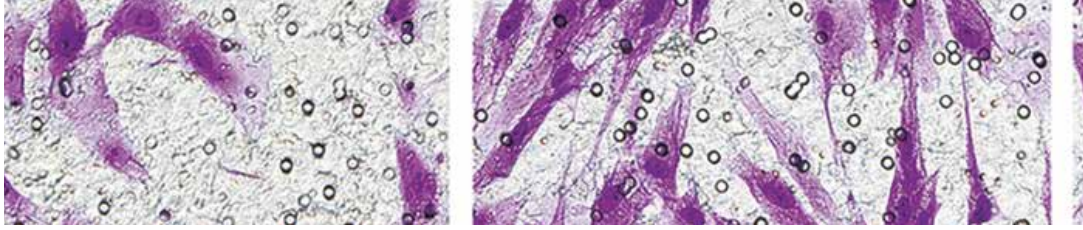
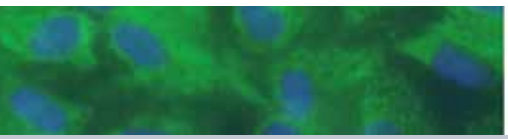


Rejuvenating Facial Esthetics *with* Regenerative and Biocompatible Techniques



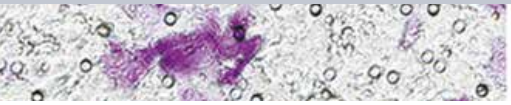
Part 2: Laser Fundamentals and Platelet-Rich Fibrin Protocols

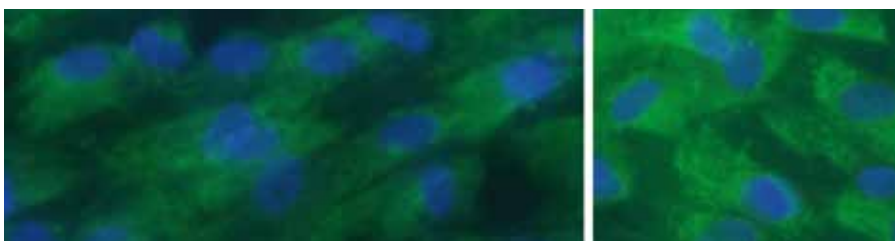
Richard J. Miron, DDS, MSc, PhD
Catherine Davies, MBBCh, MBA

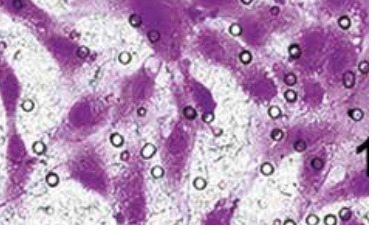
Abstract

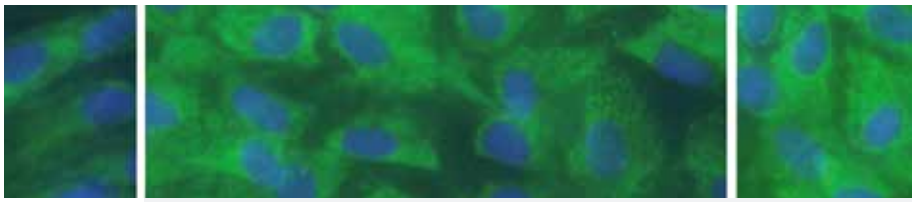
This article—the second in a two-part series combining text, photographic elements, and QR links to video tutorials and explanations—focuses on the fundamentals of lasers and the development and research-supported protocols for platelet concentrates (e.g., liquid platelet-rich fibrin [liquid-PRF]) in facial esthetics anti-aging applications. A review of lasers' role in facial esthetics—along with background information about applicable laser types, wavelengths, and anti-aging/rejuvenation indications—is presented. Additionally, the evolution of an enhanced liquid-PRF formulation and its newer centrifugation and heating protocols for isolating and procuring PRF-based injectables will be discussed in greater detail.

Key Words: platelet-rich fibrin, laser therapy, natural facial esthetics









Introduction

As people become more esthetically conscious, strive to maintain a youthful appearance, and explore opportunities to reverse the visible signs of aging, the demand for all-natural and biologically based facial rejuvenation treatments continually grows. Among the more biocompatible therapies available today are various laser regimens and autologous platelet concentrate injections, with or without adjunctive procedures (e.g., micro-needling). The materials/devices used in these alternative anti-aging approaches differ in their therapeutic mechanisms (i.e., laser light, growth factor delivery and stimulation), but their underlying commonality is an ability to harness the body's own healing and regenerative capabilities at a cellular level, thereby addressing the causes of the visible signs of aging.

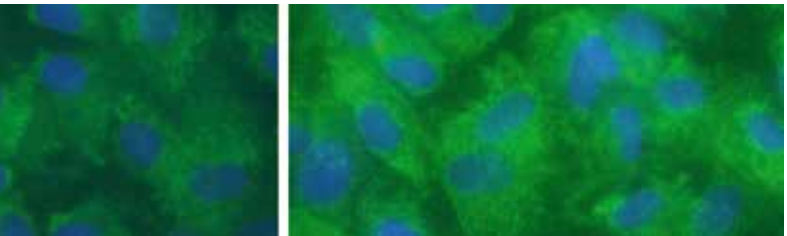
However, it is important to note that within each respective category—lasers and platelet concentrates—are:

- various types and iterations developed in response to the need for greater efficacy
- easier and more cost-effective delivery to targeted sites
- faster recovery time for specific indications.

Some product evolution involved the manufacturing and application of devices with wavelengths and components more suitable for anti-aging and regenerative indications, or the materials themselves and the manner by which they are procured and delivered, respectively.

Complementing Part 1 of this two-part series (which appeared in Volume 38, Issue 3 of the *Journal of Cosmetic Dentistry*), this article reviews the fundamentals of laser devices that indicate their use for facial esthetics applications, as well as the evolution, development, and use of liquid platelet-rich fibrin (liquid-PRF) in regenerative medicine and facial rejuvenation. Combined, laser therapy and liquid-PRF have been shown to effectively stimulate collagen synthesis and activate fibroblast proliferation/activity, respectively, to achieve facial rejuvenation and enhanced facial esthetics.

“LASER THERAPY AND LIQUID-PRF HAVE BEEN SHOWN TO EFFECTIVELY STIMULATE COLLAGEN SYNTHESIS AND ACTIVATE FIBROBLAST PROLIFERATION/ ACTIVITY, RESPECTIVELY, TO ACHIEVE FACIAL REJUVENATION AND ENHANCED FACIAL ESTHETICS.”



