

1. Arora, S., et al. (2016). "Quantification of platelets and platelet derived growth factors from platelet-rich-plasma (PRP) prepared at different centrifugal force (g) and time." Transfus Apher Sci.

**INTRODUCTION:** Platelet derived biomaterials represent a key source of cytokines and growth factors extensively used for tissue regeneration; wound healing and tissue repair. Our study was to quantify platelets and growth factors released by PRP when prepared at different centrifugal force (g) and time. **MATERIAL AND METHODS:** Our study was approved by the institutional ethical committee. One hundred millilitres of whole blood (WB) was collected in bag with CPDA as the anticoagulant(AC); (14 mL for 100 mL WB ratio). Nine aliquots of 10 mL each were made from the bag and set of three aliquots were made a group. PRP was prepared at varying centrifugal force (group A: -110 g, group B: -208 g & group C: -440 g) & time (1: -5 min, 2: -10 min & 3: -20 min). Contents of each PRP prepared were analysed. Commercial sandwich ELISA kits were used to quantify the concentrations of CD62P (Diacclone SAS; France), Platelet derived growth factors-AB (Qayee-Bio; China), transforming growth factor-beta1 (DRG; Germany) and vascular endothelial growth factor (Boster Immuno Leader; USA) released in each PRP prepared. **RESULTS:** Eight volunteers were enrolled in the study (24-30 years). The baseline blood counts of all the volunteers were comparable ( $p \geq 0.05$ ). Mean +/- SD of platelet yield of all nine groups ranged from 17.2 +/- 4.2% to 78.7 +/- 5.7%. Each PRP was activated with calcified thromboplastin to quantify the growth factors released by them. Significantly higher ( $p < 0.05$ ) transforming growth factor-beta1 and vascular endothelial growth factor were released compared to the baseline. **CONCLUSION:** Our study highlights the variation in both force (g) and time results in changes at cellular level and growth factor concentrations.

2. Asif, M., et al. (2016). "Combined autologous platelet-rich plasma with microneedling verses microneedling with distilled water in the treatment of atrophic acne scars: a concurrent split-face study." J Cosmet Dermatol.

**BACKGROUND:** Acne scarring causes cosmetic discomfort, depression, low self-esteem and reduced quality of life. Microneedling is an established treatment for scars, although the efficacy of platelet-rich plasma (PRP) has not been explored much. **OBJECTIVE:** The objective of this study was to evaluate the efficacy and safety of platelet-rich plasma (PRP) combined with microneedling for the treatment of atrophic acne scars. **METHODS:** Fifty patients of 17-32 years of age with atrophic acne scars were enrolled. Microneedling was performed on both halves of the face. Intradermal injections as well as topical application of PRP was given on right half of the face, while the left half of the face was treated with intradermal administration of distilled water. Three treatment sessions were given at an interval of 1 month consecutively. Goodman's Quantitative scale and Quantitative scale were used for the final evaluation of results. **RESULTS:** Right and left halves showed 62.20% and 45.84% improvement, respectively, on Goodman's Quantitative scale. Goodman's Qualitative scale showed excellent response in 20 (40%) patients and good response in 30 (60%) patients over right half of the face, while the left half of the face showed excellent response in 5 (10%) patients, good response in 42 (6%) patients and poor response in three patients. **CONCLUSION:** We conclude that PRP has efficacy in the management of atrophic acne scars. It can be combined with microneedling to enhance the final clinical outcomes in comparison with microneedling alone.

3. Bioulac, B., et al. (2015). "[Transfer of autologous fat and plasma : The future of anti-aging medicine?]." HNO 63(7): 497-503.

**BACKGROUND:** Aging is a complex process driven by endogenous and exogenous stimuli. The distinct cellular and noncellular components of skin and adjacent connective tissue are

constantly and irreversibly degraded during aging. OBJECTIVES: The aim was to provide an overview of the biology of skin aging and the therapeutic options for rejuvenation. METHODS: A review of the current literature and a demonstration of autologous fat transfer and platelet-rich plasma (PRP) are presented from a clinical perspective. RESULTS: The aging process affects cellular components and the extracellular matrix (ECM); thus, the first stage is the degradation of the ECM. The loss of skin elasticity is induced by a breakdown of fibers such as collagen, elastin, or reticulin, whereas the degradation of proteoglycans results in decreased turgor and skin hydration. Synthetic filling agents primarily compensate for volume loss, but do not rejuvenate biologically. In contrast, the transfer of autologous fat and PRP is based on activating stem cell populations and growth factors, in addition to providing volume to target regions. CONCLUSIONS: A profound comprehension of the cellular and molecular mechanisms of aging is important in anti-aging medicine. The transfer of autologous fat and PRP offers interesting alternatives in the sense of more biological skin rejuvenation.

