different particulate dermal filler substances by human macrophages and skin cells. Dermatol Surg.2002;28(6):484-90.
13. Nicolau PJ. Long-Lasting and Permanent Fillers: Biomaterial Influence over Host Tissue Response. Plast Reconstr Surg. 2007;119(7):2271-86.
14. Motta AC, Duek EAR. Síntese, Caracterização e Degradação "in vitro" do Poli (L-acido lactico). Polímeros: Ciênciae Tecnologia. 2006;16(1):26-32.
15. Machado Filho CM. et al. Acido PoliLLactico: um agente bioestimulador. Surg Cosmet Dermatol. 2013;5(4):345-50.
16. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol. 2008;20(2):86-100.
17. Junge K. et al. Mesh biocompatibility: effects os cellular inflammation and tissue remodelling. Langenbecks Arch Surg. 2012;397(2):255-70.
18. Wilson CJ. Et al. Mediation of biomaterials- cell interactions by adsorbed proteins: a review. Tissue Eng. 2005;11(1-2):1-10.
19. Jenney CR, Anderson JM. Adsorbed serum proteins responsible for surface dependent human macrophage behavior. J Biomed Mater Res. 2000;49(4):435-47.
20. Tang L, Jennings TA, Eaton JW. Mast cells mediate acute inflammatory responses to implanted biomaterials. Proc Natl Acad Sci USA. 1998;95(15):8841-6.
21. Berton G, Lowell CA. Integrin signalling in neutrophils and macrophages. Cell Signal.1999;11(9):621-35.
22. Zdolsek J, Eaton JW, Tang L. Histamine release and fibrinogen adsorption mediate acute inflammatory responses to biomaterial implants in humans. J Transl Med. 2007;5:31.
23. Stein P, Vitavska O, Kind P, et al. The biological basis for poly-L-lactic acid-induced augmentation. J Dermatol Sci. 2015;78(1):26-33.
24. Williams DF. On the mechanisms of biocompatibility. Biomaterials. 2008;29(20):2941-53.
25. Berlin A, Hussain M, Goldberg DJ. Calcium hydroxylapatite filler for facial rejuvenation: a histologic and immunohistochemical analysis. Dermatol Surg. 2008;34(Suppl 1):S64-7.
26. Yutskovskaya Y, Kogan E, Leshunov E. A randomized, split-face, histomorphologic study comparing a volumetric calcium hydroxylapatite and a hyaluronic acid-based dermal filler. J Drugs Dermatol. 2014;13:(9):1047-52.
27. Coleman KR, Carruthers J. Combination therapy with BOTOXTM and fillers: the new rejuvenation paradigm. Dermatol Ther. 2006;19(3):177-88.
28. Goldberg D, Guana A, Volk A, et al. Single-arm study for the characterization of human tissue response to injectable poly-L-lactic acid. Dermatol Surg. 2013;39(6):915-22.

29. Yutskovskaya YA, Jogan A. Improved Neocollagenesis and Skin Mechanical Properties After Injection of Diluted Calcium Hydroxylapatite in the Neck and Décolletage: A Pilot Study. J Drugs Dermatol. 2017;16(1):68-74.
30. Jacovella PF. Calcium hydroxylapatite facial filler (Radiesse): indications, technique, and results. Clin Plast Surg. 2006;33(4):511-23.
31. Coleman KM, Voigts R, DeVore DP, et al. Neocollagenesis after injection of calcium hydroxylapatite composition in a canine model. Dermatol Surg. 2008;34(Suppl 1):S53-5.
32. Marmur ES, Phelps R, Goldberg DJ. Clinical, histologic and electron microscopic findings after injection of a calcium hydroxyapatite filler. J Cosmet Laser Ther. 2004;6(4):223-6.
33. Goldberg DJ, Flaharty P. Radiance. Facial Plast Surg. 2000;20(2):165-9.
34. Marmur ES, Phelps R, Goldberg DJ. Clinical, histologic and electron microscopic findings after injection of a calcium hydroxylapatite filler. J Cosmet Laser Ther. 2004;6(4):223-6.
35. Bioform Medical Inc. Regulatory issues. Available at: http://www.radiesse.com, Accessed June 12, 2006.
36. Rhoda S, Narins MD. Minimizing adverse events associated with poly-l-lactic acid injection. Dermatol Surg. 2008;34 (Suppl 1):S100-4.
37. Lam SM, Azizzadeh B, Graivier M. Injectable poly-L-lactic acid (Sculptra): technical considerations in soft-tissue contouring. Plast Reconstr Surg. 2006;118(3 Suppl):55S-63S.
38. Fitzgerald R, Vleggaar D. Facial volume restoration of the aging face with poly-l-lactic acid. Dermatol Ther. 2011;24(1):2-27.
39. Bauer U, Graivier MH. Optimizing injectable poly-L-lactic acid administration for soft tissue augmentation: The rationale for three treatment sessions. Can J PlastSurg. 2011;19(3):e22-7.
40. Hevia O. A retrospective review of calcium hydroxylapatite for correction of volume loss in the infraorbital region. Dermatol Surg. 2009;35(10):1487-94.
41. Breithaupt A, Fritzgerald R. Collagen Stimulators: Poly-L-Lactic Acid and Calcium Hydroxylapatite. Facial Plast Surg Clin North Am. 2015;23(4):459-69.
42. Eviatar J, Lo C, Kirszrot J. Radiesse: Advanced Techniques and Applications for a Unique and Versatile Implant. Plast Reconstr Surg. 2015;136(5 Suppl):164S-70S.
43. Kulichova D, Borovaya A, Ruzicka T, et al. Understanding the safety and tolerability of facial filling therapeutics. Expert Opin Drug Saf. 2014;13(9):1215-26.