

New Frontiers in Skin Rejuvenation, Including Stem Cells and Autologous Therapies



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KEYWORDS

- Stem cells • Rejuvenation • Aesthetic • Cosmetic • Fat transfer • Platelet therapy • Adipose
- Thread lift

KEY POINTS

- Minimally invasive cosmetic procedures are increasing in demand and popularity with a recent trend toward a more natural look.
- Autologous therapies, such as adipose-derived stem cells, stromal vascular fraction, microfat, nanofat, and platelet therapies, have been shown to effectively rejuvenate the skin.
- Innovations in botulinum toxin, fillers, and thread lifts parallel the increasing trends in autologous therapy use in aesthetic medicine.
- A combination approach using both autologous and traditional aesthetic therapies can provide optimal aesthetic outcomes.



Video content accompanies this article at <http://www.facialplastic.theclinics.com>.

INTRODUCTION

Minimally invasive cosmetic procedures continue to dominate the aesthetic arena. There are a large number of younger patients requesting cosmetic procedures with a focus on maintaining a youthful, natural look.¹ For this reason, so-called prejuvenation has become a popular aesthetic goal for many.

There is nothing more natural than a person's own tissues. Autologous therapies are increasingly being implemented for skin rejuvenation purposes in individuals of all ages. Using an individual's own fat, yielding nanofat, adipose-derived stem cells (ASCs), and stromal vascular fraction, as well as

platelets and fibrin from the person's blood, aging can be delayed or "reversed" with relative safety and efficacy. Much of the research on autologous therapy is in its infancy, but this revolutionary technology holds great promise.

Noninvasive cosmetic procedures, in general, continue to dominate aesthetics. New developments in technologies of botulinum toxin, fillers, and threads provide patients with multiple options. A combination approach of all available interventions can be used in clinical practice to provide patients with optimal, tailored skin rejuvenation.

Disclosure: K. Karimi is the medical director of CosmoFrance, Inc, which manufactures and distributes platelet-rich fibrin centrifuges and tubes as well as polydioxanone (PDO) threads. A. Pourang has served as a faculty member for LearnSkin.com. H. Rockwell has no relevant financial disclosures.

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Regenerative Medicine

The therapeutic potential for autologous therapy is an area of medicine that continues to be explored in many fields, from orthopedics to dermatology and plastic surgery.² An individual's tissues and cells are processed outside of the body and reintroduced back into the donor, with minimal risk of hypersensitivity reactions. Autologous therapies vary and involve different cell types and growth factors that help regenerate tissues.

Stem Cells

The regenerative potential of stem cells has expanded beyond the treatment of chronic degenerative diseases and into aesthetic medicine. Compared with embryonic stem cells or induced pluripotent stem cells, mesenchymal stem cells (MSCs) are preferred for use in clinical practice given the high availability, ability to differentiate into many cell types, and relative lack of ethical concerns.²

ASCs are the multipotent MSC population found in the stromal vascular fraction (SVF) of fat tissue, with the ability to differentiate into mesoderm, ectoderm, and endoderm lineages.^{3–5} ASCs also show regenerative and wound healing properties.^{6,7} They can be obtained from adipose tissue in large quantities using a standard wet liposuction procedure under local anesthesia, with minimal discomfort and morbidity of the patient, and without the need for expansion in culture, in contrast with bone marrow MSCs (BM-MSCs).^{8,9} Other sources of stem cells include amniotic fluid stem cells, umbilical cord blood stem cells, and Wharton jelly, which have greater proliferative and differentiation potential compared with ASCs and BM-MSCs, but are limited in cell availability after *in vitro* expansion.^{10–12}

Different methods of ASC isolation have been described in the literature.^{2,13–15} Lipoaspirate is harvested by using tumescent abdominal liposuction techniques or surgical resection.¹⁶ ASCs are usually isolated by collagenase digestion of isolated white adipose tissue, followed by centrifugation to separate the SVF-containing ASCs in the pellet fraction from floating adipocytes and blood.^{8,17–19} SVF is the heterogeneous mixture of cells obtained by enzymatic separation of adipocytes, and contains fibroblasts, endothelial cells, monocytes, macrophages, granulocytes, and lymphocytes.^{20,21}

ASCs can ultimately be isolated from SVF after separation from adipocytes and can be cultured to form fibroblast-like colonies.²⁰ ASCs used alone after expansion *in vitro* or with SVF are the most common MSCs used in aesthetic dermatology

and plastic surgery practice.² Because pure ASCs alone require *in vitro* expansion, which can be time consuming and labor intensive, SVF is often used because it already contains ASCs.