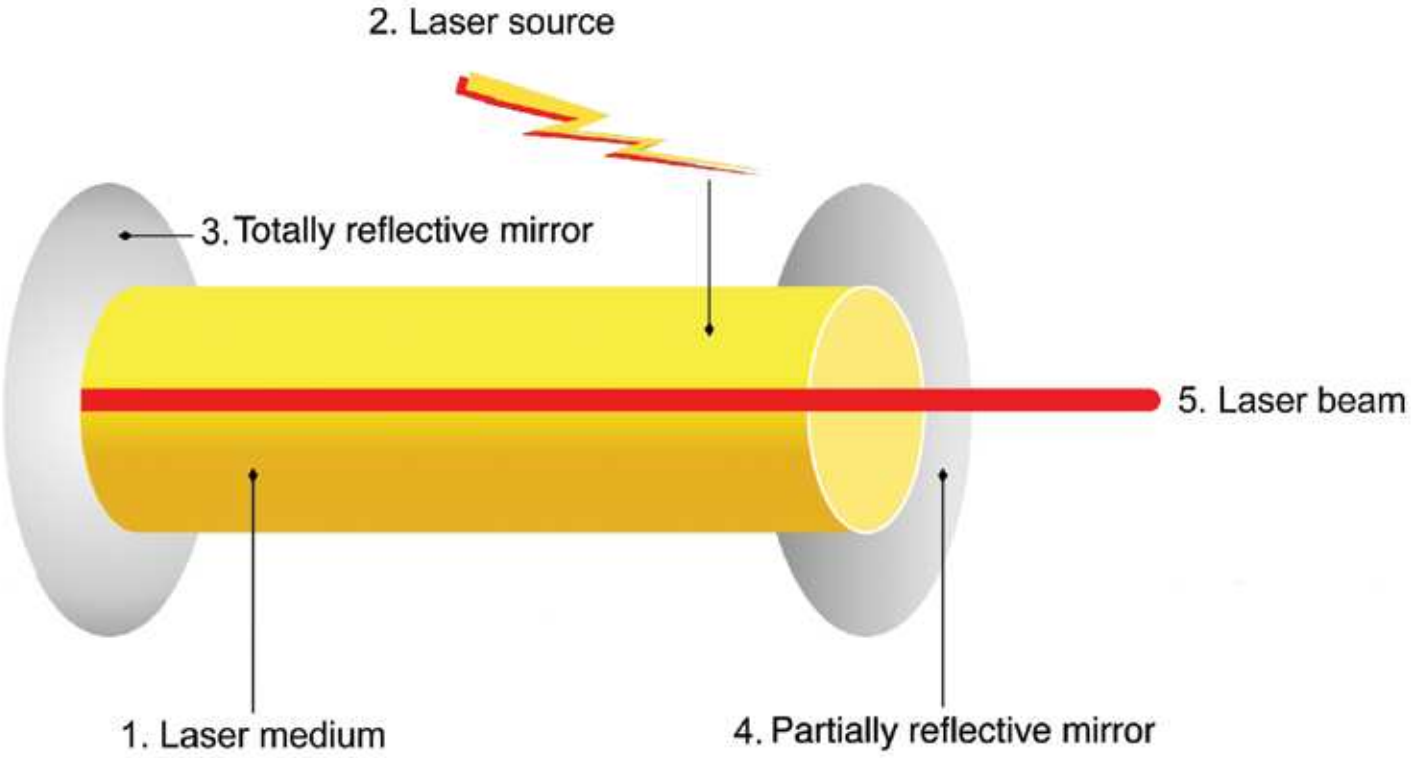


Figure 1: Laser light is characterized by a single wavelength of coherently sized and shaped waves that emit high-energy concentrations in a collimated and directional way.

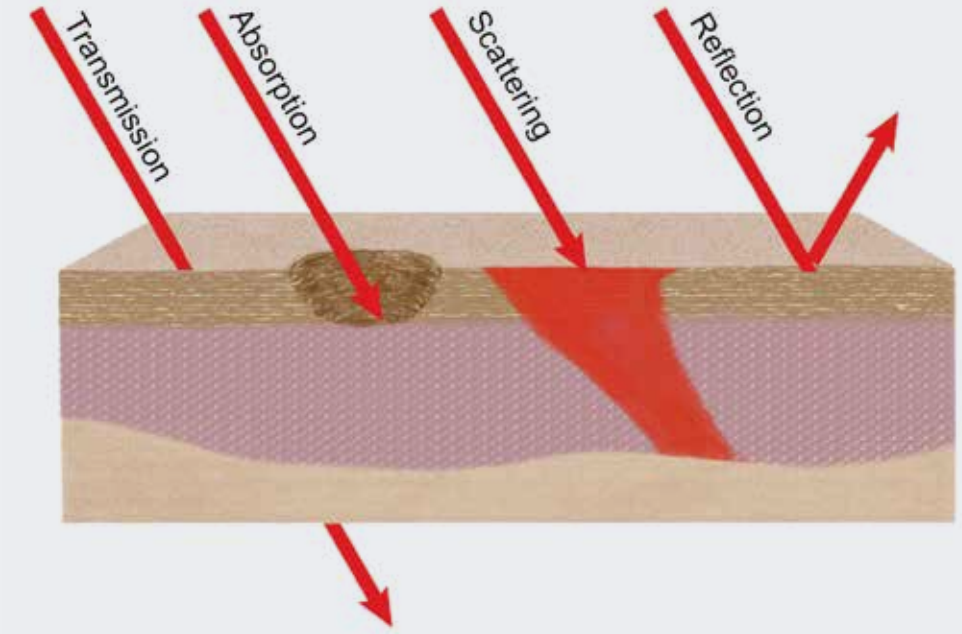


Figure 2: Laser light that reaches the skin surface is either reflected, scattered, absorbed, or transmitted.

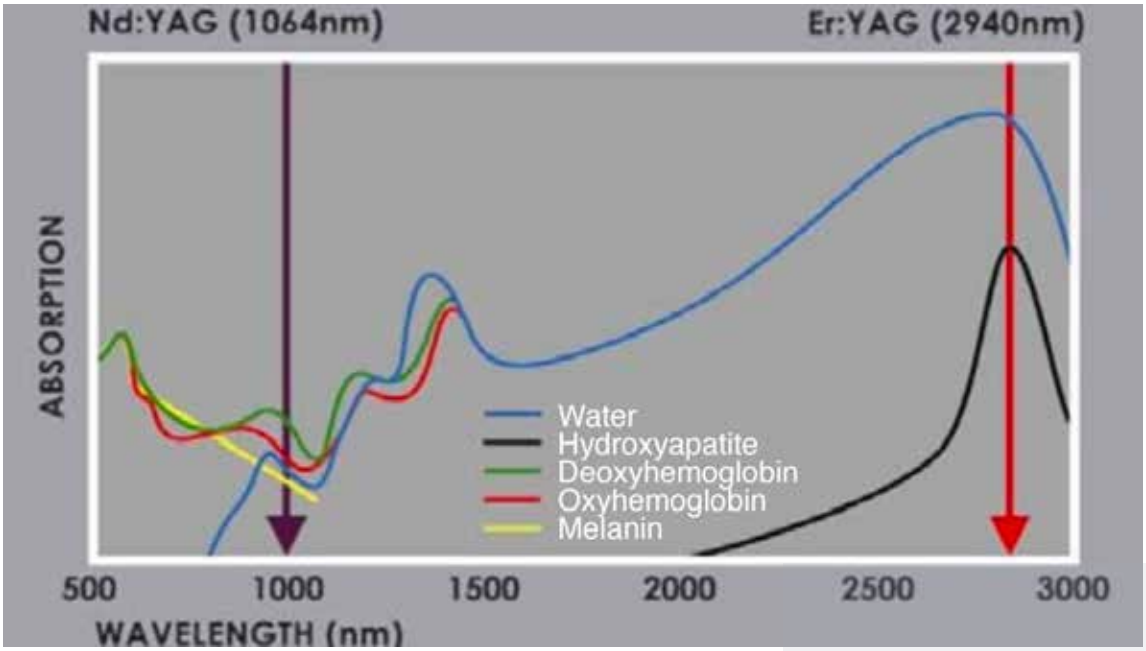


Figure 3: The absorption coefficient in human tissues as a function of laser wavelength is at a minimum with the Nd:YAG laser (e.g., 1064 nm), and at a maximum with the Er:YAG laser (e.g., 2940 nm).

