

“RichBlend” protocol for full-face filling and collagen biostimulation

Protocolo “RichBlend” para preenchimento facial e bioestimulação de colágeno

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ABSTRACT

The “RichBlend” protocol was designed for facial filling and collagen biostimulation, by means of a mixture of calcium hydroxyapatite (CaHA), hyaluronic acid (AH) and autologous platelet concentrates. This work reports the case of a 53-year-old patient with cutaneous photoaging, loss of facial volume, multiple rhythms in the frontal and periorbital regions, also marked skin flaccidity, especially the eyelid. The treatment was done with botulinum toxin (65 U) and the “RichBlend” protocol. Venipuncture was performed and the blood was centrifuged to obtain i-PRF (injectable platelet-rich fibrin) and plasma gel. After venipuncture and blood centrifugation, i-PRF and plasma gel were obtained. CaHA (Radiesse®) was diluted: a) in saline solution + i-PRF (hyperdilution) for biostimulation of the lower third of the face; and b) in AH (Juvederm Ultraplus XC®) + plasma gel, for hydrolifting on the forehead and dark circles, malar and temples. Plasma gel was applied to the nasogenian grooves and then the entire face was properly massaged. The “RichBlend” protocol rejuvenated the patient, as it promoted filling, volumizing, collagen formation (biostimulation), reduction of flaccidity, in addition to skin whitening. Since HA and CaHA are high-cost products, their mixture with autologous platelet concentrates, in liquid or gel form, allows the use of a greater amount of filled and biostimulator material on the face, at a more affordable cost.

Indexing terms: Durapatite. Hyaluronic acid. Rejuvenation.

RESUMO

O protocolo “RichBlend” foi idealizado para preenchimento facial e bioestimulação de colágeno, por meio da mistura de hidroxiapatita de cálcio (CaHA), ácido hialurônico (AH) e concentrados plaquetários autólogos. Este trabalho relata o caso de um paciente de 53 anos, com fotoenvelhecimento cutâneo, perda de volume facial, múltiplas ritides nas regiões frontal e periorbital, apresentando também acentuada flacidez cutânea, especialmente palpebral. Foi feito o tratamento com toxina botulínica (65 U) e protocolo “RichBlend”. Foi realizada a venopunção e o sangue foi centrifugado para obtenção da i-PRF (fibrina rica em plaquetas injetável) e do plasma gel. Após venopunção e centrifugação sanguínea, obtiveram-se a i-PRF e o plasma gel. A CaHA (Radiesse®) foi diluída: a) em soro + i-PRF (hiperdiluição) para bioestimulação do terço inferior da face; e b) em AH (Juvederm Ultraplus XC®) + plasma gel, para hidrolifting na frente e preenchimentos de olheira, malar e têmporas. Plasma gel foi aplicado nos sulcos nasogenianos e, em seguida, toda a face foi devidamente massageada. O protocolo “RichBlend” rejuvenesceu o paciente, pois promoveu preenchimento, volumização, formação de colágeno (bioestimulação), redução da flacidez, além do clareamento cutâneo. Uma vez que o AH e a CaHA são produtos

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How to cite this article

Almeida EPM, Levy FM, Buzalaf MAR. “RichBlend” protocol for full-face filling and collagen biostimulation. RGO, Rev Gaúch Odontol. 2023;71: e20230014. <http://dx.doi.org/10.1590/1981-86372023001420210069>

de alto custo, sua mistura com os concentrados plaquetários autólogos, na forma líquida ou gel, permite a utilização de uma maior quantidade de material preenchedor e bioestimulador na face, com custo mais acessível.

Termos de indexação: Durapatita. Ácido hialurônico. Rejuvenescimento.

INTRODUCTION

With the aging process, there is an alteration of the dermal extracellular matrix, losing the viscoelasticity induced by breakdown of collagen and elastin fibers, degradation of proteoglycans and lack of skin hydration, leading to the appearance of fine wrinkles and flaccidity [1].

Facial filling procedures with hyaluronic acid (HA) and the application of collagen biostimulators in patients with sagging skin and loss of facial volume have become part of the routine of professionals who work with orofacial harmonization (HOF) [2,3].

HA is one of nature's most hydrophilic molecules. It is biocompatible, viscoelastic and acts as an injectable facial filler material, capable of hydrating the extracellular matrix and increasing tissue volume [1].

Calcium hydroxyapatite (CaHA) is a biodegradable filling material, composed of CaHA microspheres, with a size between 25 to 45 µm, dispersed in a gel-based vehicle, composed of water, glycerin and sodium carboxymethylcellulose. This carrier gel is gradually absorbed and collagen formation is induced between the hydroxyapatite spheres. However, the gel is quickly absorbed, which leads to the loss of the product's initial volume and the neocollagenesis process occurs slowly, usually 4 months after its injection [2], improving skin flaccidity and ensuring a lasting result.