Mechanisms of action and clinical applications

The regenerative potential of SVF and ASCs is thought to be caused by various mechanisms. The regenerative ability of the skin is maintained by the stem cells that are present in the hair follicle, interfollicular epidermis, and sebaceous glands, as well as being influenced by mesenchymal-epithelial crosstalk through secreted stimulatory factors.^{22–25} Intradermal adipocyte lineage cells have been found to be necessary in driving hair follicle stem cell activation and likely play a role in other epithelial stem cell functions.^{24,26} ASCs are also thought to stimulate the recruitment of endogenous stem cells and promote their differentiation to cells that are needed, such as at a site of tissue injury.¹³ In addition, stem cells, in general, have antioxidant capabilities that likely mitigate inflammation and wound healing.²⁷ Some investigators suggest that the preadipocytes and macrophages in SVF confer regenerative properties through enhanced immune response or removal of dying cells, leading to tissue remodeling.^{28,29} Both SVF and ASCs are thought to have properties that increase vascularization, the secretion of growth factors, vascular endothelial growth factor (VEGF), hepatocyte growth factor, and insulinlike growth factor.^{30–34} Such properties are also likely responsible for enhancing fat graft survival.

Cultured ASCs have been shown to improve scar outcomes of full-thickness skin defects.³⁵ Cultured ASCs have also been found to reduce wrinkles through collagen and elastic fiber production and other antiaging effects in the skin through glycation suppression, antioxidation, and trophic effects.^{36,37} SVF, which contains ASCs, has also been used to treat necrosis resulting from facial filler injections.^{38,39}

Scientific evidence and regulatory issues

It is important to keep in mind that stem cell technology is still in its infancy, with US Food and Drug Administration (FDA)-approved trials in early phases.^{2,24,40} Potential side effects of stem cells such as rejection, hyperimmune response, neoplasm, cross contamination with other stem cell lines, and uncontrolled differentiation have been proposed.^{41–44} Human ASCs that have been cultured *in vitro* for long periods of time have been found to produce tumors in immunodeficient mice.⁴⁵ There is also a question as to whether or not the donor's age affects the regenerative potential of ASCs.⁴⁶ The lack of safety

and potential side effects data are limited and further randomized clinical trials are necessary.² Furthermore, there is no single standard protocol for obtaining ASCs, which can lead to regulatory and quality issues.^{6,47}

Some clinicians are even calling on the FDA to expedite the oversight of companies and clinics offering stem cell-based treatments.⁴⁸ Procedures are being offered in some spallike settings and are at risk for contamination and infection. Professional groups are requesting that stem cell products be regulated like drugs, that the scope of practice for such procedures be regulated, and that these procedures be performed in state or national facilities accredited by surgical associations.

Fat Transfer

Fat transfer procedures (**Table 1**) are becoming increasingly popular given it is a relatively safe autologous therapy, helping reverse volume loss with the added benefit of removing unwanted fat. Lipofilling procedures are often used to correct dark circles and hollows around the eyes, volumize the midface, and augment the chin^{20,49–51} (**Fig. 1**). Adipose tissue is not only an ideal filler because of its ability to integrate into a donor's tissues with minimal risk of immunogenicity but also contains several cell types with regenerative potential, as discussed earlier, which can rejuvenate the skin of the face.^{52–54} The ASCs in the fat have been shown to promote new collagen deposition, local hypervascularity, and dermal hyperplasia.^{55,56}

Microfat, superficial enhanced fluid fat injection, and nanofat

Successful fat grafting depends on several factors, including proper procedural technique, the possibility of needing multiple treatments, and optimization of the recipient site's capacity to support the graft.⁵⁹ Disadvantages of traditional fat grafting, which uses large blunt cannulas, include the risks of irregular fat accumulation, visible lumpiness, fat necrosis, and poor fat graft survival.^{20,60}

Mechanical and enzymatic disruption of fat has been shown to improve the viability of adipocytes and graft retention.^{20,61–63} Microfat is generated by using a smaller multiport cannula, as small as 0.7 mm in diameter, and is then injected intradermally to treat fine wrinkles.^{64–66} Superficial enhanced fluid fat injection (SEFFI) is a procedure that was developed to overcome manual centrifugation's effects on adipocyte viability.⁵⁷ Micro side-port cannulae are used to harvest microfat rich in stem cells and viable adipocytes, which is then enhanced with autologous platelet-rich plasma (PRP) and injected

superficially with syringe needles. This treatment has been shown to result in lump-free skin rejuvenation and volume enhancement.⁵⁸ Micro-SEFFI (M-SEFFI) is a refined version of the SEFFI procedure, obtaining smoother fat, harvested with a multiperforated cannula with extremely small ports (0.3 mm).²¹

Nanofat is generated by further processing of fat via mechanical emulsification. Tonnard and colleagues²⁰ describe their procedure for nanofat production in which standard high-negative-pressure liposuction is used to harvest fat using a multiport 3-mm cannula with sharp side holes of 1 mm in diameter. After saline rinsing and filtering, adipose tissue is then emulsified by shifting the fat 30 times between two 10-mL syringes connected by a female-to-female Luer-Lok connector to create an emulsion, which is filtered over a sterile nylon cloth. The remaining effluent without connective tissue is called nanofat, yielding 1 mL of nanofat per 10 mL of liposyringe (Video 1). Nanofat is layered fanwise intradermally using a 27-gauge needle in delicate areas such as superficial rhytids and eyelids, with a delayed effect usually appearing within 4 weeks to 3 months. Nanofat does not contain viable adipocytes, limiting its ability to volumize tissue, but retains high levels of ASCs, which can be used for skin rejuvenation purposes. It is thought that increased collagen and elastin formation and skin remodeling occurs because of the ASCs.²⁰