Laser Fundamentals & Facial Esthetics Applications

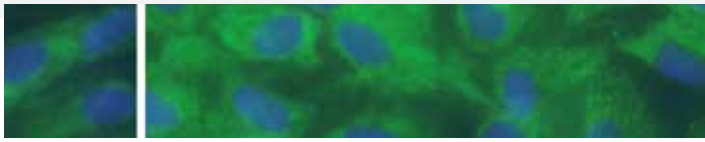
Since their introduction in the 1960s, laser devices have been used increasingly in medicine, dentistry, and facial esthetics for various surgical, therapeutic, and regenerative applications.[1,2] Unlike an ordinary light beam, lasers radiate high-energy, collimated, and monochromatic light wavelengths that are identical in size and shape and directionally focused **(Fig 1)**.[3,4]

A laser's active medium (e.g., argon, erbium/yttrium, neodymium, CO2) determines its light wavelength, and this wavelength subsequently dictates the tissue components with which the laser light will interact, the type of such interaction or noninteraction and, therefore, the specific laser's indications. For example, interactions between lasers and facial tissues include reflected and transmitted (i.e., noninteractions), as well as scattered and absorbed **(Fig 2)**.[2]

The combination of wavelength type (i.e., infrared, visible, ultraviolet) and molecular (i.e., water) or macromolecular (i.e., proteins, oxyhemoglobin, deoxyhemoglobin, pigments [melanin, carotenes]) tissue components determine the laser's photochemical, photothermal, and ultimately therapeutic/regenerative effects.[1,2] In particular, water molecules absorb infrared light, while protein and pigment macromolecules absorb UV and visible light **(Fig 3)**.[1,4] The resulting photochemical and/or photothermal effects of laser light absorption include vaporization/ablation for skin imperfection removal or stimulation/healing for tightening and rejuvenation, respectively.[5,6]

Although the first generations of lasers originally limited facial esthetic treatments to ablative therapies that required extensive recovery times for complete re-epithelialization,[5,6] recent technological advances have led to the commercial availability of more than 150 laser devices approved for medical and dental

> "THE PLATELET CONCENTRATE FORMULATIONS DEVELOPED… DEMONSTRATE SPECIFIC CHARACTERISTICS CONTRIBUTING TO THEIR SUITABILITY FOR TISSUE REGENERATION AND ANTI-AGING APPLICATIONS, BUT WHICH ALSO LIMIT THEIR POTENTIAL FOR SPECIFIC INDICATIONS."



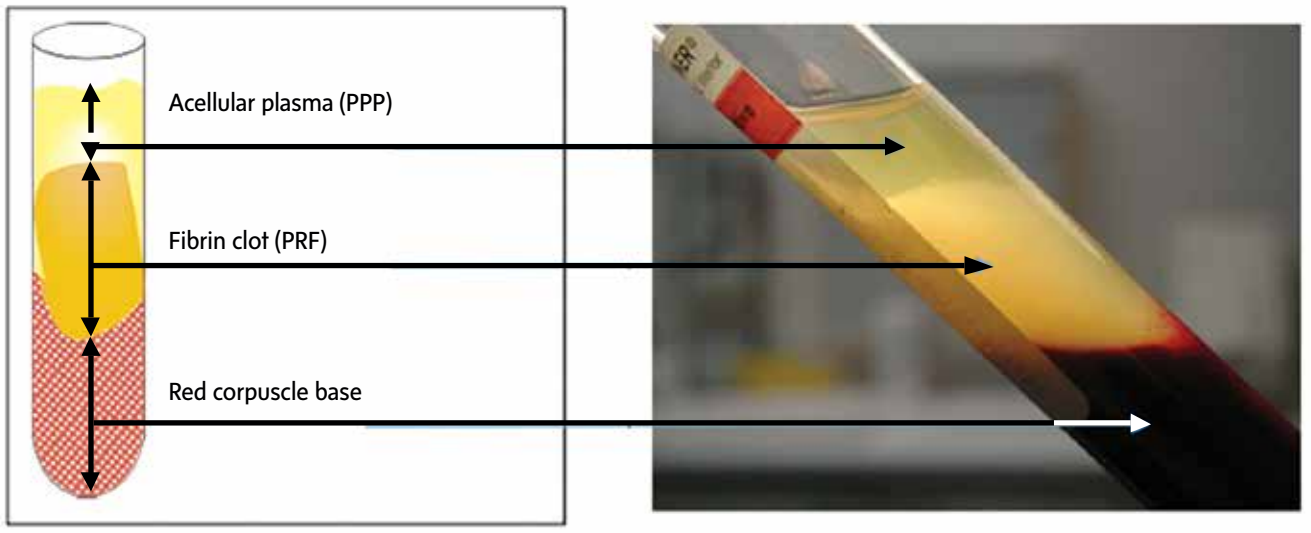


Figure 4: Centrifugation of whole blood produces three layers, with the platelet-rich fibrin (PRF) clot forming in the upper third of glass tubes.[14]

Table 1. Laser Characteristics & Indications for Facial Esthetics

Laser	Wavelength	Indications	Notes
Alexandrite	755 nm	• Vascular lesion removal	• Red light laser removal also induces hair removal and pigmented lesions
Argon	488 nm 515 nm	• Vascular lesion removal	• Blue/green laser light • Works on the principle of selective thermolysis
CO_2	10600 nm	• Skin resurfacing • Laser peels	• Ablative • Generates substantial heat
Er:YAG	2940 nm 2790 nm	• Skin rejuvenation • Collagen stimulation and synthesis • Skin tightening • Laser peels • Treatment of superficial age spots	• Simpler, more controlled, precise ablative therapy • Less aggressive
Nd:YAG	1032 nm 1064 nm	• Vascular lesion removal • Pigmented lesion removal • Tissue regeneration • Rejuvenation of deep nasolabial folds and marionette lines	• Absorbed by deeper levels of tissue • Intraoral energy application promotes better penetration into nasolabial/marionette tissues through thinner mucosal tissue, less downtime, no visible treatment signs
Ruby	532 nm	• Pigmented lesion removal • Tattoo removal • Hair removal	• For hair removal, targets melanin pigment in hair bulbs, thereby destroying bulb to achieve permanent epilation
Q-Switched	High-energy Pulsed	• Pigmented lesion removal	• Selectively targets melanosomes within melanocytes and keratinocytes • Requires sound diagnostic and histopathologic classification

applications. As a result, lasers are widely used for a variety of facial esthetics indications, including scar revisions;[7] pigmented and vascular lesion removal;[8,9] facial resurfacing;[10] facial rejuvenation;[7,10] and other esthetic therapies **(Table 1)**.[11,12] As an all-natural regenerative option for facial anti-aging therapies, laser treatments can readily complement platelet concentrate therapies.[13]

Evolution of Platelet Concentrate Formulations & Production Methods

Since their introduction in the 1970s and subsequent evolution in the past five decades, platelet concentrates have been used increasingly to produce supra-physiological doses of blood growth factors to regenerate various human tissues.[13] The platelet concentrate formulations developed—and which evolved to include, sequentially, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and liquid-PRF—demonstrate specific characteristics contributing to their suitability for tissue regeneration and anti-aging applications, but which also limit their potential for certain indications. Therefore, researchers continuously endeavored to improve processes for acquiring growth-factor containing platelet concentrates and delivering them to affected sites to maximize their benefits.

