

Platelet-rich fibrin and calcium hydroxyapatite (CaHA): The perfect combination to accelerate, potentiate, and extend skin rejuvenation

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Abstract. *Background:* calcium hydroxyapatite (CaHA), classified as a collagen biostimulator, known commercially as Radiesse® (Merz Pharma GmbH & Co. KGaA, Frankfurt, Germany) contains 30% synthetic CaHA suspended in a 70% aqueous sodium carboxymethylcellulose gel matrix. It provides both volume replacement and collagen biostimulation as a primary mechanism of action and has become one of the most effective treatments to rejuvenate the face. However, after the treatment it is necessary to wait for approximately 4 to 6 weeks to achieve any results. *Methods:* we included 100 patients, all adults and divided into two groups: 50 patients treated with CaHA filler (Radiesse, Merz North America, Inc., Raleigh, NC) in a “traditional dilution” with 0, 5 ml of 2% lidocaine and 2 ml of standard saline solution, so we have 4 ml in total to use 2 ml per side, and 50 patients treated with our new technique with 1.5 ml of CaHA filler diluted with 2.5 ml of Platelet-rich fibrin (PRF) for a total of 4 ml. *Results:* Platelet-rich fibrin used as a diluent for Radiesse in facial bio stimulation treatments produces superior results in a shorter timeframe, as demonstrated in this case series. Patients experience a unique radiance in their skin during treatment and can expect a safe profile with no severe complications. *Conclusion:* Using platelet-rich fibrin and Radiesse together is a simple and effective way to boost the product's bio-stimulatory effect. This combination could become a key element in anti-ageing medicine to promote cell regeneration.

Key words: radiesse, platet-rich fibrin, face biostimulation

Introduction

The perception of beauty has been influenced worldwide and throughout history by many factors, including ethnic conditions, geographic location, socio-economic situation, and lifestyles¹ During the second half of the last decade, a phenomenon called “*Snapchat Dysmorphia*” was recognized in some patients who were influenced via filters to alter their bodies². This phenomenon will evolve into what we know now as “*Zoom Dysmorphia*”. The pandemic induced

by Covid-19 brought about several changes in everyday lifestyles such as work-from-home culture, online classes, prompting an abundance in video conference calls. These extended hours of video web interaction can lead to unsettling changes and perceptions of how one views their self-image³. With this exponential increase in the self-perception of beauty focused on the face, physicians performing aesthetic medicine treatments were led to improve their injectable treatments.

Collagen biostimulators as calcium hydroxyapatite (CaHA), known commercially as Radiesse®

(Merz Pharma GmbH & Co. KGaA, Frankfurt, Germany), a biocompatible and biodegradable medical device that offers the proven benefit of a collagen and elastin biostimulator at dermal and subdermal tissue levels^{4,5} approved by the United States Food and Drug Administration (FDA) and CE-certified in Europe⁶, it contains 30% synthetic CaHA suspended in a 70% aqueous sodium carboxymethylcellulose gel matrix¹⁻³. This provides both volume replacement and collagen biostimulation as a primary mechanism of action and has become one of the most effective treatments to rejuvenate the face and achieve a tightened appearance of the skin, as well as an increase in dermal thickness and improvement in the skin's glow. The result is a long-lasting aesthetic improvement for ≥ 18 months with tight and elastic skin and increased skin thickness⁴⁻⁶.

Based on our extensive clinical expertise using hydroxyapatite (CaHA), we have identified that after the treatment it is necessary to wait for approximately 4 to 6 weeks to achieve results of the product in terms of quality of the skin, skin thickness and brightness⁷⁻¹². During this time, the molecules are grouped in the extracellular matrix to stimulate collagen and elastin production, unfortunately, this time-out makes patients anxious about the results and some patients indicated that they had the perception of "*losing the initial effect*": After receiving the treatment, patients typically notice a slight increase in volume and a glowing appearance of their skin. However, this effect tends to fade within a few weeks. Therefore, to achieve and maintain the desired results of biostimulation, patients are instructed to wait from 4 to 6 weeks. Recognizing this issue, we sought alternatives to complement calcium hydroxyapatite (CaHA) and address this problem.