Nanofat injection may be a less expensive, less time-consuming way of introducing beneficial stem cells to surrounding tissues, because SVF would need to be further isolated from the nanofat's dead adipocyte fraction.²⁰ The fragmented adipocyte portion may even be beneficial because it can induce cytokine release and growth factors, which can help regenerate tissue.^{20,67}

Combination therapies

ASCs from processed adipose tissue are often combined with macrofat grafts to improve outcomes. Although nanofat alone is typically used for skin rejuvenation, it is often combined with macrofat grafts. Gu and colleagues⁶⁸ used condensed nanofat, removing oil that was thought to be too bulky to inject into scars, combined with fat grafts to effectively treat atrophic facial scars.

Cell-assisted lipotransfer (CAL), a technique in which fat grafts are enriched with SVF, has been shown to improve fat transfer in facial lipoatrophy compared with conventional lipoinjection, along with a decreased risk of fibrosis, pseudocyst formation, and calcification as seen with traditional lipoinjection.^{69,70}

Table 1
Types of autologous fat therapy

Cell Type	Mechanism of Extraction	Pros	Cons	Contain Viable Adipocytes?	Contains ASCs?
Macrofat	Standard liposuction using large multiport cannula	Good filler	Granuloma formation Lumps Fat necrosis Calcification Poor graft retention possible May need multiple treatments	Yes	Yes
Microfat	Liposuction using small multiport cannula	Good filler Rejuvenates skin	Same as macrofat	Yes	Yes
SEFFI ⁵⁷	Liposuction using small multiport cannula (0.8 and 0.5 mm) + PRP	Finer than microfat Good filler Rejuvenates skin Does not require further tissue manipulation Includes PRP growth factors	Oil cyst formation reported in some patients Requires additional procedure (PRP)	Yes	Yes
M-SEFFI ⁵⁸	Liposuction using small multiport cannula (0.3 mm) + PRP	Finer than SEFFI Good filler Rejuvenates skin Does not require further tissue manipulation Includes PRP growth factors	Requires additional procedure (PRP)	Yes	Yes
Nanofat ²⁰	Liposuction using multiport 3-mm cannula with sharp side holes of 1 mm in diameter with subsequent emulsification of microfat, filtered to remove connective tissue	Rejuvenates skin	Suboptimal filler Requires additional processing after liposuction	No	Yes
SVF ²	Lipoaspirate undergoes processing using enzymatic or mechanical separation and is then washed, treated with collagenase, centrifuged, and red blood cells are removed	Inexpensive Applied during 1 surgical procedure	Not purely ASCs (heterogeneous cell fraction), which is still beneficial Requires additional processing after liposuction	No	Yes
ASC (alone, after expansion in vitro) ²	Culture from SVF	Obtain homogeneous cell fraction with fully defined phenotype	Cost and time intensive Potential tumorigenic ability Requires additional processing and culturing after liposuction	No	Yes

Abbreviations: PRP, platelet-rich plasma; M-SEFFI, micro-superficial enhanced fluid fat injection; SEFFI, superficial enhanced fluid fat injection.

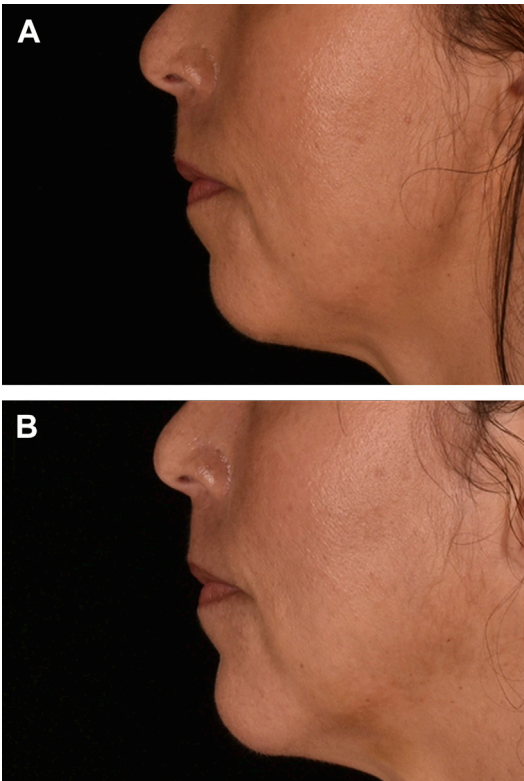


Fig. 1. (A) Before and (B) after chin augmentation with autologous microfat graft purified by PureGraft (Bimini Technologies, Solana Beach, CA) mixed with autologous PRF obtained using ezPRF System.