From PRP to PRF

For example, when the anticoagulants typically necessary for PRP production (e.g., bovine thrombin [CaCI2] are eliminated, blood more readily and naturally clots during a typical 8-to-12-minute centrifugation cycle, producing three distinct layers with unique characteristics: an upper platelet-poor plasma layer, a platelet-rich plasma layer, and a red corpuscle base layer **(Fig 4)**.[14] The stable, three-dimensional clot formed from this process is now referred to as PRF, which contains a high concentration of platelets, leukocytes, and growth factors entrapped within a fibrin matrix **(Fig 5)**.[15]

Compared to PRP, PRF significantly improves not only the host immune system defense against incoming pathogens,[16-21] but also secretion of the growth factors and cytokines responsible for tissue regeneration over an extended period of time.[22,23] This is attributed to the concentrate fibrin's function as the required scaffold surface material; its cells (e.g., leukocytes, macrophages, neutrophils, and platelets) attract and recruit future

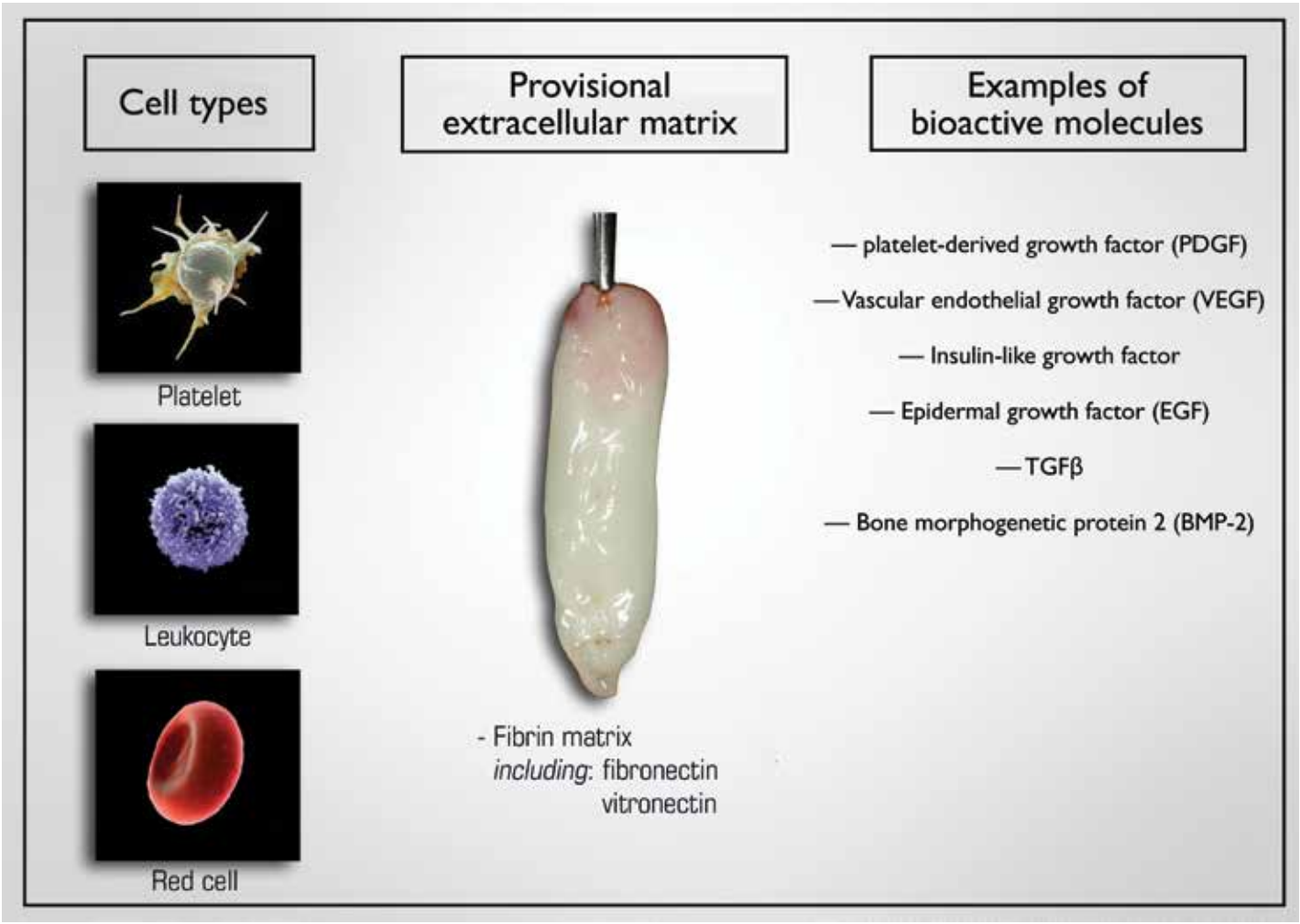
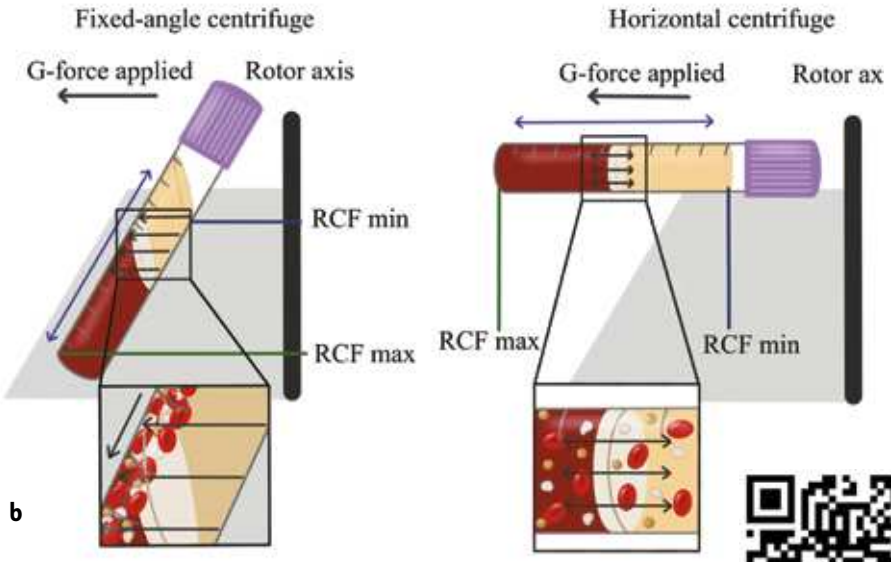


Figure 5: Natural components of PRF include cell types; a provisional, three-dimensional extracellular matrix scaffold fabricated from autologous fibrin; and more than 100 bioactive molecules.[13]

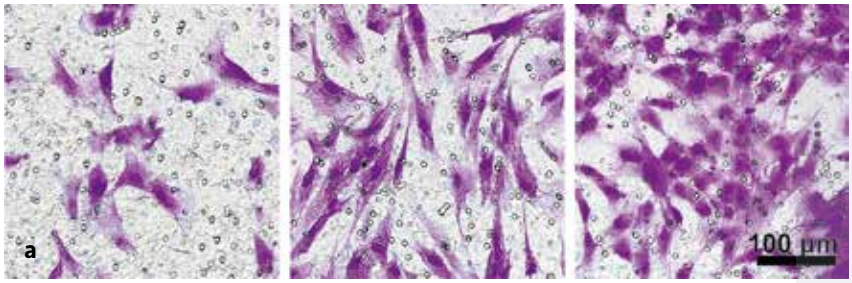
6b

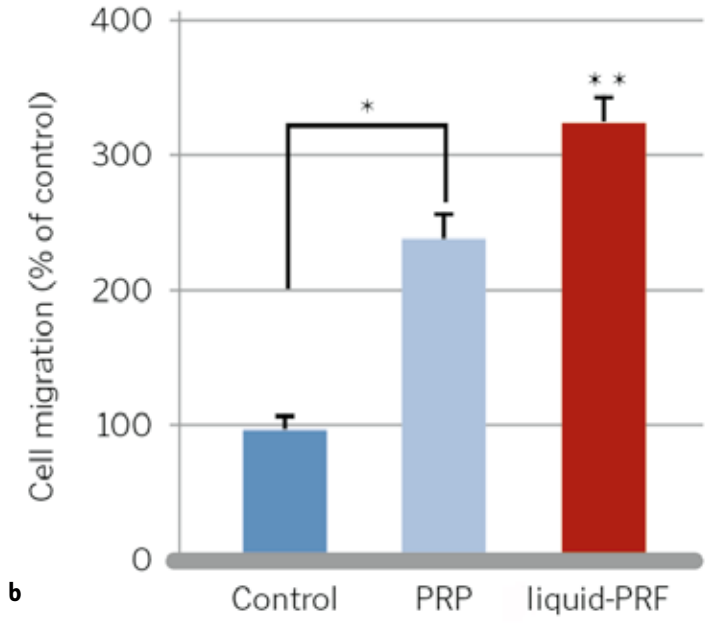
Figures 6a & 6b: (a) A centrifugation spin cycle separates whole blood into a plasma component rich in growth factors, platelets, and leukocytes useful as a regenerative agent in facial esthetics. (b) QR code to video explaining the advance from PRP to PRF.



