

Platelet-Rich Plasma with Basic Fibroblast Growth Factor for Treatment of Wrinkles and Depressed Areas of the Skin

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Background: There are several treatments for wrinkles and depressed areas of the face, hands, and body. Hyaluronic acid is effective, but only for 6 months to 1 year. Autologous fat grafting may cause damage during tissue harvest.

Methods: In this study, patients were injected with platelet-rich plasma plus basic fibroblast growth factor (bFGF). Platelet-rich plasma was prepared by collecting blood and extracting platelets using double centrifugation. Basic fibroblast growth factor diluted with normal saline was added to platelet-rich plasma. There were 2005 patients who received platelet-rich plasma plus bFGF therapy.

Results: Of the 2005 patients treated, 1889 were female and 116 were male patients; patients had a mean age of 48.2 years. Treated areas included 1461 nasolabial folds, 437 marionette lines, 1413 nasojugal grooves, 148 supraorbital grooves, 253 midcheek grooves, 304 foreheads, 49 temples, and 282 glabellae. Results on the Global Aesthetic Improvement Scale indicated that the level of patient satisfaction was 97.3 percent and the level of investigator satisfaction was 98.4 percent. The period for the therapy's effectiveness to become apparent was an average of 65.4 days. Platelet-rich plasma plus bFGF therapy resulted in an improved grade on the Wrinkle Severity Rating Scale. Improvement was 0.55 for a Wrinkle Severity Rating Scale grade of 2, 1.13 for a Wrinkle Severity Rating Scale grade of 3, 1.82 for a Wrinkle Severity Rating Scale grade of 4, and 2.23 for a Wrinkle Severity Rating Scale grade of 5.

Conclusions: Platelet-rich plasma plus bFGF is effective in treating wrinkles and depressed areas of the skin of the face and body. The study revealed that platelet-rich plasma plus bFGF is an innovative therapy that causes minimal complications. (*Plast. Reconstr. Surg.* 136: 931, 2015.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

Various therapies are available for treatment of wrinkles and depressed areas of the skin. The world's most preferred therapy for treatment of wrinkles and depressed areas of the skin is hyaluronic acid injection.¹⁻³ However, hyaluronic acid is only effective for 6 to 12 months; therefore, periodic treatment is required for the treatment to remain effective.⁴

Another cosmetic surgery option for treating wrinkles or depressed areas of the skin is fat injection. Fat injection is already a standard surgical treatment,^{5,6} but it requires liposuction, which causes internal bleeding and swelling that lasts 2 to 3 weeks.

Action and Therapeutic Effectiveness of Platelet-Rich Plasma

Platelet-rich plasma is widely used to promote tissue repair because it contains and releases various growth factors that heal tissue.⁷⁻¹⁰ Platelet-rich plasma is also used to rejuvenate the skin. Platelet-rich plasma improves the texture of the skin, but there are limits to the effect it has on deep wrinkles. Platelet-rich plasma must be combined with fat grafting to stimulate tissue growth.^{7,11}

Several studies have indicated the usefulness of basic fibroblast growth factor (bFGF).¹²⁻¹⁵ Clinical studies involving the skin used bFGF in combination with skin grafts to treat burns and to treat

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chronic skin ulcers.^{16–18} However, very few clinical studies have reported using bFGF to treat wrinkles and depressed areas of the skin.

No studies have reported adding bFGF to platelet-rich plasma to treat wrinkles or depressed areas of the skin in more than 1000 patients. The purpose of the current study was to verify the safety and efficacy of using platelet-rich plasma plus bFGF to treat wrinkles and depressed areas of the skin.

PATIENTS AND METHODS

This study was conducted according to the principles of the Declaration of Helsinki. Informed consent for platelet-rich plasma plus bFGF therapy was obtained from all patients before treatment. This is a retrospective study that included primarily patients with wrinkles and depressed areas. Cancer patients or patients who had a personal history of cancer, blood platelet disorders, or coagulation disorders were excluded.

From January of 2008 to December of 2011, we evaluated and treated 2005 patients who had wrinkles or depressed areas of the skin with platelet-rich plasma plus bFGF. Of these 2005 patients, 1889 were female and 116 were male patients. Patients had an average age of 48.2 years (range, 17 to 84 years). All patients had only one treatment within 6 months. A total of 4815 areas treated, with an average treatment number of 1.33, highlighting that different areas were treated at the same time (Table 1).