4. Bulam, H., et al. (2015). "The Inhibitory Effect of Platelet-Rich Plasma on Botulinum Toxin Type-A: An Experimental Study in Rabbits." *Aesthetic Plast Surg* **39**(1): 134-140.

BACKGROUND: Combination treatments of botulinum toxin type-A and other rejuvenation agents or instruments are gradually becoming more popular. After observing a high incidence of therapy failure following simultaneous applications of botulinum toxin type-A and platelet-rich plasma mesotherapy, we aimed to investigate whether PRP has an inhibitory effect on botulinum toxin type-A. METHODS: Twenty-four New Zealand white rabbits were divided into 4 groups, and the anterior auricular muscle and overlying skin were used for injections. Groups I and II both received onabotulinumtoxinA intramuscular injections. In addition, autologous platelet-rich plasma mesotherapy was performed in Group I while Group II received saline mesotherapy. Group III was designed as the in vitro mixture group in which onabotulinumtoxinA and platelet-rich plasma were mixed and then administered intramuscularly. Group IV received saline within the mixture instead of platelet-rich plasma. The contralateral ears of all the rabbits served as control and were only treated with onabotulinumtoxinA. Visual evaluation of ear positions and electroneuromyographic studies were done prior to all procedures and at day 14. Anterior auricular muscles were harvested at day 14 and were evaluated with quantitative real-time PCR. RESULTS: Visual and electroneuromyographic studies revealed less onabotulinumtoxinA activity in Groups I and III. When platelet-rich plasma was administered through skin mesotherapy, onabotulinumtoxinA activity failure was more severe in comparison with direct contact. No significant difference in SNAP-25 mRNA expression through quantitative real-time PCR was observed between groups. CONCLUSION: Although we could not explain the exact mechanism underlying this interaction, platelet-rich plasma applications result in less onabotulinumtoxinA muscle paralysis activity.

5. Centeno, C. J., et al. (2011). "Is cosmetic platelet-rich plasma a drug to be regulated by the Food and Drug Administration?" *J Cosmet Dermatol* **10**(3): 171-173.

In the area of cosmetic dermatology, some physicians have been injecting activated platelet-rich plasma into the face to promote cosmesis or using it to enhance fat grafts. However, subtle changes to the federal drug code (21 CFR 1271.1) made in 2004, when applied to activated PRP, purport to make this autologous substance a federally regulated drug requiring an extensive and costly Biologics License Application. Are autologous cells drugs? Many physicians believe there are significant problems with this regulatory paradigm.

6. Cervelli, V., et al. (2014). "The effect of autologous activated platelet rich plasma (AA-PRP) injection on pattern hair loss: clinical and histomorphometric evaluation." Biomed Res Int **2014**: 760709.

To investigate the safety and clinical efficacy of AA-PRP injections for pattern hair loss. AA-PRP, prepared from a small volume of blood, was injected on half of the selected patients' scalps with pattern hair loss. The other half was treated with placebo. Three treatments were given for each patient, with intervals of 1 month. The endpoints were hair re-growth, hair dystrophy as measured by dermoscopy, burning or itching sensation, and cell proliferation as measured by Ki-67 evaluation. At the end of the 3 cycles of treatment, the patients presented clinical improvement in the mean number of hairs, with a mean increase of 18.0 hairs in the target area, and a mean increase in total hair density of 27.7 ( number of hairs/cm<sup>2</sup>) compared with baseline values. Microscopic evaluation showed the increase of epidermis thickness and of the number of hair follicles two weeks after the last AA-PRP treatment compared to baseline value ( $P < 0.05$ ). We also observed an increase of Ki67(+) keratinocytes of epidermis and of hair follicular bulge cells and a slight increase of small blood vessels around hair follicles in the treated skin compared to baseline ( $P < 0.05$ ).

7. Cervelli, V., et al. (2009). "Application of platelet-rich plasma in plastic surgery: clinical and in vitro evaluation." Tissue Eng Part C Methods **15**(4): 625-634.