7c

Figures 7a-7c: (a) Tubes are placed vertically into the horizontal configuration device, but gradually rotate horizontally during the spin cycle, which promotes better layer separation. (b) Due to differences in minimum and maximum relative centrifugal force (RCF) between fixed-angle and horizontal centrifuges, greater separation of blood layers based on density is achieved with the latter. (c) QR code to video explaining horizontal centrifugation.





Figures 8a & 8b: (a) Migration assay of human skin fibroblasts cultured with liquid-PRF and PRP after 24 hours. (b) Scale bars = 100 µm.
* Denotes significant difference between two groups p<0.05.
** Denotes significantly higher than all other treatment groups p<0.05.
Assay performed in triplicate with three independent experiments.[25]

“THE ABILITY TO PROCURE AND DELIVER AN INJECTABLE, LIQUID NONADDITIVE PLATELET DERIVATIVE (E.G.,LIQUID–PRF) WAS CONSIDERED AN ENHANCEMENT, SINCE IT ENABLED LIQUID–PRF´S EXTENSIVE USE FOR A VARIETY OF REGENERATIVE PROCEDURES.”

regenerative cells to treatment sites, while its fibrin also serves as a reservoir for growth factors released over 10 to 14 days.

Enhanced PRF Procurement & Delivery

PRF clots/membranes are typically produced using glass or silica-coated plastic tubes to promote faster clotting in dentistry and medicine. However, the ability to procure and deliver an injectable, liquid version of a nonadditive platelet derivative (e.g., liquid-PRF) was considered an enhancement since it enabled liquid-PRF's extensive use for various regenerative procedures, including knee injections to manage osteoarthritis; injections to treat temporomandibular joint disorders; and facial injections to improve esthetics through natural collagen synthesis. Interestingly—and particularly relevant to anti-aging efforts—upon injection, liquid-PRF subsequently clots, boosting its ability to maintain volume over time (e.g., in facial wrinkles and nasolabial folds).

To create liquid-PRF, blood is drawn into a plastic tube incorporating new surface technology, then processed at a very low speed (i.e., 300 g) for an even shorter centrifugation time (i.e., three to eight minutes) to maintain cells in the upper layer. The ability to use more hydrophobic materials (i.e., plastic) enables the separation of the blood into two layers **(Figs 6a & 6b)**, with cells maintained in the upper layer. The same type of tube can also be centrifuged at higher speeds (i.e., 2000 g) for eight minutes to create a denser liquid-PRF more suitable for use as a biological filler (i.e., bio-filler) to replace chemical fillers.

Additionally, centrifugation methods for procuring PRF evolved from historically fixed-angle rotors—which cause angled, uneven blood separation—to horizontal centrifugation, which promotes optimal cell separation into their appropriate layers based on density **(Fig 7a)**.[24] The high g-forces of fixed-angle centrifuges push cells toward the back of tubes, then downward/upward based on cell density, and place additional shear stress on cells during separation; larger cells (e.g., red blood cells) typically trap and pull smaller platelets to the bottom of the PRF tubes **(Fig 7b)**. Horizontal centrifugation produces PRF with up to four times greater accumulation of cells and growth

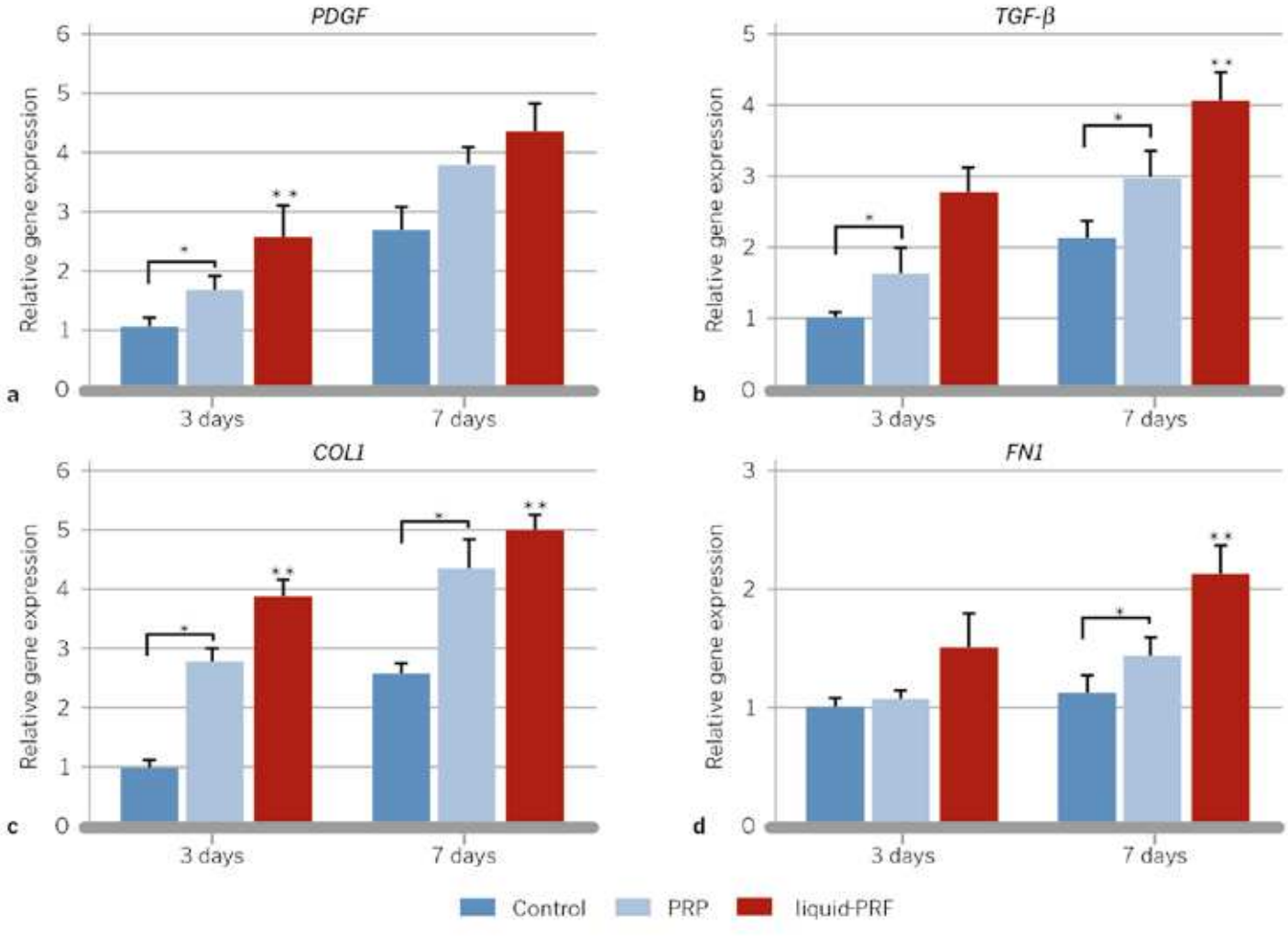
factors by enabling free cell mobility, reducing cell trauma/shear stress, and preventing cells from amassing along outer tube walls **(Fig 7c)**.[24]