Methods

Platelet-rich plasma was prepared by collecting blood and extracting platelets using double centrifugation. We collected 9 ml of blood and created 1 ml of platelet-rich plasma. In the first round, centrifugation was performed at 1800 rpm

for 10 minutes, after which the top layer of plasma was collected. In the second round, centrifugation was performed at 3200 rpm for 10 minutes, after which the buffy coat and bottom layer of plasma were collected. The bFGF used was Trafermin from Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Two hundred micrograms of bFGF was diluted with normal saline, and 10 to 20 μ g of bFGF was added per milliliter of platelet-rich plasma.

Evaluation and Statistical Analyses of Results

The Global Aesthetic Improvement Scale was used to assess patient and physician satisfaction with the treatment. Global Aesthetic Improvement Scale scores were determined up to 180 days after treatment. A score of 3 or higher was deemed to indicate that the treatment was effective and met with satisfaction. If the patient was treated in multiple areas, each area was assessed separately. This study was performed within the nine clinics in our practice by multiple physicians. Satisfaction was measured by each physician and their patients. To minimize physician bias, the authors reviewed patient satisfaction.

The effectiveness of platelet-rich plasma plus bFGF becomes evident over time, and the inflection point at which that effectiveness becomes apparent varies from patient to patient and becomes clearer over time. However, as patient visits occur at irregular intervals, verification that the treatment has been successful becomes more difficult. Assessment of such clinical outcomes after treatment is similar to calculating the survival rate after cancer therapy. Thus, the Kaplan-Meier method was used to determine the pattern in which the therapy's effectiveness became evident. The generalized Wilcoxon test (Peto-Prentice method) was used to test for statistically significant differences with respect to age and areas treated.

Table 1. Number of Areas Treated with Platelet-Rich Plasma plus bFGF Therapy

| Site | No. of Cases | Typical Injection Volume (ml) |
|----------------------|--------------|-------------------------------|
| Nasolabial fold | 1461 | 2.0–2.5 |
| Marionette line | 433 | 1.0–1.4 |
| Nasojugal groove | 1413 | 0.5–0.8 |
| Supraorbital groove | 148 | 1.0–2.0 |
| Midcheek groove | 253 | 1.0–1.6 |
| Forehead | 304 | 2.0–4.0 |
| Temple | 49 | 2.0–3.0 |
| Glabella | 282 | 0.4–0.8 |
| Perioral lip wrinkle | 134 | 0.4–0.6 |
| Neck | 54 | 1.2–2.4 |
| Back of the hand | 27 | 4.0–8.0 |
| Other | 257 | Dependent on area |

RESULTS

Patient satisfaction was 97.3 percent and physician satisfaction was 98.4 percent (Fig. 1). A Global Aesthetic Improvement Scale score of 3 or higher was deemed to indicate that the treatment was effective. Patients were compared by the rate at which the therapy's cumulative effectiveness became evident, which was defined as the time from first treatment to the first sign of clinical effect. As mentioned above, the therapy's cumulative effectiveness became more evident over time (Fig. 2).