The CaHA-based filler, also known as collagen biostimulator, initially stimulates the formation of type III collagen, which is then replaced, through remodeling, by type I collagen, at approximately 6 months, which contributes to improved rhytids and the appearance of the skin [4]. Considering that HA promotes volume gain with water incorporation and CaHA undergoes initial volume loss due to the absorption of the carrier gel, the combined use of these two products is interesting [2].

At the same time, it is necessary to emphasize the importance and increase in the use of regenerative therapies in the field of facial aesthetics, and with that of platelet concentrates [5], since it is an autologous raw material, which therefore does not cause reactions adverse effects after its injection, at no cost to the patient, which presents excellent results in angiogenesis and stimulation of fibroblast differentiation and skin regeneration [6].

In dentistry and medicine, autologous platelet concentrates have been used for decades to treat wounds, soft tissue injuries, in the field of maxillofacial surgery, bone grafts, oral and periodontal surgeries, orthopedics, gastrointestinal surgeries and burns. In dermatology and HOF, they have been used for the treatment of bedsores, facial healing, facial swelling, alopecia and skin rejuvenation [5].

There are two generations of autologous platelet concentrates: the first generation, PRP (Platelet Rich Plasma), uses an anticoagulant (sodium citrate) in the tube used for blood collection and centrifugation, which makes it stable for about 8 hours, that is, it has a longer working time [5, 7]; and the second generation, PRF (Fibrin Rich in Platelets), consists of the extraction of platelets and blood growth gains, through the use of tubes without anticoagulants, which makes the work time much shorter (15-20 minutes) [5]. It resembles a three-dimensional fibrin mesh, like that of a PRF clot [8].

Some authors have used the association of these filler materials considered safe in HOF, both the mixture of HA with CaHA [2,11] and the mixture of HA with PRP [1,12], to add the advantages and favorable properties of each material in the rejuvenation of the patient.

Based on these studies and considering the favorable characteristics of each material, we designed the "RichBlend" Protocol, which consists of treating the skin with collagen biostimulation and restoring the lost facial volume inherent to age, with a mixture, in appropriate proportions, of materials already widely used in HOF: CaHA, AH and autologous

platelet concentrates. This protocol associates the advantages of each of these materials, according to the need of each facial region.

This paper aims to show a case report of a patient treated with the "RichBlend" protocol and to detail each phase of this innovative, effective and low-cost protocol.

CASE REPORT

Patient MAM, 53 years old, male, with skin photoaging, loss of volume in the frontal region, temples and premaxilla, abundance of skin rhytids in the frontal and periorbital region, also presenting marked flaccidity on the side of the face and eyelids and some spots in the region of dark circles, he came to us for orofacial harmonization (figure 1).



Figure 1. Initial (left) and final (right) appearance of the patient.

Given the patient's needs, we proposed the treatment of dynamic wrinkles with the application of toxin on the forehead, glabella and periorbital region, the treatment of flaccidity with collagen biostimulation with CaHA (Radiesse®, Merz North America, Inc, Franksville, Wisconsin, USA) associated with i-PRF in the lower lateral third of the face and restoration of facial volume loss, with filling with AH (Juvederm Ultraplus XC®, Allergan, Pringy, Annecy, France), associated with CaHA (Radiesse®) and plasma gel, forehead, temples and premaxilla and zygomatic arch regions.

Initially, botulinum toxin (Xeomin®, MERZ Pharma, Dessau-Rosslau, Germany) was applied on the forehead, glabella and periorbital region (total 65 U). Figure 2 shows the marking for toxin application, which is necessary for better maintenance, absorption and stabilization of the product to be used for filling. Fifteen days after the application of the toxin, the patient was scheduled to control the toxin and perform the "RichBlend" protocol.

Before performing the anesthesia, venipuncture was performed with a 21G needle scalp, the patient's blood was collected in 2 plastic tubes with a white cap of 9 ml (without anticoagulant) and in 6 plastic tubes with a blue cap of 3 ml (with citrate sodium). The white cap tubes were centrifuged at 208 g for 8 minutes (KASVI K14-4000PRF centrifuge, São José dos Pinhais, PR, Brazil) [10] for the production of i-PRF (liquid PRF). The blue cap tubes were centrifuged at 580 g for 8 minutes (KASVI K14-4000PRF centrifuge, São José dos Pinhais, PR, Brazil) for the production of PRP [13].