Autologous Platelet Therapies

Despite origins in oral maxillofacial surgery,^{71,72} autologous platelet therapies have been found to be useful in several other clinical fields, including aesthetics. This usefulness is largely caused by the existence of parallels between the body's innate mechanisms of wound healing, in which platelets play a major contributing role, and the pathways necessary for rejuvenation. First, there was platelet-rich plasma (PRP)^{71,72} but, with the turn of the century, came the emergence of platelet-rich fibrin (PRF).⁷³

Mechanism of action

As cellular constituents of whole blood, platelets are among the first responders to sites of tissue and vascular injury. In such events, platelet aggregation and activation result in the release of several critical growth factors from platelet alpha granules, including platelet-derived growth factor, fibroblastic growth factor, epithelial growth factor, insulinlike growth factor,

transforming growth factor, and VEGF,^{74–77} which are further described in **Table 2**.

The chemotactic properties of these growth factors serve, in part, to recruit MSCs, which then differentiate at the site of injury.^{77–79} Furthermore, during this process of response to injury, the enzyme thrombin converts the soluble blood protein fibrinogen into insoluble fibrin. Fibrin then acts as a binding scaffold for erythrocytes and platelets to stabilize clot formation, establishing a fibrin matrix for subsequent remodeling, and sustaining growth factors.^{77,80–83} It is these growth

Table 2
Growth factors found in platelet therapies and their functions

Growth Factor	Functions
PDGF	<ul style="list-style-type: none"> • Stimulates fibroblasts and leukocytes such as neutrophils and macrophages • Chemotactically recruits MSCs, endothelial cells, and fibroblasts, and stimulates their replication • Important for blood vessel maturation • Collagen production
FGF	<ul style="list-style-type: none"> • Mitogenic for fibroblasts and endothelial cells • Facilitates repair of soft tissues • Angiogenic effects lay groundwork for the synthesis, deposition, organization, and ultimate formation of extracellular matrix • Hyaluronic acid production • Collagenesis
EGF	<ul style="list-style-type: none"> • Regulates proliferation, growth, and the migration of epithelial cells • Angiogenic
IGF	<ul style="list-style-type: none"> • Promotes cell growth
TGFβ	<ul style="list-style-type: none"> • Mitogenic and morphogenic functions • Promotes wound healing • The TGFβ3 isoform inhibits haphazard scarring and promotes cellular differentiation and replication • Stimulates collagenesis
VEGF	<ul style="list-style-type: none"> • Angiogenic • Facilitates extracellular matrix synthesis and deposition

Abbreviations: EGF, epithelial growth factor; FGF, fibroblastic growth factor; IGF, insulinlike growth factor; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor beta.

factors that ultimately orchestrate the healing, regenerative, and rejuvenating properties of platelets. Thus, by using blood concentrates such as PRP and PRF, clinicians can selectively implement the clinically relevant effects of platelet growth factors.

Platelet-rich plasma and platelet-rich fibrin

PRP and PRF are acquired by centrifuging whole blood for product-specific durations of time and either with or without additives (Fig. 2). PRP and PRF primarily differ in their respective preparation, rate of growth factor release, and mode of activation for clot formation. These basic differences and similarities and several others are summarized in Table 3.

Platelet-rich plasma versus platelet-rich fibrin

The preferred use of PRF compared with PRP is well justified. First, without the need for additives, as is the case with PRP, preparation and use of PRF confers reduced costs for patients and providers as well as a more standardized protocol that is less susceptible to human error. Furthermore, PRF is a completely autologous product, whereas PRP, as a result of its preparation, is not; thus, PRP presents the risk of inducing an adverse immune response.⁸⁸

With continued consideration of their differences in preparation, the high-speed versus low-speed centrifugation parameters of PRP and PRF preparation, respectively, diversify their composition. As a result of low-speed

centrifugation, a greater proportion of the beneficial cellular content of blood is preserved within the resulting PRF layer,^{84,91} whereas the high-speed centrifugation of PRP pushes many cells toward the hematocrit,^{86,91} which is ultimately unused and discarded.

PRF further surpasses PRP with regard to growth factor release. The rapid activation and release of growth factors in PRP has been noted to yield short-term benefits without long-term advances in wound healing.⁹⁰ Contrastingly, the natural, physiologic activation of PRF and its prolonged growth factor release sustains healing and regenerative signals for a longer period of time.⁹⁰ In addition, in 1 particular study, PRF was found to yield higher overall concentrations of growth factors than PRP.⁹²

Although the growth factor signaling of both PRP and PRF attracts MSC migration to sites of implementation and injury, PRF has been shown to contain multipotent stem cell markers and carry cells that bear phenotypic features that are characteristic of MSCs.⁹³ As a result, PRF serves not only to attract and sustain MSCs with its autologous and naturally forming fibrin matrix but may also serve as a reservoir of these regenerative and rejuvenating cells.