This article presents a new technique that seeks to potentiate and accelerate the results of calcium hydroxyapatite (CaHA), known commercially as Radiesse® (Merz Pharma GmbH & Co. KGaA, Frankfurt, Germany) by mixing it with Platelet-rich fibrin (PRF), an autologous blood product concentrate prepared through centrifugation, mainly composed of platelets, fibrin, and leukocyte, cytokines, and stem cells within a fibrin matrix.

Materials and Methods

Retrospective analysis and technique

A retrospective and observational study was made in Colombia with 100 patients treated with Radiesse® (Merz Pharma GmbH & Co. KGaA, Frankfurt, Germany) for facial biostimulation from January 2019 to January 2022. The inclusion criteria were patients of any gender, older than 18 years, with different grades of face sagginess, seeking an improvement in their skin quality, skin glow and tightening. The exclusion criteria included pregnancy, local infections, previous facial surgery, or hyper sensibility to lidocaine. All patients signed a written informed consent for using their data and photographs, and they agreed to use their data in future studies and scientific publications.

We used CaHA filler (Radiesse, Merz North America, Inc., Raleigh, NC), seeking its benefits as a collagen biostimulator to improve the patients' dermal thickness, sagginess, and volume loss in the face. We divided the group in two: 50 patients treated with CaHA filler (Radiesse, Merz North America, Inc., Raleigh, NC) in a "*traditional dilution*" with 0, 5 ml of 2% lidocaine and 2 ml of standard saline solution, having collectively 4 ml in total to use 2 ml per side, and 50 patients treated with our new technique of 1.5 ml of CaHA filler diluted with 2.5 ml of Platelet-rich fibrin (PRF), for a total of 4 ml. This will be further developed throughout this study.

The treatment was performed by two expert injectors using the same labelling and distribution of the product in all patients covering the middle and lower third of the face, using the same amount of volume for all patients. Following antisepsis and disinfection protocols, no adverse events occurred during or after the treatment.

Technique Description

Step 1: Platelet-rich Fibrin (PRF)

The first part of our technique consists of obtaining the platelet-rich Fibrin (Figure 1). Usually this procedure is performed by obtaining a blood sample, which

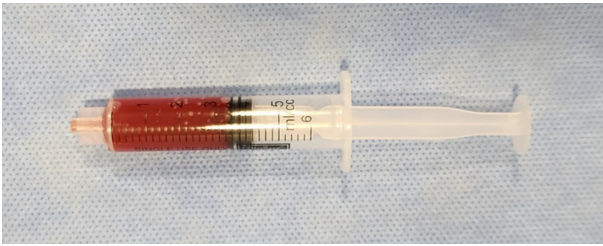


Figure 1. Platelet-rich Fibrin (PRF) result.

is introduced into 4.5 ml test tubes and immediately centrifuged at 2700 revolutions per minute for 12 minutes at 280 G (gravitational force of the centrifugation). After a few minutes, the absence of an anticoagulant allows the activation of most of the platelets in the sample to trigger the coagulation cascade. Fibrinogen is initially concentrated in the upper part of the tube until the effect of circulating thrombin transforms into a fibrin network. The result is a fibrin clot containing platelets in the middle of the tube, just between the red blood cell layer at the bottom and the acellular plasma at the top. This clot is removed from the tube, and the red blood cells are discarded. The clot is placed in the FRP box and covered with the compressor and lid. This produces an inexpensive autologous fibrin membrane in approximately one minute⁹⁻¹².

For regenerative medicine in aesthetic treatments and based on the experience of the authors, we decided to adjust the technique:

- Using a tube with sodium citrate to centrifuge the blood sample, that will prevent clot formation, and we can obtain more than just platelets important products like fibrin growth factors, stem cell and proinflammatory cells such as leukocytes that play a very important role in aesthetics treatments.
- We decrease the number of revolutions per minute to 800 then to 1,000 for 5 minutes, so that the cells are not denatured, preserving the active form of the hemoderivatives.
- By eliminating calcium gluconate from the mixture so that the products do not separate, we maintain a compact and complete result with all the elements necessary for cell regeneration.