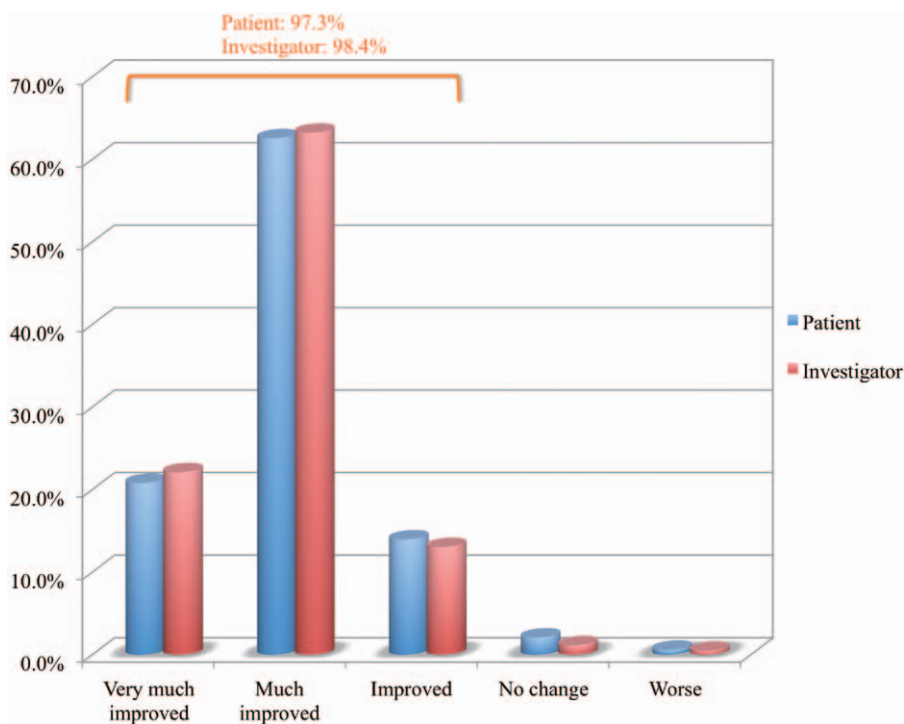


Fig. 1. Chart showing patient satisfaction (blue) and investigator satisfaction (red).

The Wrinkle Severity Rating Scale was used to evaluate wrinkles before treatment, and the period for the therapy's effectiveness to become evident was evaluated by Wrinkle Severity Rating Scale grade. The therapy's effectiveness became evident in an average of 60.4 days for wrinkles with a grade of 2, in an average of 64.8 days for wrinkles with a grade of 3, in an average of 66.6 days for wrinkles with a grade of 4, and in an average of 70.8 days for wrinkles with a grade of 5. There was no significant difference, but the lower the Wrinkle Severity Rating Scale grade, the earlier the patient satisfaction (Fig. 3).

Changes in the Wrinkle Severity Rating Scale grade before and after treatment were examined. The endpoint for treatment was determined to be when patient satisfaction was a Global Aesthetic Improvement Scale score of 3 or more within 6 months. Improvement for wrinkles with a Wrinkle Severity Rating Scale grade of 2 before treatment was 0.55, improvement for wrinkles with a Wrinkle Severity Rating Scale grade of 3 before treatment was 1.13, improvement for wrinkles with a Wrinkle Severity Rating Scale grade of 4 before treatment was 1.82, and improvement for wrinkles with a Wrinkle Severity Rating Scale grade of 5 before treatment was 2.23. These results revealed that the therapy provided acceptable improvement even

for wrinkles with a high Wrinkle Severity Rating Scale grade (Table 2).

Typical results are shown in Figures 4 through 8. This treatment appears to have an early expression period and a long-lasting effect.

A known material complication of treatment is overcorrection, and this tended to increase over time. Nonetheless, the overall incidence of overcorrection has decreased steadily over the observed years. By 2011, the incidence had decreased to approximately 1.5 percent (Fig. 9). This decrease was mainly attributable to greater ability to adjust the amount of bFGF, the platelet-rich plasma plus bFGF dose, and the injection site.

DISCUSSION

Various treatments are used to treat wrinkles or depressed areas of the skin that accompany aging.¹⁻³ The world's preferred treatment is hyaluronic acid. However, hyaluronic acid is only effective for 6 to 12 months; thus, periodic treatment is required for the treatment to remain effective.⁴

Fat injection is a standard surgical option for treating wrinkles or depressed areas of the skin.^{5,6} However, liposuction is required to collect fat for injection, which can cause internal