PRF vs. PRP for Facial Esthetics & Skin Fibroblasts

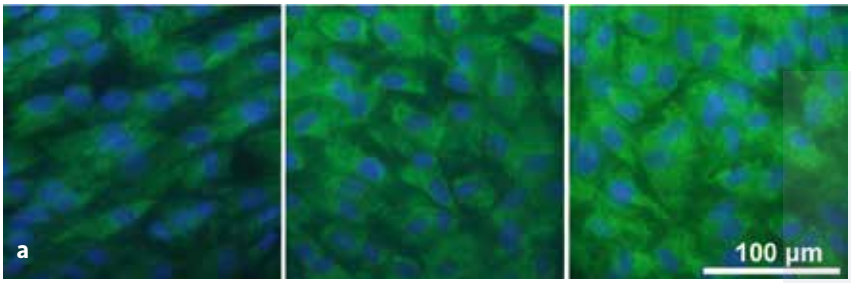
Research comparing skin cell behavior and regeneration with liquid-PRF and PRP found that while all platelet concentrates were nontoxic and demonstrated high cell survival, skin fibroblasts migrated more than 350% in liquid-PRF compared to control and PRP, a 200% increase **(Figs 8a & 8b)**.[25] Liquid-PRF also induced more significant cell proliferation at five days. Additionally, although both PRP and liquid-PRF induced significantly elevated cell messenger RNA (mRNA) levels of platelet-derived growth factor (PDGF), considerably higher transforming growth factor beta (TGF-β), Type I collagen (COL1), and fibronectin (FN1) mRNA levels were observed in the liquid-PRF group **(Figs 9a-9d)**.[25] Finally, liquid-PRF demonstrated a significantly greater ability to induce collagen matrix synthesis compared to PRP **(Figs 10a & 10b)**.[25] It was therefore concluded that liquid-PRF has greater regenerative potential on human skin fibroblasts than PRP, and that because PRF tubes contain no additives, PRF is a more natural approach to tissue regeneration and is also less expensive.[25]

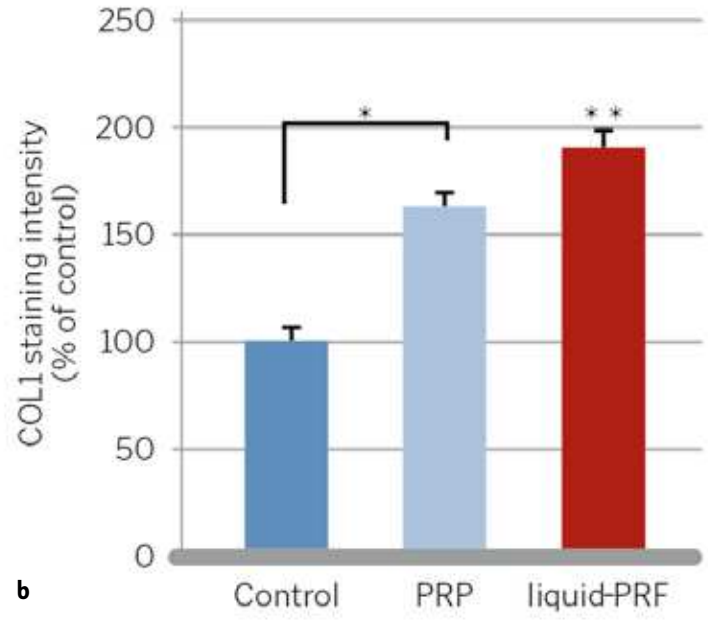
Expanding PRF Applications with Heat Processing

A limitation of PRP and PRF is their relatively fast resorption time (i.e., typically 10 to 14 days). This often precludes their use as typical "barrier membranes" to protect guided bone regeneration from faster-growing soft tissues in dental procedures,[26] or as facial fillers when augmenting deep folds and tissues—both of which require a long-lasting solution. Fortunately, a number of developments involving blood-derived growth factors, heating plasma and PRF membranes, and specialized cooling tech-



Figures 9a-9d: Expression of regeneration-related and extracellular matrix-related genes of gingival fibroblasts cultured with PRP and liquid-PRF at 3 days and 7 days: (a) PDGF; (b) TGF-β; (c) COL1; (d) FN1.
* Denotes significant difference between two groups $p<0.05$.
** Denotes significantly higher than all other treatment groups $p<0.05$.
Assay performed in triplicate with three independent experiments.[25]





Figures 10a & 10b: Immunofluorescent COL1 staining of skin fibroblasts cultured with PRP and liquid-PRF at 7 days: (a) COL1 staining (green) merged with DAPI staining (blue). (b) Scale bars = 100 µm; COL1 staining quantification. * Denotes significant difference between two groups $p<0.05$. ** Denotes considerably higher than all other treatment groups $p<0.05$. Assay performed in triplicate with three independent experiments.[25]

> “A NUMBER OF DEVELOPMENTS INVOLVING BLOOD–DERIVED GROWTH FACTORS, HEATING PLASMA AND PRF MEMBRANES, AND SPECIALIZED COOLING TECHNIQUES ENABLES THE EXTENSION OF PRF’S RESORPTION TIME AND WORKING PROPERTIES FROM TWO TO MORE THAN THREE WEEKS, AND EVEN UP TO SIX MONTHS.”

niques enables the extension of PRF's resorption time and working properties from two to more than three weeks and even up to six months.[27-31] In particular, to overcome their rapid degradation properties and better maintain volume stability, a protocol was developed to heat and then cool the platelet-poor plasma (PPP) layer, which contains ~60% albumin; mix a concentrated PRF layer (C-PRF)[32,33] taken from the buffy coat back into the now more densely organized gel; and ultimately produce albumin gel PRF (Alb-PRF), also called extended PRF (E-PRF).[34]

Alb-PRF & Facial Esthetics Bio-Filler Protocol

To produce Alb-PRF **(Fig 11)**, peripheral blood is collected using 9- to 10-ml additive-free tubes that are then placed in a horizontal centrifuge (Bio-PRF; Venice, FL), which is set at 2000 g for an eight-minute protocol **(Fig 12)**. After processing, separate blood layers (i.e., yellow plasma upper layer and remaining decanted red cells) can be observed.

Two to four milliliters of the uppermost layer of PPP (e.g., 2 cc) is then collected with a syringe, and other blood portions (i.e., buffy coat, liquid-PRF, and red blood cells) are placed in a specialized cooling device (Bio-Cool, Bio-PRF) to prevent clotting **(Fig 13)**. The syringes containing PPP are then inserted into a specifically designed heating device (Bio-Heat, Bio-PRF) at 75°C for 10 minutes for human serum albumin denaturation and to produce the albumin gel **(Fig 14)**. The syringes are removed and cooled to room temperature in the cooling unit for 10 minutes, resulting in noticeable color and texture changes between the Alb-PRF and standard liquid-PRF **(Fig 15)**.[30]

The albumin gel and liquid-PRF are then mixed between syringes using a female-female luer-lock connector **(Fig 16)**. Adequate mixing is ensured by passing back and forth between syringes approximately 10 times. Once thoroughly mixed, the Alb-PRF/facial esthetics bio-filler can be utilized as an injectable autologous concentration of growth factors, cells, and heated albumin **(Fig 17)**.