Step 2: CaHA filler (Radiesse, Merz North America, Inc., Raleigh, NC) dilution with PRF

Once the Platelet-rich Fibrin (PRF) is ready for application we take 2.5 ml of this and mix it with 1.5 ml of Radiesse Classic (Merz North America, Inc., Raleigh, NC). For a total of 4 ml (2.5 ml of PRF + 1.5 Radiesse Classic) (Figure 2) using a 2 lines connector and 2 syringes of 5 ml we combine the two products by passing one to the other 30 times, until a homogeneous mixture is obtained and then we divided it in 4 syringes of 1 ml each (Figure 3).

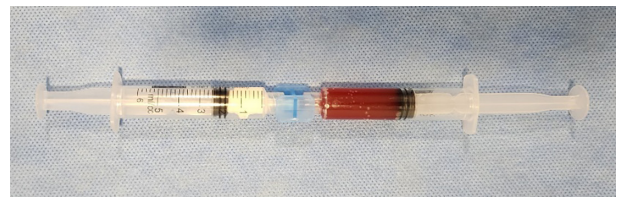


Figure 2. Radiesse dilution with PRF for a total mixture of 4 ml.



Figure 3. Radiesse + PRF dilution, ready to use, total of 4 ml distributed in 4 × 1 ml syringes.

Step 3: Application technique

According to the dilution we previously explained, we have 2 ml to use on each side of the face. As an Anatomical reference, we look for the lateral limit of the zygomatic bone between the malar head and the zygomatic apophysis. Here we made the first entry point. With a 22G x 50 mm cannula, we can reach the nasolabial folds to the front and back to the tragus, covering these two areas of the face; furthermore, using this entry point, we lower the cannula down to the angle of the mouth.

The second entry point is located between the mandibular angle and the most prominent part of the chin; with this point, we can perform the whole approach of the mandibular contour. Using these two entry points, we can distribute the entire product on the face, covering the anterior and lateral area and the jaw contour, with less invasive trauma.

To distribute the product in the two groups, we used a 22G cannula, using the fan application method and retrograde linear deposition. Small amounts of product were deposited in multiple vectors, covering the entire face.

Results

Patients complete an online survey six months after the treatments, in order to determine their perception on the results of the treatment. The main inquiries revolved around how long it took for them to see results in terms of skin glow, thickness and the lifting effect, and whether they were satisfied with the overall results. The authors received the results directly and organized the data in SPSS for analysis.

In the PRF group, 42 % and 24% of the patients said that the results were evident one week or two weeks after the treatment, respectively (Figure 4).

In contrast to the traditional dilution group, 80% of the patients said the results were only evident more than four weeks after the treatment, and none of the 50 patients treated responded that they could see results in 1 to 2 weeks (Figure 5).

Regarding the question of whether they lost the initial effect obtained from the treatment on the face,

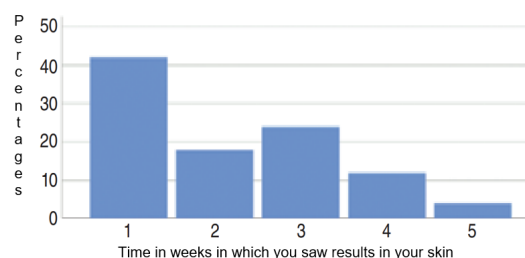


Figure 4. These are the survey findings on the wait time for obtaining the results on patients treated with Platelet Rich Fibrine dilution. 1 = less than one week. 2 = After one-week 3 After three weeks, 4 = After four weeks 5 = After six weeks.

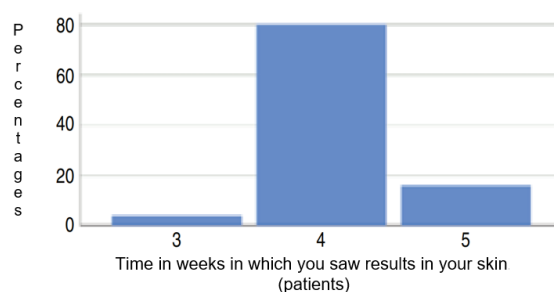


Figure 5. These are the survey findings on the wait time for obtaining the results on patients treated with Standard dilution. 1 = less than one week. 2 = After one-week 3 After three weeks, 4 = After four weeks 5 = After six weeks.

a significant difference was found since in the group treated with PRF, 70% of the patients responded that they did not find a loss of this effect compared to the group that received treatment with standard dilution, where 84% answered that they had lost the initial effect.