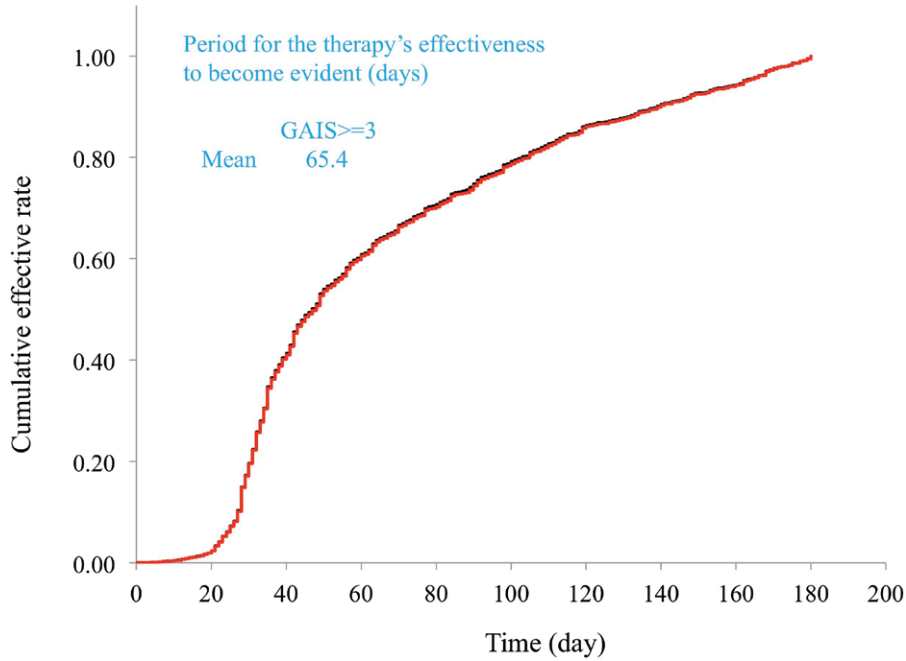


Fig. 2. Graph showing the time after treatment and changes in patient satisfaction. *GAIS*, Global Aesthetic Improvement Scale.

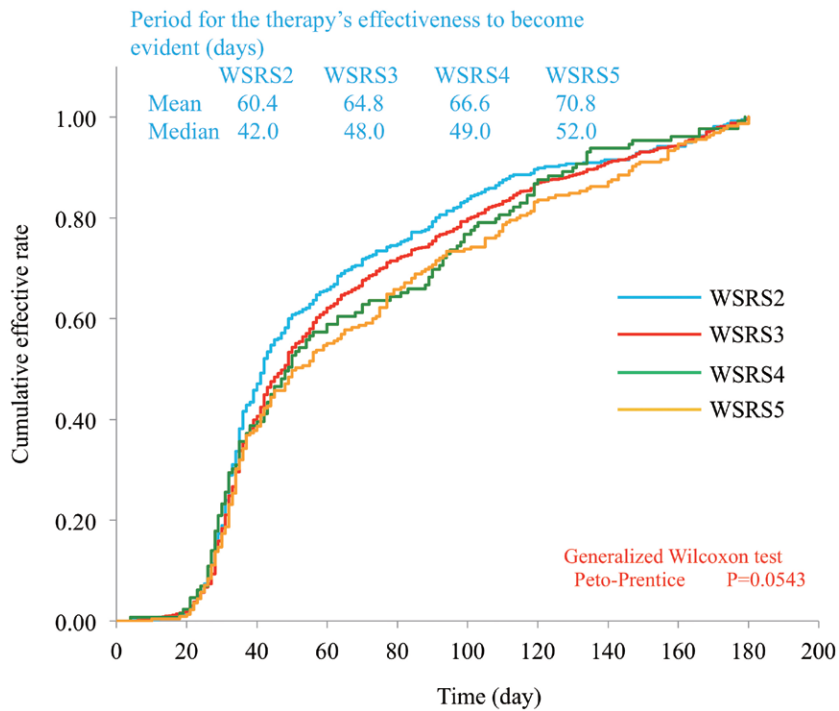


Fig. 3. Graph showing the time after treatment and satisfaction by grade on the Wrinkle Severity Rating Scale (*WRSR*) before treatment. A Wrinkle Severity Rating Scale grade of 2 is shown in *blue*, a grade of 3 is shown in *red*, a grade of 4 is shown in *green*, and a grade of 5 is shown in *yellow*.