Collectively, these findings, as well as other comparative studies and reviews documented in the literature, support PRF's superiority to PRP for use as a wound healing and regenerative aid.^{86,94-96}

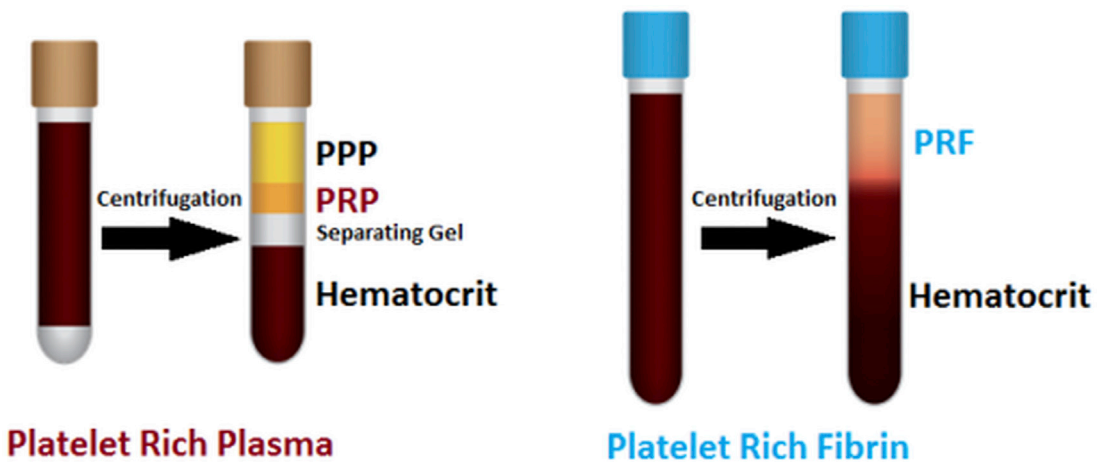


Fig. 2. The generation of PRP from whole blood (*left*) and PRF from whole blood (*right*) from centrifugation. The supernatant contains the desired product, whereas the hematocrit, consisting of concentrated red blood cells, constitutes the inferior subsection of the solution, and is discarded. PPP, platelet-poor plasma. (Courtesy of CosmoFrance Inc., Miami, Florida and modified from Karimi K, Rockwell H. The Benefits of Platelet Rich Fibrin (PRF). Facial Plastic Surgery Clinics. 2019;27(3); with permission.)

Table 3
Comparison between platelet-rich plasma and platelet-rich fibrin

	PRP	PRF
Preparation: Centrifugation Speed ^{84,85}	Generally higher speeds	Generally lower speeds
Preparation: Additives ^{86,87}	Whole blood is spun with anticoagulant and separating gel	Whole blood is spun without any additives
Activation and Coagulation (Fibrin Polymerization/Clot Formation) ^{86–89}	Induced by the addition of thrombin (often bovine derived) and calcium chloride or entrusted to be stimulated by endogenous coagulation factors after application/injection	Spontaneous; natural cascade of coagulation and fibrin clot formation based on intrinsic processes
Growth Factor Release Rate ^{85,90}	Rapid release of growth factors on implementation (~24 h)	Prolonged duration of growth factor release (~7–10 d)
Leukocyte Content ^{86,87}	0%–50% of cells in supernatant product; lower retention	~65% of cells in supernatant product; higher retention
MSC Recruitment ^{77,87}	Chemotactically attracts MSC migration	Chemotactically attracts MSC migration
Lifespan of Fibrin Matrix Clot After Application ⁸⁶	Dissolves quickly after application	Processed slowly; gradually, physiologically remodeled

APPLICATIONS OF PLATELET-RICH PLASMA AND PLATELET-RICH FIBRIN

PRP and PRF boast an array of surgical and nonsurgical aesthetic applications, including improved retention of fat grafts, hair restoration, optimizing cartilage grafts in rhinoplasty, improving scar appearance, collagen induction therapy, ablative laser resurfacing, and volumization both independently and in conjunction with hyaluronic acid filler.^{97,98}

Implications as an Injectable

As people age, the collagen, elastin, subcutaneous fat, and hyaluronic acid content of the facial skin declines,⁹⁹ which manifests as facial hollowness, drooping skin, and the formation of rhytids. It has been suggested that injection of growth factors stimulates dermal collagen synthesis.⁷⁵ PRP's target site-specific growth factor release has been shown to promote fibroblast proliferation and stimulate both type I collagenesis¹⁰⁰ and hyaluronic acid production.⁹⁸ Although PRP is known to enhance the duration of the effects from hyaluronic acid filler treatments,⁹⁸ the prolonged release of growth factors⁸⁵ and physiologic rate of remodeling of the PRF fibrin matrix⁸⁶ is hypothesized to further improve the duration of hyaluronic acid filler

treatments. Author K.K. has observed this effect in his own practice with patients who have received both treatment with filler alone as well as filler combined with PRF for injection. Injecting PRF provides an immediate volumization effect that diminishes over the following few weeks; however, anecdotal evidence from repeat treatments of PRF by author K.K. has suggested long-term improvements in facial volume, skin texture, and skin pigmentation when used as a lone autologous dermal filler (**Fig. 3**).⁹⁷

Microneedling

Microneedling, or collagen induction therapy, is a popular, minimal-downtime procedure known to improve skin texture, the presence of fine lines and scars, and enhance skin appearance without risks of hyperpigmentation.¹⁰¹ This superficial, controlled puncturing of the skin by very fine needles stimulates the wound healing response, ultimately leading to the release of growth factors by platelets and neocollagenesis. Topical application of growth factors has been shown to improve skin texture and appearance¹⁰²; thus, applying either PRP or PRF topically during and immediately after microneedling treatment can improve results by saturating the newly created fine, porous wounds with concentrated growth factors