For the level of satisfaction with the results that the patients could see on their skin 15 and 60 days after treatment, the group that received PRF treatment, 74% of patients reported excellent improvement 15 days after treatment, and 90% reported excellent improvement by the 60th day after treatment. None of the 50 responses indicated “none” or “slight improvement” (Figure 6). Compared to the group that received the treatment with the standard dilution, concerning the improvement at 15 days of treatment responded, only 10% perceived excellent results; for the progress at 60 days, 75% responded with significant improvement (Figure 7). This shows that the perception of results in the group treated with fibrin is significantly higher.

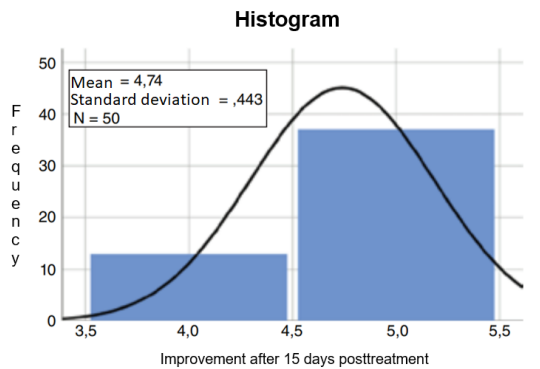


Figure 6. Final box chart of the answer obtained on the question: How much improvement did you notice 15 days after the treatment? On patients treated with Platelet Rich Fibrine dilution. 1 = None 2 = Little bit 3= good, 4 = really good 5 = very much better.

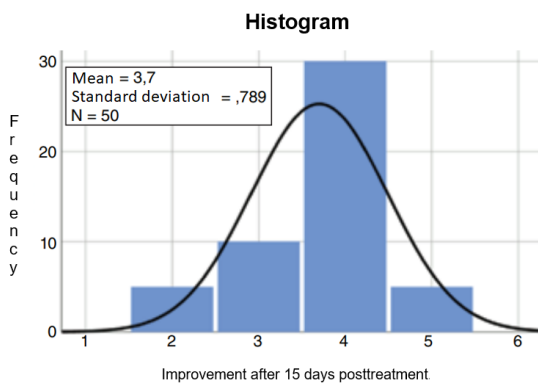


Figure 7. Final box chart of the answer obtained on the question: How much improvement did you notice 15 days after the treatment? On patients treated with standard dilution. 1 = None 2 = Little bit 3= good, 4 = really good 5 = very much better.

PRF group

“Standard dilution” group

When the patients were asked if they considered it was necessary to wait a long time to see the results, the group treated with PRF answered 92% that it was not required to wait too long, compared to 42% of the group of patients treated with standard dilution, who responded affirmatively to this question (Figure 7). Therefore, the group that most perceived the waiting time as being prolonged was treated WITHOUT fibrin.

Discussion

Numerous scientific studies have conclusively demonstrated the efficacy of collagen biostimulators containing calcium hydroxyapatite for anti-ageing treatments¹⁸⁻²². However, we have developed a technique for those seeking quicker results that combines collagen biostimulators, specifically calcium hydroxyapatite, with platelet-rich fibrin. Through our extensive experience with this method, we have consistently achieved exceptional results devoid of any adverse events such as bruising, swelling, or mild pain. In addition, our survey of 100 patients has indicated that those undergoing treatments with PRF witnessed visible improvements within the first two weeks, with enhanced skin quality reported after just 15 days. Based on these remarkable outcomes, our new dilution technique utilizing Radiesse with platelet-rich fibrin holds immense promise.

Why choose Platelet-rich fibrin (PRF)?

Platelet-rich fibrin (PRF) is an autologous blood product concentrate prepared by centrifugation mainly composed of platelets, fibrin, and leukocyte, cytokines and stem cells within a fibrin matrix. Leukocytes appear to strongly influence growth factor release, immune regulation, anti-infective activities, and matrix remodeling during healing⁸.