Table 2. Improvement in Wrinkle Severity Rating Scale Grade after Platelet-Rich Plasma plus bFGF Treatment Compared with before Treatment

| WSRS Grade before Treatment | Change in WSRS Grade |
|-----------------------------|----------------------|
| 2 | 0.55 |
| 3 | 1.13 |
| 4 | 1.82 |
| 5 | 2.23 |

WSRS, Wrinkle Severity Rating Scale.

bleeding and swelling that lasts for up to 2 to 3 weeks. As other lasting fillers have complications, risk becomes an issue.¹⁹ Platelet-rich plasma plus bFGF can cause internal bleeding and swelling for a period of less than 1 week and the therapy is demonstrating a lasting effect. These qualities make platelet-rich plasma plus bFGF an extremely exceptional therapy.

Platelet-rich plasma is widely used to promote tissue repair, as it contains and releases various growth factors.⁷⁻¹⁰ Studies have shown that platelet-rich plasma improves the texture of the skin and helps skin to recover after being subjected to a fractional laser.²⁰⁻²² Nevertheless, there are limits to the effect that platelet-rich plasma alone can have on deep wrinkles, and platelet-rich plasma often is combined with fat grafting to treat skin defects or depressed areas of the skin.^{7,11}

The current study combined and injected bFGF and platelet-rich plasma to repair wrinkles and depressed areas of the skin. The current authors previously treated patients with platelet-rich plasma alone, but found that platelet-rich plasma alone was often ineffective. Furthermore, if platelet-rich plasma alone was effective, it failed to treat deep wrinkles or depressed areas of the skin. Fibroblast growth factor has an effect on the dermis and fat areas and increases the volume of collagen, hyaluronic acid, and fat. Kawazoe and Kim reported that platelet-rich plasma plus bFGF effectively stimulated tissue growth.¹⁵ Nevertheless, few clinical studies have reported use of platelet-rich plasma plus bFGF.

Platelet-rich plasma plus bFGF is not instantaneously effective, and its effectiveness becomes evident over time. Overall, patient treatment took an average of 65 days (median, 47 days) until patients were satisfied. The point of patient satisfaction differed depending on treatment area and the patient's age and sex, but approximately 98 percent of patients were satisfied after 6 months of treatment. Although