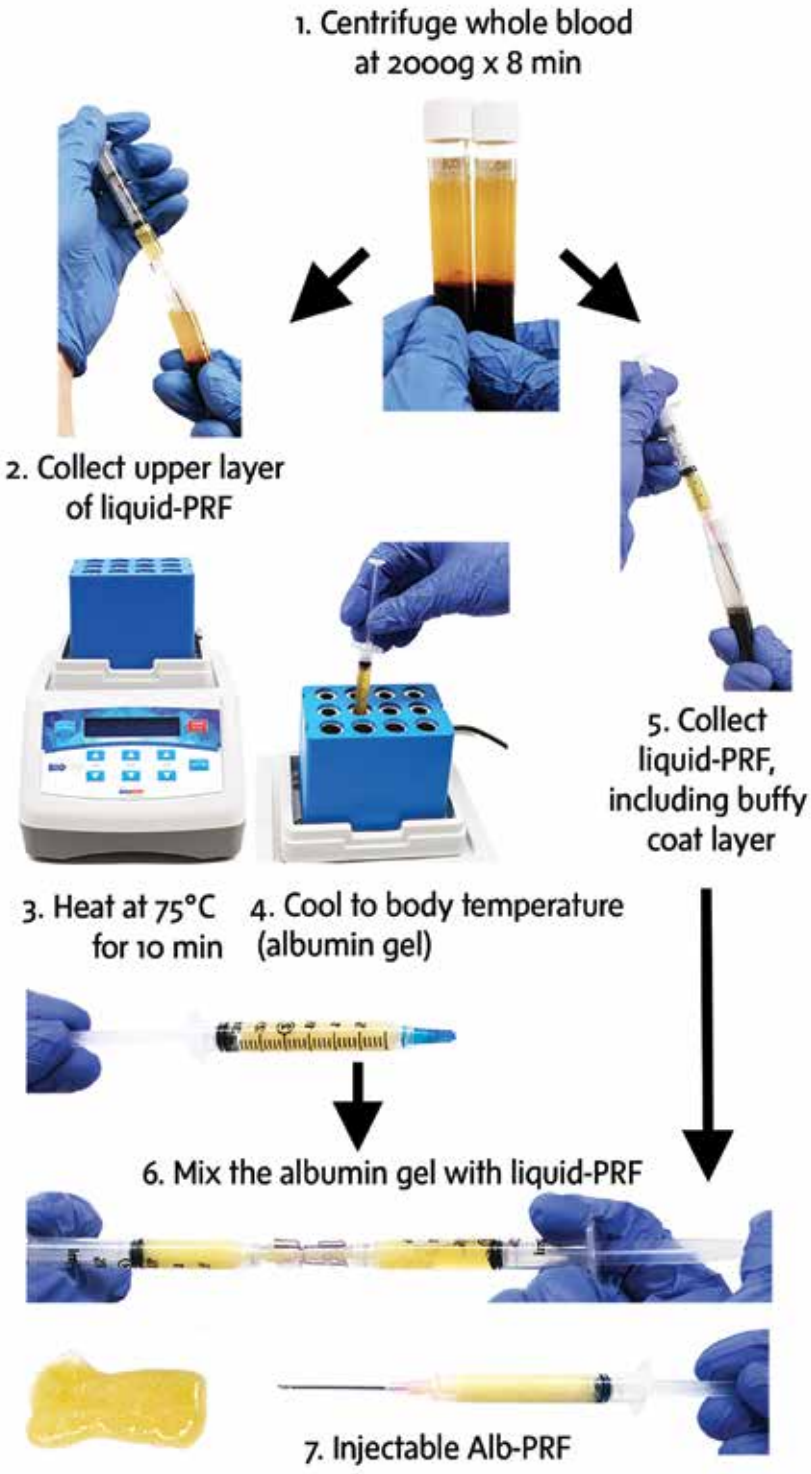


Figure 11: Diagram outlining steps required to prepare Alb-PRF (i.e., E-PRF).[34]

> “ADVANCES IN HOW LIQUID–PRF AND PLATELET CONCENTRATES ARE PROCURED, PROCESSED, AND ADMINISTERED HAVE ENHANCED THEIR REGENERATIVE AND REJUVENATION APPLICATIONS IN FACIAL ESTHETIC THERAPIES.”

Summary

Due to their natural regenerative approach, lasers and platelet concentrates have grown in popularity and are used in the ever-growing field of facial esthetics and, as described in this article, for good reason. Laser device fundamentals, evolution, and development of treatment-specific techniques have broadened the range of anti-aging indications for which these technologies can be applied. Simultaneously, advances in how liquid-PRF and platelet concentrates are procured, processed, and administered have enhanced their regenerative and rejuvenation applications in facial esthetic therapies. Collectively, they present a compelling case for using both to provide safer, more natural, faster, and economical anti-aging solutions.

Independently and in combination, laser therapy and liquid-PRF effectively stimulate collagen synthesis and activate fibroblast proliferation/activity to achieve enhanced facial esthetics, all by harnessing the body’s own healing and regenerative capabilities at a cellular level. As people become more esthetically conscious, strive to maintain a youthful appearance, and explore opportunities to reverse the visible signs of aging, the demand for all-natural and biologically based facial rejuvenation treatments such as lasers and liquid-PRF will likely continue to increase.

Acknowledgment

Figures 1-5, 6a, 7a-7c, 8a-8b, 9a-9d, and 10a-10d originally appeared in the authors’ book, PRF in Facial Esthetics, *©2020, Quintessence Publishing Co. Inc. Chicago. Figures 11 and 12 originally appeared in Dr. Miron’s book,* Understanding Platelet-Rich Fibrin, *©2021, Quintessence Publishing Co. Inc. Chicago. Reprinted with permission from the publisher.*

Figure 12: Blood collected in additive-free tubes is centrifuged horizontally.

Figure 13: The remaining platelet-rich layer is kept in a specialized cooling device to extend clotting time.

Figure 14: After centrifugation, the upper 2 cc of plasma is placed into specifically designed medical heating device to heat the serum and PPP. (Note: the machine must be preheated before use at 75°C for 10 minutes.)

Figure 15: Following 10 minutes of heating, clinical differences in color are observed between the liquid-PRF (top syringe) and the albumin gel (bottom syringe).

Figure 16: A luer-lock mixer device is attached to the liquid-PRF and albumin gel syringes to mix both components and create Alb-PRF (i.e., E-PRF).

Figure 17: View of Alb-PRF (i.e., E-PRF) ready for use and immediate injection following adequate mixing.

for Clinicians Tips

BEGINNER

» Use only hydrophobic plastic tubes when centrifuging liquid-PRF to prevent coagulation into a PRF fibrin matrix.

» Remember that once PRF is drawn into a syringe, it will clot within 20 to 40 minutes and even more rapidly if exposed to oxygen.

INTERMEDIATE

» PRF may be injected into facial tissues or the scalp, similar to PRP.

» Because PRF growth factors are released over a 10- to 14-day period, a 14- to 28-day treatment cycle of three to four therapies spread evenly one month apart for the first three to four months is the typical initial skin regeneration regimen.

ADVANCED

» PRF may be used as an autologous growth factor applied to the face before/after micro-needling *(see Part 1 of this series).*

» Remember that when it comes to liquid-PRF and PRF bio-filler injections, the severity and location of the problem areas will determine dosage, needle size, and injection depth. A thorough knowledge of facial anatomy is essential.