PRF can be utilized as a single biomaterial or with a grafting material to accelerate bone regeneration⁹. Platelet Rich Fibrin simultaneously promotes angiogenesis, immunity, and epithelialization as the healing process and soft tissue maturation¹⁰ and has been widely used in different types of dental treatments, where a high capacity of tissue regeneration is required with very satisfactory results^{8,17,25}.

Furthermore, PRF has the potential to be widely used in the medical field due to the advantages of low cost, high safety, and easy extraction. Whitman and Marx took the lead in applying PRF in the treatment of bone tissue repair in the 1990s, even as a drug/cell carrier for a controlled release and achieved positive effects¹²⁻¹⁷ during the past decades, PRF has been used in dermatology, orthopedics, and plastic surgery, particularly in treating all kinds of chronic wounds. Some

studies have suggested that PRF can promote the connection of microcirculation, inhibit inflammation and reduce the rate of apoptosis. Yuliya Menchisheva has confirmed the substantially positive effects of the PRF by applying it to the treatment of wounds within a month in a clinic¹⁴⁻¹⁸.

For this study, this publication made a review of 100 treatments performed on different patients in Colombia, 50 of them treated with our new protocol of platelet-rich Fibrin as the diluent, and it is essential to note that in all patients treated with this technique, no adverse event or complication associated with the treatment occurred, our team found that adding PRF

to CaHA filler (Radiesse, Merz North America, Inc., Raleigh, NC)²¹⁻²³ potentiated its biostimulating characteristics, accelerating the results and providing a greater capacity to replenish volume. As for hands, it also behaves as a structural support for the fascia, allowing much more harmonious results²⁴⁻²⁷ (Figures 8 and 9).

After seeing the results of the surveys, they show a superiority in the onset of action of the outcomes in the group treated with platelet-rich Fibrin. Although, in contrast, none of the 50 patients treated with the standard dilution reported seeing results in the first 3 weeks after treatment, 65% of the patients treated



Figure 8. Patient treated with Radiesse + PRF, A. Before / B. 3 weeks after the treatment, especially on oblique position we can appreciate how much have improve in terms of skin quality and jaw line. oblique position.



Figure 9. Patient treated with Radiesse + PRF, A. Before / B. 3 months after the treatment. We can appreciate skin thickness improvement and nasolabial folds and skin tone for this patient, as well as jaw line improvement.

with the new protocol perceived results in the same period.

Furthermore, when asked how much improvement they could see in their skin 15 days after treatment, 74% of the patients treated with Fibrin reported excellent results compared to only 10% in the group of patients treated with standard dilution, and when asked if they had noticed an improvement three days after the treatment, 86% of the patients treated with our new technique answered affirmatively.

According to these results and the experience of the authors, we can conclude that there is a significant superiority in terms of 4 fundamental factors when implementing the technique described here of platelet-rich Fibrin as a diluent for classic Radiesse in facial biostimulation:

1. The results are evident faster compared with the standard dilution.
2. Dead time or waiting time for the biostimulation effects is eliminated.

3. The duration of the results is maintained in better condition over a more extended period.
4. Fibrin may enhance a bio-stimulatory development since it provides growth factors and pro-inflammatory proteins that can favor the effect of the hydroxyapatite molecules on the fibroblast.

Conclusion

Platelet-rich Fibrin is an intelligent and very effective alternative to enhance the results of calcium hydroxyapatite-based injectable products, has proven to have a high safety and patient satisfaction profile, and is an easy-to-replicate technique.

We would like to mention some remarkable findings that we were able to demonstrate during the application of this technique: improving skin color, helping to eliminate blemishes, improving healing processes and sun damage, improving vascularization, and improving the skin's elasticity, this technique allows us to use more total volume, as in traditional dilution normally uses 3 ml in total. Furthermore, by treating these 50 patients with PRF + Radiesse we did not get and infections or other adverse events, allowing us to consider it a safe technique which can give patients faster results compared to the traditional dilution group, as was evident at the survey results, we are presenting in Figures 6 and 7.

the clinical results also show a clear superiority consistent with the results of the patient survey, as shown in Figures 8 and 9, skin quality, skin tightening and improvement in mandibular contour is more evident with the patients with PRF + Radiesse.

Conflict of Interest: The authors in this article declare that they have no conflict of interest.

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