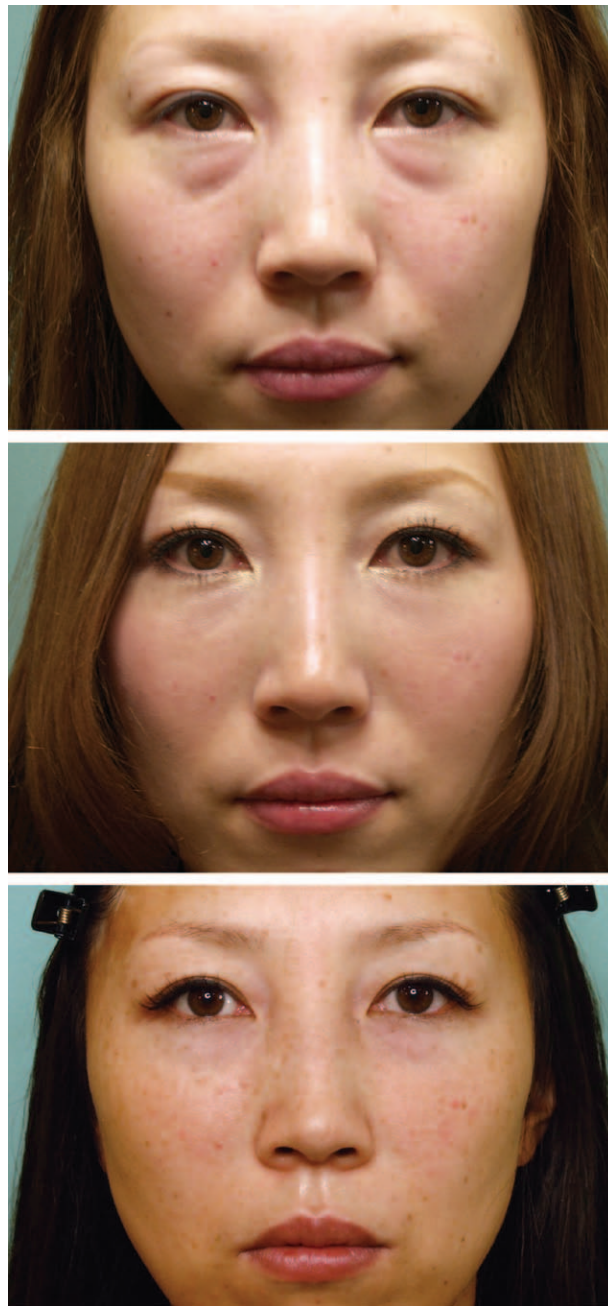


Fig. 4. Patient 1, a 39-year-old woman. Platelet-rich plasma plus bFGF was injected into the patient's nasojugal grooves and midcheek grooves (right, 1.3 ml; left, 1.5 ml). (Above) Before treatment. (Center) One month after treatment. (Below) Four years 7 months after treatment. The grooves were deep before treatment, but 1 month after treatment, the therapy's effectiveness was already evident. The patient's grooves were effectively filled. Moreover, the treatment remained effective 4 years 7 months later, and there was no combination treatment performed during this period.

there are individual differences in the point at which treatment is determined to be effective,



Fig. 5. Patient 2, a 51-year-old man. Platelet-rich plasma plus bFGF was injected into the patient's nasojugal grooves and midcheek grooves (right, 1.5 ml; left, 1.7 ml). Hyaluronic acid was injected simultaneously for rhinoplasty. (Left) Before treatment. (Right) One and one-half months after treatment. This patient underwent rhinoplasty using hyaluronic acid at the same time. Deep grooves were apparent before treatment, and 1.5 months after treatment the therapy was already effective.