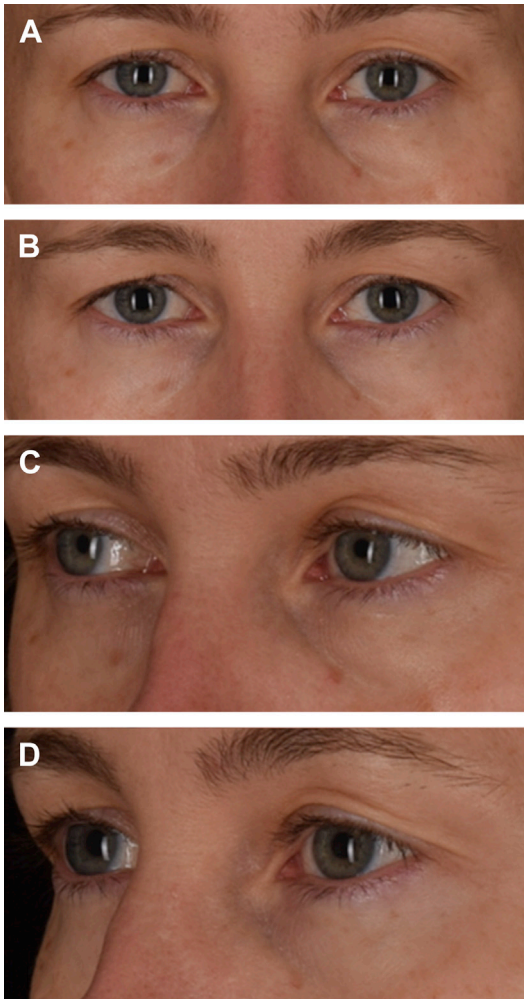


Fig. 3. (A and C) Before and (B and D) after 3 treatments of PRF alone injected to the infraorbital hollows of this 45-year-old female patient to correct pigmentation irregularities and provide subtle improvement in volumization.

(Video 2).⁹⁸ In 2 split-face studies comparing the effects of microneedling with topically applied vitamin C versus PRP, and distilled water versus PRP, respectively, the PRP-treated side showed better improvement in the presence of scars¹⁰³ and yielded improved overall skin texture,¹⁰⁴ more so than the respective non-PRP-treated side.

Lasers

Laser resurfacing serves to retexturize the skin by stimulating the body's wound healing and tissue regeneration response. Topical application of PRP or PRF following such treatments may serve to both reduce healing time and enhance results by further supplementing the tissue injury

response with concentrated platelets and their multifunctional growth factors (**Fig. 4**). In split-face studies comparing the efficacy of PRP versus saline after fractional skin resurfacing, the PRP-treated side visually healed more rapidly than the saline-treated side.¹⁰⁵ Skin biopsies revealed improved collagen bundle thickness on the PRP-treated side compared with the saline-treated side.¹⁰⁶ Although the complementary effects of PRF with ablative lasers have yet to undergo extensive analysis, anecdotal evidence in author K.K.'s practice suggests that PRF may yield similar, if not superior, benefits after laser resurfacing because of its prolonged growth factor release.

FUTURE DIRECTIONS

Further research on PRP and PRF is warranted to better elucidate their functional roles in medical cosmetic rejuvenation. Although PRP has a more extensive history of applied use, research on the functionality and sustainability of growth factors and other regenerative cells in purely autologous PRF justifies its continued use. Comparative studies including both treatments may provide

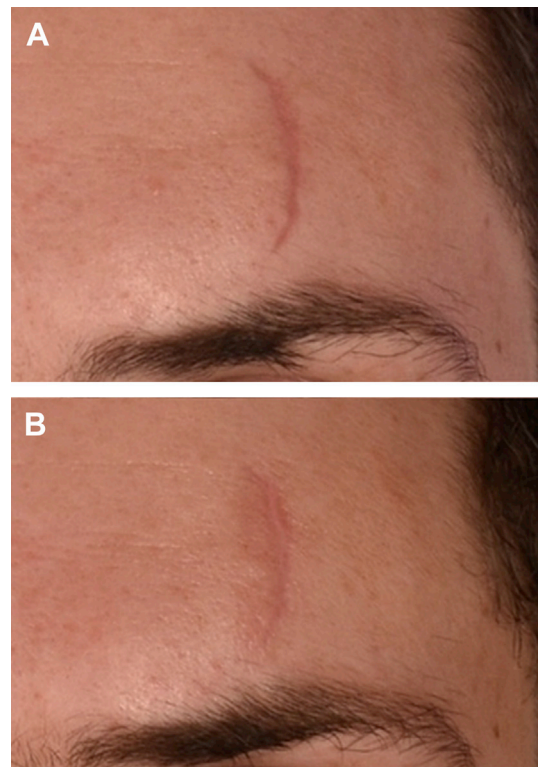


Fig. 4. (A) Before and (B) after 2 treatments of a fractionated laser combined with topical PRP to improve the presence of this 25-year-old male patient's forehead scar.

additional insight into the preferential implications of each.