The clinical use of platelet-rich plasma (PRP) for a wide variety of application has been reportedly employed most prevalently in problematic wounds, maxillofacial and hemi-facial atrophy, Romberg Syndrome, and diabetic foot ulcers. To our knowledge, PRP has never been described in the enhancement of fat grafting during tissue-engineering application in vivo. The authors describe the preparation of PRP and its use in a series of 43 patients who underwent plastic, reconstructive, and maxillofacial surgery for chronic lower extremity ulcers ( $n = 18$ ) and multiple facial applications ( $n = 25$ ). PRP mixed with fat grafting was used in 76% patients affected by multiple facial diseases and in 88.9% patients affected by lower extremity ulcers. PRP injection alone was used in the remaining patients. The authors observed that after a 7.1-week and 9.7-week (average) course of twice-daily wound treatment with PRP suspended on a collagen base, 61.1% and 88.9% of chronic lower extremity ulcers underwent to 100% reepithelization compared with 40% and 60% of controls ( $n = 10$ ) treated with hyaluronic acid and collagen medication. In patients treated with reconstructing three-dimensional projection of face by fat grafting and PRP, we observed a 70% maintenance of contour restoring and three-dimensional volume after 1 year compared to only 31% of controls ( $n = 10$ ) treated with fat grafting alone. In vitro, PRP induced a significant increase in the number of adipose-tissue-derived stem cells compared to control cultures. These results documented that PRP accelerates chronic skin ulcer reepithelization and improves maintenance and function of fat graft in patients who underwent plastic reconstructive surgery, possibly by stimulating adipose-tissue-derived stem cell proliferation.

8. Cervelli, V., et al. (2011). "Use of platelet-rich plasma and hyaluronic acid in the loss of substance with bone exposure." Adv Skin Wound Care **24**(4): 176-181.

OBJECTIVE: : In lower-extremity surgery, the complex wound with bone exposure remains a challenging problem for the plastic surgeon. The purpose of this study was to describe a new therapeutic approach to stimulate the regeneration of the lower-extremity complex wounds based on a combined treatment composed of platelet-rich plasma (PRP) and hyaluronic acid (HA) dressing. DESIGN: : Wounds with posttraumatic bone exposure have been treated with HA dressing alone or in combination with PRP. PATIENT: : Fifteen patients affected by lower-extremity wound with posttraumatic bone exposure have been treated at the Department of

Plastic and Reconstructive Surgery, University of Tor Vergata, Rome, Italy. RESULTS: : After a single treatment, the authors observed that the mean re-epithelialization time was 8.1 weeks in 73.3% patients treated with PRP and HA dressing versus the 30% patients treated with HA dressing only. CONCLUSION: : These data confirm the evidence of using PRP technology in the healing of both soft- and hard-tissue wounds. Moreover, the satisfaction of the patient confirms the quality of this study's results.

9. Chawla, S. (2014). "Split Face Comparative Study of Microneedling with PRP Versus Microneedling with Vitamin C in Treating Atrophic Post Acne Scars." *J Cutan Aesthet Surg* 7(4): 209-212.

INTRODUCTION: Acne scars are largely preventable complications of acne. 95% of the scars occur over the face thus impacting the quality of life. Correction of scars is the priority for acne patients. MATERIALS AND METHODS: Thirty patients with post acne atrophic facial scars attending the OPD during the period from April to October 2013 were offered four sittings of microneedling with PRP on one side and microneedling with vitamin C on other side of the face at an interval of 1 month. RESULTS: Twenty-seven out of the total 30 patients completed the treatment schedule. Two patients were lost to follow up and one dropped out of the study due to severe PIH. Mean age of the patients was 27.5 years. Out of 30 patients, 23 achieved reduction in scarring by one or two grades. Excellent response was seen in five (18.5%) patients with platelet-rich plasma (PRP) as compared to two (7%) patients who received treatment with vitamin C according to physician's assessment. As far as up gradation by 1 score is considered, i.e., good response, it was similar in both cases. Vitamin C did not prove to be as efficacious as PRP since 10 (37%) patients had poor response in vitamin C-treated area compared to only 6 (22.2%) patients who underwent PRP therapy, but vitamin C proved to be efficacious in dealing with post inflammatory hyper-pigmentation secondary to acne. Patients were more satisfied with PRP as compared to vitamin C. The results were evaluated and statistical analysis was done using SPSS 16.0.2. CONCLUSIONS: Overall results were better with microneedling and PRP. Vitamin C combined with microneedling also showed improvement with respect to firmness and smoothness of skin; as well as post inflammatory hyper-pigmentation. Microneedling combined with PRP proved to be good in treating boxcar and rolling scars but had limited efficacy in dealing with ice pick scars.