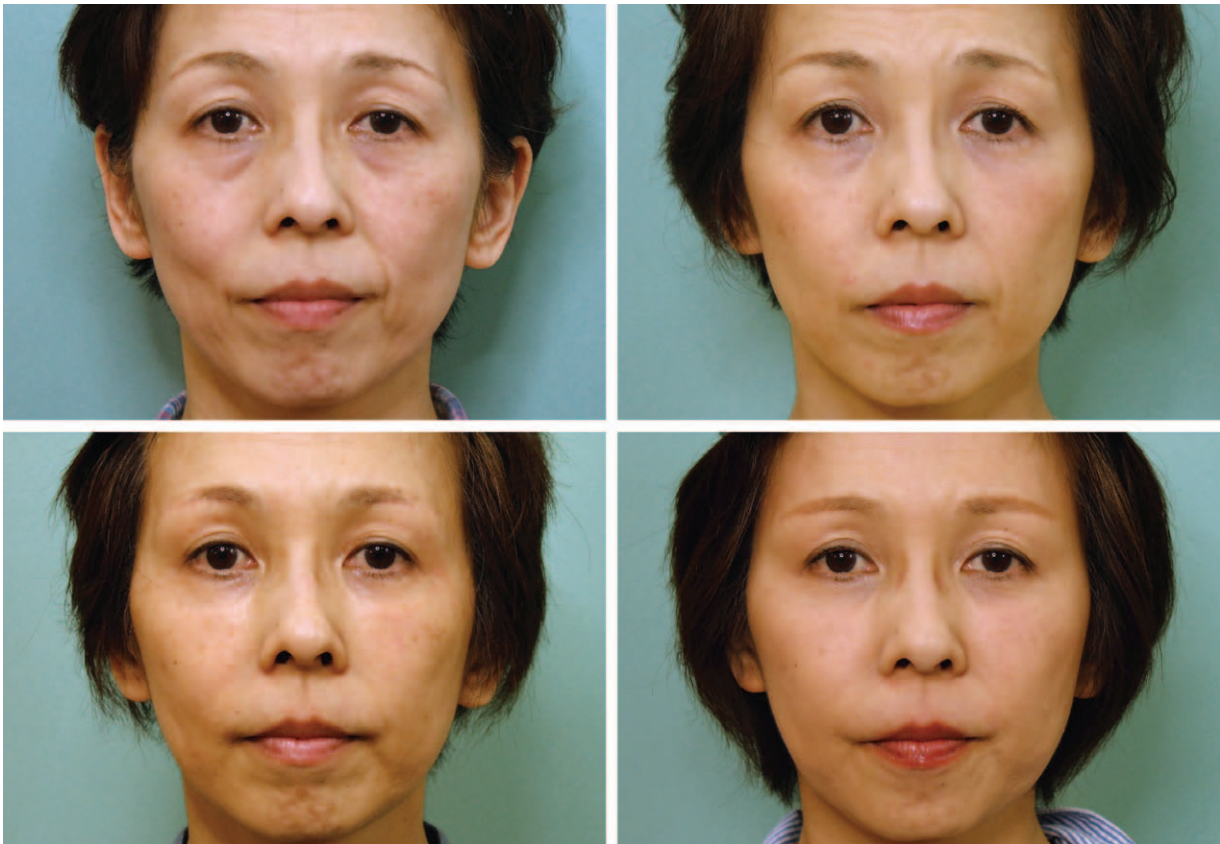


Fig. 6. Patient 3, a 50-year-old woman. Platelet-rich plasma plus bFGF was injected into the patient's upper eyelids (right, 0.3 ml; left, 0.3 ml), nasojugal and midcheek grooves (right, 0.6 ml; left, 0.7 ml), and nasolabial folds and marionette lines (right, 1.0 ml; left, 1.0 ml). (Above, left) Before treatment. (Above, right) Two and one-half months after the first treatment. The patient underwent subbrow blepharoplasty 1 year 2 months after initial treatment. (Below, left) One year 5 months after the first treatment and before the second treatment. This patient received platelet-rich plasma plus bFGF for the temple (right, 1.0 ml; left, 1.0 ml), forehead (1.5 ml), glabella (0.5 ml), and left nasolabial fold (0.3 ml) as a second treatment 1 year 5 months after the first treatment. Those grooves and depressed areas were obvious before the second treatment. (Below, right) Nine months after the second treatment.



Fig. 7. Patient 4, a 29-year-old woman. Platelet-rich plasma plus bFGF was injected into the nasojugal grooves (right, 0.6 ml; left, 0.6 ml). (Above) Before treatment. (Center) Fifteen days after the first treatment. (Below) Five years 7 months after the first treatment.

most patients were ultimately satisfied with treatment effectiveness.

This study verified the safety and efficacy of platelet-rich plasma plus bFGF therapy within a 6-month period. The therapy was markedly effective in most patients, displayed exceptional efficacy, and was deemed safe. The current authors have used this therapy for over 6 years. Based on clinical experience, platelet-rich plasma plus bFGF therapy remains effective for 3 years or longer. Further studies are needed to verify this duration of action and to assess the long-term safety of the therapy.