After centrifugation, the blood was separated into two portions inside each tube, the red portion (red blood cells) and the yellow portion (plasma containing leukocytes and platelets). Approximately 1.5 ml of i-PRF was collected from each white tube by aspiration of the yellow portion through an 18 ½ G 40X1.2 mm needle, attached to a 5 ml syringe.

The needle was positioned close to the red portion, being very careful not to touch it, in order to avoid aspiration of red blood cells, due to its inflammatory potential. The syringe containing i-PRF was kept inside a styrofoam containing ice until used as a biostimulator. The collection of the yellow portion of the blue tubes (containing anticoagulant) was performed with a similar syringe and needle, but the platelet-poor plasma (PPP, located in the upper 2/3, fraction F1) was initially collected, which was placed in a heater (VULCAN, digital plasma gel, Araucaria – PR, Brazil) at 76°C for 12 minutes, to obtain the precursor gel. Platelet-rich plasma (PRP, lower 1/3) was also collected and used to homogenize the plasma gel after heating, in the proportion of 5(PPP):1(PRP) (v:v) [13]. After this homogenization, the material was cooled in the freezer for 10 minutes, obtaining the plasma gel, which was used as a filler and biostimulator.

During the time of centrifugation and preparation of the iPRF and plasma gel, the patient's skin was aseptized with a 0.2% chlorhexidine solution, anesthetized on the face (infiltrative terminal anesthesia of mental and infraorbital nn, with 2% lidocaine with epinephrine), markings were carried out in all areas that would be filled or would receive biostimulator injection (the technique of transference of facial marking with contact paper was used, as shown in figures 2, right and 3), and then, anesthesia was completed in the points of holes, where the 22 G cannula would be introduced for application of the products.



Figure 2. Marking for application of botulinum toxin (left) and biostimulators/fillers by transfer with contact paper (right).



Figure 3. Marking for application of fillers and biostimulators bilaterally.

After preparing the platelet concentrates, the Radiesse® was diluted and the products were mixed in 3 phases for its application (figure 4), as detailed below: - 0.5 ml CaHA diluted in 1 ml serum + 0.5 ml lidocaine and 3 ml i-PRF (hyperdilution - figure 4, above) was used to make a range of 6 retroinjections (about 0.4 ml each) of biostimulator on the lower side of the face bilaterally - Treatment of the lower third of the face (Figure 5, below).



Figure 4. Hyperdilution of CaHA + i-PRF (above) and mixture of CaHA with HA and plasma gel (below).

- 0.5 ml of CaHA in 0.5 ml of HA and 2 ml of plasma gel = 3 ml (figure 4, below), to perform the Hydrolifting or Hydrodissection on the forehead; in this technique, the tissue from the frontal region is detached (according to the design of the marking) with a cannula, while the anesthetic lidocaine without vasoconstrictor is injected, diluted in saline solution at the supraperiosteal level, and, subsequently, the mixture of CaHA with plasma gel and HA in this hydrodissected region – Treatment of the upper third of the face (figure 5).

- 0.5 ml of CaHA in 0.5 ml HA, 2 ml of plasma gel and 1 ml of lidocaine, totaling 4 ml, which were divided between the regions below the dark circles, malar and temples (2 ml on each side) - Treatment of the middle third of the face (figure 6).

First, collagen biostimulation was performed in the lower lateral third of the face (Figure 5, below), due to the shorter work time with the i-PRF. Then, the forehead was hydrolifted (figure 5), massaged with a pink jade stone roller and, finally, the middle third was filled, in the dark circles, malar and temples region (figure 6), according to the markings previously made (figures 2, right and 3).

It is important to remember that, before every application, the products must be well homogenized to properly distribute the CaHA particles in the mixture. The plasma gel remaining in the syringe was applied to the nasolabial sulcus and glabella (in small amounts) and then massaged over the entire face.

At the end of the treatment, it can be seen that the RichBlend protocol rejuvenated the patient because it promoted filling, volumization, collagen formation (biostimulation), reduction of flaccidity, in addition to skin lightening (figure 1, right). Furthermore, due to the association of platelet concentrates with commercial biomaterials, there was a considerable reduction in the cost of treatment.



Figure 5. Treatment of the lower third with hyperdiluted CaHA in serum and i-PRF for collagen biostimulation and treatment of the upper third (hydrofilling on the forehead).

DISCUSSION

It is important to understand how the tissue repair process happens after facial fillings using the different materials used in the “RichBlend” technique. Studies comparing the rheological characteristics of AH- and CaHA-based facial fillers show that it has higher G' and \ast than AH-based fillers, therefore, more desirable characteristics of a dermal filler in terms of viscosity and elasticity [14,15]. Thus, it is expected that the tissue reaction against these materials is different.