10. Cho, J. M., et al. (2011). "Effect of platelet-rich plasma on ultraviolet b-induced skin wrinkles in nude mice." *J Plast Reconstr Aesthet Surg* 64(2): e31-39.

BACKGROUND: Platelet-rich plasma (PRP) is researched and used in many clinical fields as it contains an abundance of various growth factors. Recently, a topical injection of PRP has been clinically tried for treatment of photoageing-related skin wrinkles. Nevertheless, there have been only a few studies including objective data or explaining the mechanisms of PRP. Therefore, the authors performed animal experiments to collect laboratory data and to infer the basal mechanism of the effect of PRP on skin rejuvenation. METHODS: Mice photoaged by ultraviolet B (UVB) irradiation for 8 weeks were divided into three groups (no-treatment group, saline injected group and PRP-injected group) with 10 mice in each group. After 4 weeks, the degree of wrinkle formation was compared among three groups by replica analysis, and skin biopsies were performed. An additional in vitro assay with growth-factor-neutralising antibodies was performed to evaluate whether growth factors contained in PRP could accelerate fibroblast proliferation and collagen production, which may play a major role in skin rejuvenation. RESULTS: The wrinkles in the PRP-injected group were significantly reduced than in the other groups. Biopsy results indicated that the dermal layer was remarkably thicker in the PRP-injection group. In in vitro assay, fibroblast proliferation and

collagen production were increased in the experimental group through growth factors in the PRP. CONCLUSION: Although more in vivo studies and research about the mechanism of PRP are required, the results of this study indicate that PRP is effective in the rejuvenation of photoaged skin.

11. Cieslik-Bielecka, A., et al. (2012). "L-PRP/L-PRF in esthetic plastic surgery, regenerative medicine of the skin and chronic wounds." *Curr Pharm Biotechnol* **13**(7): 1266-1277.

The use of platelet concentrates for topical use is of particular interest for the promotion of skin wound healing. Fibrin-based surgical adjuvants are indeed widely used in plastic surgery since many years in order to improve scar healing and wound closure. However, the addition of platelets and their associated growth factors opened a new range of possibilities, particularly for the treatment of chronic skin ulcers and other applications of regenerative medicine on the covering tissues. In the 4 families of platelet concentrates available, 2 families were particularly used and tested in this clinical field: L-PRP (Leukocyte- and Platelet-rich Plasma) and L-PRF (Leukocyte- and Platelet-Rich Fibrin). These 2 families have in common the presence of significant concentrations of leukocytes, and these cells are important in the local cleaning and immune regulation of the wound healing process. The main difference between them is the fibrin architecture, and this parameter considerably influences the healing potential and the therapeutical protocol associated to each platelet concentrate technology. In this article, we describe the historical evolutions of these techniques from the fibrin glues to the current L-PRP and L-PRF, and discuss the important functions of the platelet growth factors, the leukocyte content and the fibrin architecture in order to optimize the numerous potential applications of these products in regenerative medicine of the skin. Many outstanding perspectives are appearing in this field and require further research.

12. Fabi, S. and H. Sundaram (2014). "The potential of topical and injectable growth factors and cytokines for skin rejuvenation." *Facial Plast Surg* **30**(2): 157-171.

Growth factors and cytokines (referred to collectively hereafter as GFs) control cell growth, proliferation, and differentiation via a network of inter and intracellular signaling pathways. There are striking parallels between the pathways involved in skin wound healing and those implicated in photoaging of the skin. In recent years, topical and injectable GFs have emerged as an intriguing therapeutic modality that can be harnessed for aesthetic and medical purposes. This article provides a review of available evidence for the role in skin regeneration of topical GFs, and of injectable GFs contained in autologous platelet-rich plasma (PRP). It presents data from recent studies of GFs, offers a discussion of their potential to serve as antiaging actives, and includes safety considerations. As studies of injectable GFs typically assume preexisting familiarity with PRP protocols and the theory behind them, explanatory notes are provided. An assessment is provided of the evidence gaps that exist currently between experimental observations regarding GFs and their proven clinical benefits. Data of evidence levels II and III support the use for skin rejuvenation of topical GFs derived from sources including secretions or lysate of human dermal fibroblasts, and secretions of the snail *Cryptomphalus aspersa*. GFs with associated stem cell proteins, secreted by human dermal fibroblasts under hypoxic stress, can accelerate skin healing after laser resurfacing. In vitro and animal studies, small case series of PRP-treated patients and one prospective clinical study of its variant, platelet-rich fibrin matrix (PRFM), suggest the value of injectable GFs for skin rejuvenation. However, data of higher power are required to expand this proof of concept into an evidence-based paradigm. The clinical applications of topical and injectable GFs are promising, and remain to be fully defined. With continued study, data of higher evidence level can be accrued and formulations can be developed that