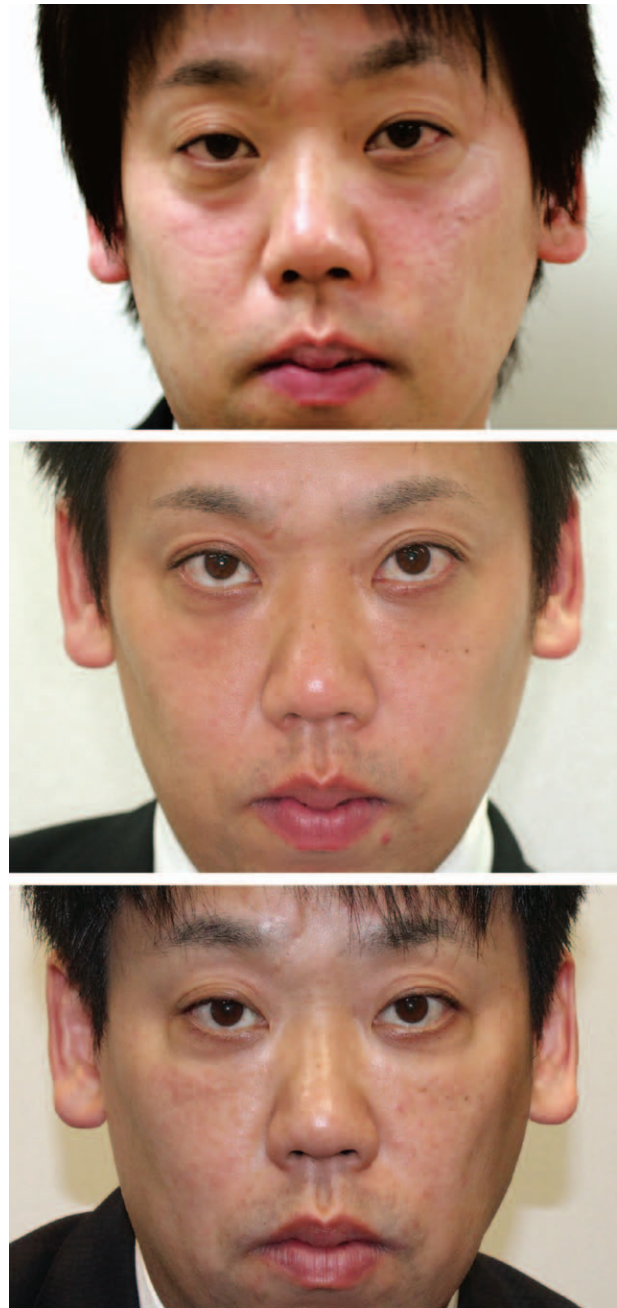


Fig. 8. Patient 5, a 35-year-old man. Platelet-rich plasma plus bFGF was injected into the nasojugal grooves (right, 0.5 ml; left, 0.5 ml). (Above) Before treatment. (Center) Four months after the first treatment. (Below) Seven years after the first treatment.

Injecting a mixture of platelet-rich plasma and fibroblast growth factor increases the volume of not only the skin but also adipose and the subcutaneous tissue. This treatment is therefore assumed to last a long period because of the large volume increase to different tissue planes. We plan to report medium- and long-term results in the next phase of the report.

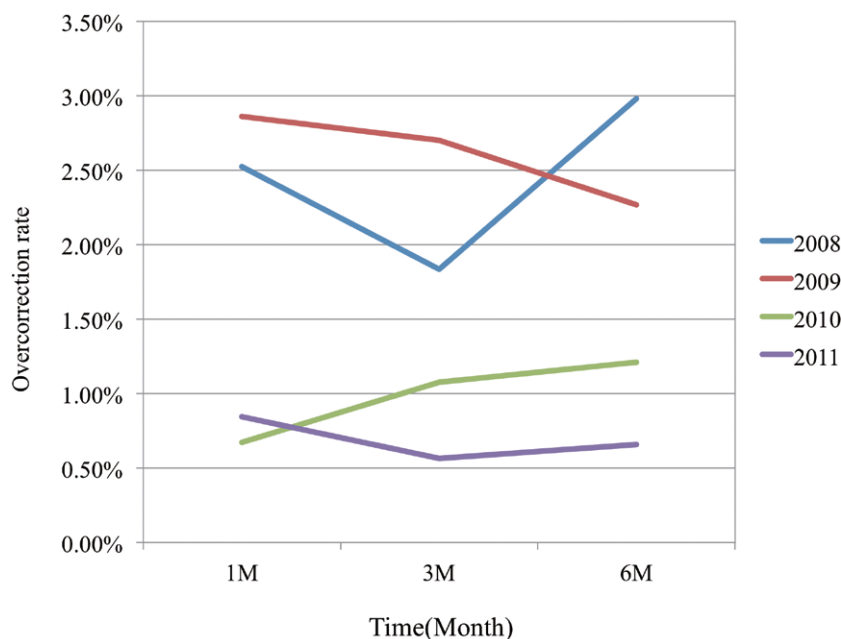


Fig. 9. Changes in the incidence of overcorrection by year.

CONCLUSIONS

A main complication of this treatment was overcorrection. The dose of platelet-rich plasma plus bFGF is crucial to prevent overcorrection. With hyaluronic acid use, the volume of injected hyaluronic acid is roughly equivalent to the volume of tissue growth. For platelet-rich plasma plus bFGF therapy, predicting the amount of tissue growth in response to the amount of platelet-rich plasma plus bFGF is of utmost importance. The injection amount must be determined with some level of overreaction in mind, as responses will have individual differences. Physician experience is an important factor in making the determination of the correct injection amount. The amount of bFGF in platelet-rich plasma plus bFGF is the key step in preventing overcorrection, as adding more bFGF will stimulate more tissue growth, and may also therefore cause an overcorrection. As such, using a smaller amount of bFGF reduces the probability of overcorrection.

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PATIENT CONSENT

Patients provided written consent for the use of their images.

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