HA incorporates water into its molecule, resulting in an increase in volume over time; CaHA, on the other hand, presents an aqueous gel vehicle, which undergoes reabsorption and results in initial volume loss, but subsequently volume gain occurs due to the formation of collagen in the tissue. Therefore, the combined use of these materials compensates for the characteristic volume changes of the individual materials in the postoperative period [2,11].

Tissues filled with CaHA or HA also differ from a morphological point of view. In the 4th month after the application of these materials, type III collagen formed in areas filled with CaHA is higher compared to those filled with AH. In the 9th month, collagen type I is higher for CaHA and type III is higher for HA. There is also a higher content of elastin and more pronounced angiogenesis for CaHA, in both periods. These findings indicate that CaHA results in more active physiological remodeling of the extracellular matrix than HA, and that type I collagen gradually replaces type III collagen with tissue maturation [15]. CaHa may increase proteoglycans and may also have an effect on elastin, which may induce remodeling in extracellular matrix components [16].

Platelet concentrates are autologous preparations derived from whole blood with high concentrations of platelets, containing more than 800 bioactive molecules [17,18], especially growth factors, with great potential for collagen biostimulation [9]. In addition, plasma gel will also promote face volumization.



Figure 6. Treatment of the middle third with a mixture of CaHA, HA and plasma gel (filling the malar, dark circles and temples).

The centrifugation protocol is of great importance for improving the characteristics of the resulting i-PRF, increasing the amount of platelets and leukocytes, as well as the concentration of growth factors in the fibrin matrix. In Choukroun's original protocol, high centrifugation force (710 g) for 8 minutes is used [19]. With the same centrifugation time, but with low (44 g) or intermediate (177 g) forces, preparations are obtained with higher concentrations of platelets and leukocytes or growth factors (VEGF and TGF- 1), respectively [9]. For this reason, in our protocol, we used an intermediate centrifugation force (208 g) for 8 minutes, a protocol known as advanced-PRF plus (A-PRF+) [10]. There are still few clinical studies evaluating the use of i-PRF for facial rejuvenation. Good results have been reported using low (60 g) and intermediate (208 g) centrifugation forces, but further studies are needed [12,20].

To obtain the plasma gel, after centrifugation, the upper 2/3 of the yellow portion (PPP) is initially collected, and this material is heated to 76°C for 12 min. During heating, protein denaturation occurs, which facilitates the formation of the precursor gel. After heating, the lower 1/3 of the plasma, rich in platelets and growth factors [21], is then homogenized with PPP, in order to ensure the inclusion of platelets and growth factors in the plasma gel that will be used in the patient, so that there is potential for biostimulation, in addition to the volumization effect [13]. Even so, at least in vitro, the biostimulatory potential of i-PRF is greater than that of PRP. Experiments with human skin fibroblast cultures

showed greater cell migration and proliferation in the presence of i-PRF compared to PRP, as well as higher mRNA levels for type 1 collagen, TGF- and fibronectin, in addition to a greater capacity to induce collagen matrix synthesis [5]. It is possible to think about homogenizing the plasma gel with i-PRF in order to increase its biostimulating potential, but this would require a new blood collection, due to the rapid formation of the fibrin network in the i-PRF, while the plasma gel is prepared.

An important factor to be considered is that, as HA and CaHA are high-cost products, their mixture with platelet concentrates allows the use of a greater amount of volumizing and biostimulating material on the face, to reestablish lost or ptosed tissues with age, at a lower cost to the patient.

A limitation of the “RichBlend” protocol is that platelet concentrates are rapidly reabsorbed by the body. Therefore, depending on the patient’s volume requirement, filling with plasma gel will need to be repeated to maintain long-term volume results. In this case, it is also worth performing a new i-PRF session along with the filling, in order to expand the biostimulation effect, further improving skin sagging and restoring the facial volume initially achieved.

CONCLUSION

The “RichBlend” protocol combines the advantages of CaHa, AH and autologous platelet concentrates, according to the needs of the patient and each facial region, at an affordable cost.

Collaborators

E Almeida and F Levy, conceptualization (equal), data curation (equal), investigation (equal), methodology (equal), writing - original draft (supporting) and writing - review & editing (supporting). MAR Buzalaf, conceptualization (equal), formal analysis (lead), project administration (lead), resources (equal), supervision (lead) and writing - review & editing (lead).

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Received on: 14/12/2021

Final version resubmitted on: 21/8/2022

Approved on: 27/10/2022

Assistant editor: Luciana Butini Oliveira