References

1. Soleymani T, Abrouk M, Kelly KM. An analysis of laser therapy for the treatment of nonmelanoma skin cancer. Dermatol Surg. 2017 May;43(5):615-24.
2. Mahamood RM. Laser basics and laser material interactions. In: Laser metal deposition process of metals, alloys, and composite materials. New York: Springer; 2017. p. 11-35.
3. Beigvand HH, Razzaghi M, Rostami-Nejad M, Rezaei-Tavirani M, Safari S, Rezaei-Tavirani M, Mansouri V, Heidari MH. Assessment of laser effects on skin rejuvenation. J Lasers Med Sci. 2020 Spring;11(2):212-9.
4. Brazzini B, Hautmann G, Ghersetich I, Hercogova J, Lotti T. Laser tissue interaction in epidermal pigmented lesions. J Eur Acad Dermatol Venereol. 2001 Sep;15(5):388-91.
5. Goldman MP, Fitzpatrick RE. Cutaneous laser surgery: the art and science of selective photothermolysis. 2nd ed. St. Louis: Mosby; 1994.
6. Chen KH, Tam KW, Chen IF, Huang SK, Tzeng PC, Wang HJ, Chen CC. A systematic review of comparative studies of CO2 and erbium: YAG lasers in resurfacing facial rhytides (wrinkles). J Cosmet Laser Ther. 2017 Aug;19(4):199-204.
7. Robati RM, Asadi E. Efficacy and safety of fractional CO2 laser versus fractional Er:YAG laser in the treatment of facial skin wrinkles. Lasers Med Sci. 2017 Feb;32(2):283-9.
8. Goldberg DJ. Benign pigmented lesions of the skin. treatment with the Q-switched ruby laser. J Dermatol Surg Oncol. 1993 Apr;19(4):376-9.
9. Raulin C, Schöönermark MP, Greve B, Werner S. Q-switched ruby laser treatment of tattoos and benign pigmented skin lesions: a critical review. Ann Plast Surg. 1998 Nov;41(5):555-65.
10. Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N, Hamblin MR. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. Semin Cutan Med Surg. 2013 Mar;32(1):41-52.
11. Grossman MC, Dierickx C, Farinelli W, Flotte T, Anderson RR. Damage to hair follicles by normal-mode ruby laser pulses. J Am Acad Dermatol. 1996 Dec;35(6):889-94.
12. Shiffman HS, Khorsandi J, Cauwels NM. Minimally-invasive combined Nd:YAG and Er:YAG laser-assisted uvulopalatoplasty for treatment of obstructive sleep apnea. Photobiomodul Photomed Laser Surg. 2021 Aug;39(8):550-7.
13. Miron RJ, Fujioka-Kobayashi M, Bishara M, Zhang Y, Hernandez M, Choukroun J. Platelet-rich fibrin and soft tissue wound healing: a systematic review. Tissue Eng Part B Rev. 2017 Feb;23(1):83-99.
14. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Mar;101(3):e37-44.

15. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol. 2010 Apr;81(4):546-55.

16. Davis VL, Abukabda AB, Radio NM, Witt-Enderby PA, Clafshenkel WP, Cairone JV, Rutkowski JL. Platelet-rich preparations to improve healing. Part I: workable options for every size practice. J Oral Implantol. 2014 Aug;40(4):500-10.

17. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Ciol. 2005 Nov;15(11):599-607.

18. Tsirogianni AK, Moutsopoulos NM, Moutsopoulos HM. Wound healing: immunological aspects. Injury. 2006 Apr;37 Supp 1:S5-12. doi: 10.1016/j.injury.2006.02.035.

19. Adamson R. Role of macrophages in normal wound healing: an overview. J Wound Care. 2009 Aug;18(8):349-51.

20. Davis VL, Abukabda AB, Radio NA, Witt-Enderby PA, Clafshenkel WP, Cairone JV, Rutkowski JL. Platelet-rich preparations to improve healing. Part II: platelet activation and enrichment, leukocyte inclusion and other selection criteria. J Oral Implantol. 2014 Aug;40(4):511-21.

21. Ghasemzadeh M, Hosseini E. Intravascular leukocyte migration through platelet thrombi: directing leukocytes to sites of vascular injury. Thromb Haemost. 2015 Jun;113(6):1224-35.

22. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, Miron RJ. Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Investig. 2016 Dec;20(9):2353-60.

23. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. J Periodontol. 2017 Jan;88(1):112-21.

24. Miron RJ, Chai J, Zheng S, Feng M, Sculean A, Zhang Y. A novel method for evaluating and quantifying cell types in platelet-rich fibrin and an introduction to horizontal centrifugation. J Biomed Mater Res A. 2019 Oct;107(10):2257-71.

25. Wang X, Yang Y, Zhang Y, Miron RJ. Fluid platelet-rich fibrin stimulates greater dermal skin fibroblast cell migration, proliferation, and collagen synthesis when compared to platelet-rich plasma. J Cosmet Dermatol. 2019 Dec;18(6):2004-10.

26. Kawase T, Kamiya M, Kobayashi M, Tanaka T, Okuda K, Wolff LF, Yoshie H. The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation. J Biomed Mater Res B Appl Biomater. 2015 May;103(4):825-31.

27. Gheno E, Barros Mouão CF, de Mello-Machado RC, Lourenço ES, Miron RJ, Catarino KFF, Alves AT, Alves GG, Calasans-Maia MD. In vivo evaluation of the biocompatibility and biodegradation of a new denatured plasma membrane combined with liquid PRF (Alb-PRF). Platelets. 2021 May 19;32(4):542-54.

28. Jun SY, Kim HY, Oh HJ, Choi E, Cho MS, Kim HS. Feasibility of autologous plasma gel for tonsil-derived stem cell therapeutics in hyperparathyroidism. Sci Rep. 2018 Aug 9;8(1):11896.

29. Doghaim NN, El-Tatawy RA, El-Hamd Neinaa YM. Assessment of the efficacy and safety of platelet poor plasma gel as autologous dermal filler for facial rejuvenation. J Cosmet Dermatol. 2019 Feb 26. doi: 10.1111/cocd.12876. (Online ahead of print).

30. Barros Mourão CF, Gheno E, Lourenço ESM, de Lima Barbosa R, Kurtzman GM, Javid K, Mavropoulos E, Benedicenti S, Calasans-Maia MD, de Mello Machado RC, Alves GG. Characterization of a new membrane from concentrated growth factors associated with denaturized albumin (Alb-CGF) for clinical applications: a preliminary study. Int J Growth Factors Stem Cells Dent. 2018 May-Aug;1(2):64-9.

31. Garg AK. Autologous blood concentrates. Hanover Park (IL): Quintessence Pub.; 2018. p. 188.

32. Miron RJ, Chai J, Zhang P, Li Y, Wang Y, Barros Mourão CF, Sculean A, Fujioka-Kobayashi M, Zhang Y. A novel method for harvesting concentrated platelet-rich fibrin (C-PRF) with a 10-fold increase in platelet and leukocyte yields. Clin Oral Investig. 2020 Aug;24(8):2819-28.

33. Fujioka-Kobayashi M, Katagiri H, Kono M, Schaller B, Zhang Y, Sculean A, Miron RJ. Improved growth factor delivery and cellular activity using concentrated platelet-rich fibrin (C-PRF) when compared with traditional injectable (i-PRF) protocols. Clin Oral Investig. 2020 Dec;24(12):4373-83.

34. Fujioka-Kobayashi M, Schaller B, Barros Mourão CF, Zhang Y, Sculean A, Miron RJ. Biological characterization of an injectable platelet-rich fibrin mixture consisting of autologous albumin gel and liquid platelet-rich fibrin (Alb-PRF). Platelets. 2021 Jan 2;32(1):74-81. **jCD**

Dr. Miron is the lead educator and researcher at Advanced PRF (Platelet Rich Fibrin) Education in Venice, Florida, and an adjunct visiting faculty in the Department of Periodontology at the University of Bern, Switzerland.

Dr. Davies is the lead educator and lecturer for PRF (Platelet Rich Fibrin) Education in Venice, Florida, and lectures in Florida and California.

Disclosures: Dr. Miron is a stock shareholder in Miron Research and Development in Dentistry. Dr. Davies did not report any disclosures.