Platelet Therapies in Combination with Adipose-derived Stem Cells

PRF has been shown to enhance the proliferation of nanofat-derived stem cells *in vitro* in a dose-dependent manner.¹⁰⁷ ASCs cultured in PRP have also been shown to have stimulatory effects on the proliferation and migration of dermal fibroblasts and keratinocytes.¹⁰⁸ This finding suggests that ASCs support reepithelialization via paracrine pathways and help maintain epidermal homeostasis.²⁴

These synergistic relationships found *in vitro* have been shown clinically. PRP has been shown to improve fat graft outcomes as a result of enhanced survival of fat cells and ASC differentiation triggered by the growth factors present in PRP.¹⁰⁹ One study showed that patients who received nanofat, PRF, and autologous fat transplant showed improvement in soft tissue depression and skin texture along with high patient satisfaction compared with traditional autologous fat transplant.¹¹⁰ Another study compared nanofat and intradermal PRF injection with hyaluronic acid injection. Facial skin texture was improved to a greater extent and there was a higher satisfaction rate in the nanofat-PRF group.¹⁰⁷ Gentile and colleagues³⁰ found that both fat grafts enriched with SVF and fat grafts enriched with PRP were effective at improving facial scars and maintaining contour restoration and lower fat resorption after 1 year, compared with controls who received centrifuged fat injections without SVF or PRP.

Other Autologous Cell Types

Other autologous therapies, such as fibroblasts, keratinocytes, dermal papillae, and melanocytes, have also been developed for various cosmetic and medical purposes.¹¹¹ Autologous fibroblasts, in particular, are used for aesthetic purposes. Dermal fibroblasts are mesenchymal cells that synthesize collagen and glycosaminoglycans and are involved in cutaneous wound healing and skin repair.¹¹²

Autologous cultured fibroblasts are injected into the patient's dermis with resulting long-lasting filling effect up to 48 months, thought to be caused by continuous protein repair.^{113–115} Laviv (Fibrocell Technologies, Inc, Exton, PA) has been approved for use in nasolabial folds; however, 3 treatment sessions every 3 to 6 weeks are required and the fibroblasts are sourced through a postauricular biopsy.^{116,117} However, 1 study that used Laviv in the nasojugal groove

showed improvement after 1 session compared with placebo without any serious adverse events.¹¹⁸

OTHER NOVEL SKIN REJUVENATION THERAPIES

Although autologous therapies have great potential for skin rejuvenation, traditional minimally invasive cosmetic procedures continue to dominate the aesthetic industry.^{1,119} New developments in botulinum toxin, fillers, threads, and combination therapies are discussed next.

Neurotoxins

Botulinum toxin type A (BoNTA) injections using products such as Botox (onabotulinumtoxinA), Dysport (abobotulinumtoxinA), and Xeomin (incobotulinumtoxinA) continue to be the most popular noninvasive cosmetic procedures and their popularity continues to increase.^{1,119}

New neurotoxin products and techniques are entering the sphere of facial rejuvenation. Jeuveau (prabotulinumtoxinA-xvfs) (Evolus, Inc, Irvine, CA), a low-cost alternative to other neurotoxins, has been approved by the FDA for treatment of glabellar lines.^{120,121} DaxibotulinumtoxinA, a neurotoxin developed by Revance Therapeutics (Newark, CA), is a long-acting product formulated with a proprietary peptide that was found to be safe and effective for moderate or severe glabellar lines in phase 2 and 3 studies.^{122,123}

Originally approved for correcting wrinkles of the glabella and the periorbital region, the extensive off-label use of BoNTA in different areas of the face is now expanding to different methods of delivery.^{124,125} The microbotox method was developed to provide a more natural look for patients, with effects lasting up to 6 months.¹²⁶ Highly diluted onabotulinumtoxinA is injected in multiple small blebs at 0.8-cm to 1.0-cm intervals into the dermis or the interface between the dermis and the superficial surface of the muscles of the face and neck. This more superficial approach is thought to prevent a frozen appearance while also improving skin texture because of atrophy of sebaceous and sweat glands.

A recent study compared the intramuscular injection versus intradermal microdroplets injection versus nanomicroneedle delivery of BoNTA for the treatment of crow's feet.¹²⁷ For dynamic wrinkles, intramuscular injection and intradermal microdroplet injections were more effective than nanomicroneedles. For static wrinkles, nanomicroneedles and intradermal microdroplets injection were more effective. Skin elasticity, collagen content, and hydration of nanomicroneedle group

and intradermal microdroplet group increased more significantly than those of the intramuscular injection group and were highest at 12 weeks in the intradermal microdroplet group.

There are some limited data supporting the use of BoNTA for hypertrophic scars and keloids, but further studies are required to evaluate BoNTA in wound healing and scarring.^{128,129}

Fillers

The growing popularity of injectable fillers follows behind the popularity of neurotoxins. Most fillers used on the market now are absorbable fillers made up of either hyaluronic acid, polylactic acid, hydroxylapatite calcium microspheres, or collagen. Ellansé (Sinclair Pharmaceuticals, London, United Kingdom), composed of polycaprolactone microspheres in an aqueous carboxymethylcellulose gel carrier, is a collagen biostimulator with results lasting up to 4 years in certain product lines.¹³⁰ Bellafill (Suneva Medical, Inc, Santa Barbara, CA), consists of 80% bovine collagen gel and 20% polymethylmethacrylate (PMMA) microspheres, forming a matrix that supports the production of endogenous collagen over time.¹³¹ Silk Medical Aesthetics Inc has developed a biocompatible liquid silk filler made from pure silk from the thread of silkworm cocoons that will be undergoing clinical trials soon.¹³²

Diluted calcium hydroxylapatite (CaHA) has also recently been used for skin tightening purposes in individuals who have age-associated upper arm skin changes.¹³³ CaHA's microspheres have been shown to stimulate fibroblast proliferation neocollagenesis, neolastogenesis, and angiogenesis.^{133–135} Diluted CaHA is also used in the neck and décolletage to stimulate neocollagenesis by a procedure using multiple retrograde linear threading passes of diluted CaHA in the subdermal plane followed by massage with a gel or cream.¹³³

An advanced injection technique called myomodulation has also recently been introduced in the scientific literature.¹³⁶ The clinician addresses muscle movement with injectable fillers in the treatment of facial structural deficiencies by supporting muscle movement or blocking overaction. These effects can be augmented with neurotoxins, highlighting the importance of individualized, combination therapies when rejuvenating the skin.

Thread Lifts

The placement of dissolvable sutures continues to gain popularity as a minimally invasive treatment of facial ptosis that provides a temporary lifting of drooping tissues with a low risk of complications.

Dissolvable barbed sutures, most commonly made of polydioxanone (PDO), are placed under the skin of the face and neck to reduce skin laxity, creating a brow, midface, jawline, and chin and/or neck lift, while also stimulating collagen formation^{137–140} (Fig. 5). Suspension threads, which contain barbs, have the added benefit of stimulating collagen formation. On histology, fibrous capsules have been found along threads, which are thought to contribute to lifting surrounding tissues.^{1–3} This fibrosis, associated with acute inflammatory cells, is eventually replaced by type I collagen.^{4–6}

Skin rejuvenation and facial skin lifting results achieved with threads have been noted for up to 24 months.¹³⁸ However, combination therapy with dermal fillers can improve the long-term results by compounding the effects of neocollagenesis.¹⁴¹ In order to further maximize results, it is also important to carefully select patients with adequate tissue volume and for the procedure to be performed by a skilled clinician.^{137,142} Lifting of ptotic tissues with PDO threads is best recommended for patients with contraindications to invasive surgical procedures, those who are amenable to short-term results for a lower cost, or those who combine the procedure with other modalities such as dermal fillers.¹³⁸



Fig. 5. (A) Before and (B) 4 months after midface PDO thread lift. Two threads were placed on each side. Improvement of nasolabial folds is apparent.

Nevertheless, advancements continue to be made with this technology, with several thread types available, from free-standing, barbed threads that do not need to be suspended to smooth threads that need to be anchored to a stable structure of the face or scalp.

Combination Treatments

Given that aging involves changes not only in the skin and fat but also bones, muscles, and ligaments, rejuvenation techniques must address each component of the aging process. A one-size-fits-all technique only using one modality is not as effective as a treatment plan that is tailored to the individual's needs using combination therapy, all while meeting the patient's goals and expectations. Although many of these therapies are not new, the concept of combining therapies is being emphasized in clinical practice (Fig. 6).

Combination therapies (Table 4) can potentially achieve better, quicker results at a lower cost to the patient with high patient satisfaction.^{143–151} For example, accordion lines can be treated using superficial injections or microneedling application of highly diluted BoNTA and non-cross-linked hyaluronic acid.^{143,152,153} It is important to also tailor therapies to the individual, such as taking an individual's ethnicity features into consideration. It is also crucial to remember that skin rejuvenation also applies to areas other than the face affected



Fig. 6. (Top) Before and (bottom) after views of a patient who received injections of PRF mixed with dermal filler under both eyes.

Table 4
Combination therapies in aesthetic medicine

Modality	Purpose
Ablative lasers	Reverse skin
Collagen-stimulating treatments	sagging and dullness
PRP/PRF	Correct
Microneedling	pigmentation
Radiofrequency/ultrasound	
Microdermabrasion	
Skin peels	
Topical therapies; eg, retinol and hydroquinone	
Botulinum toxin	Correct dynamic wrinkles
Fillers:	Volume
Hyaluronic acid filler	augmentation
Calcium hydroxylapatite filler	
Poly-L-lactic acid filler	
Autologous fat tissue	

by aging, including the neck, décolletage, and the hands.

SUMMARY

The rapidly growing aesthetic industry is continuously evolving to meet the increasing demand for cosmetic enhancement. With this expansion has come new technology within regenerative medicine, providing natural, effective, and safe skin rejuvenation. Using a combination approach, new innovations in injectable botulinum toxin and fillers, along with state-of-the-art autologous stem cells, lipofilling, and platelet therapies, can help patients reach their aesthetic goals.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at <https://doi.org/10.1016/j.fsc.2019.09.009>.

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