offer optimal clinical efficacy, safety, tolerability, and stability. Better understanding of the mechanism of action of GFs can potentially advance our general understanding of dermal signaling pathways, and hence of hyaluronic acid and other alloplastic fillers; and allow the development of protocols for synergistic combination of GFs with other skin rejuvenation modalities.

13. Fukaya, M. and A. Ito (2014). "A New Economic Method for Preparing Platelet-rich Plasma." Plast Reconstr Surg Glob Open **2**(6): e162.

**BACKGROUND:** Although platelet-rich plasma (PRP) is nowadays a common method in various medical fields, including cosmetic surgery or dermatology, the expensiveness of the kit for processing is still a hurdle. **METHODS:** A new unique economic method for preparing PRP was reported. The method consists in a simple modification of a disposable 5-mL syringe that allows insertion into a common centrifuge and positioning of the syringe on the centrifuge so the PRP separates next to the tip of the syringe. Platelet-derived growth factor BB in PRP was measured under anticoagulant dextrose solution A (ACD-A) or heparin as anticoagulant and with or without prostaglandin E1 (PGE1) as a platelet aggregation suppressant. **RESULTS:** The new method successfully created PRP with high platelet-derived growth factor BB in all conditions, and the highest value was obtained by using ACD-A and PGE1. **CONCLUSIONS:** The new method is useful, and the use of ACD-A and PGE1 is the most recommended.

14. Gentile, P., et al. (2015). "The Effect of Platelet-Rich Plasma in Hair Regrowth: A Randomized Placebo-Controlled Trial." Stem Cells Transl Med **4**(11): 1317-1323.

Platelet-rich plasma (PRP) has emerged as a new treatment modality in regenerative plastic surgery, and preliminary evidence suggests that it might have a beneficial role in hair regrowth. Here, we report the results of a randomized, evaluator-blinded, placebo-controlled, half-head group study to compare, with the aid of computerized trichograms, hair regrowth with PRP versus placebo. The safety and clinical efficacy of autologous PRP injections for pattern hair loss were investigated. PRP, prepared from a small volume of blood, was injected on half of the selected patients' scalps with pattern hair loss. The other half was treated with placebo. Three treatments were administered to each patient at 30-day intervals. The endpoints were hair regrowth, hair dystrophy as measured by dermoscopy, burning or itching sensation, and cell proliferation as measured by Ki67 evaluation. Patients were followed for 2 years. Of the 23 patients enrolled, 3 were excluded. At the end of the 3 treatment cycles, the patients presented clinical improvement in the mean number of hairs, with a mean increase of 33.6 hairs in the target area, and a mean increase in total hair density of 45.9 hairs per cm<sup>2</sup> compared with baseline values. No side effects were noted during treatment. Microscopic evaluation showed the increase of epidermis thickness and of the number of hair follicles 2 weeks after the last PRP treatment compared with baseline value ( $p < .05$ ). We also observed an increase of Ki67(+) keratinocytes in the epidermis and of hair follicular bulge cells, and a slight increase of small blood vessels around hair follicles in the treated skin compared with baseline ( $p < .05$ ). Relapse of androgenic alopecia was not evaluated in all patients until 12 months after the last treatment. After 12 months, 4 patients reported progressive hair loss; this was more evident 16 months after the last treatment. Those four patients were re-treated. Our data clearly highlight the positive effects of PRP injections on male pattern hair loss and absence of major side effects. PRP may serve as a safe and effective treatment option against hair loss; more extensive controlled studies are needed. **SIGNIFICANCE:** Platelet-rich plasma (PRP) has emerged as a new treatment modality in regenerative plastic surgery, and preliminary evidence suggests that it might have a beneficial role in hair regrowth. Here, the results of a

randomized, placebo-controlled, half-head group study to compare the hair regrowth with PRP versus placebo are reported. Hair regrowth was quantified by a blinded evaluator using computerized trichograms. The safety and clinical efficacy of autologous PRP injections for pattern hair loss were investigated. Of the 23 patients enrolled, 3 were excluded. At the end of the 3 treatment cycles, the patients presented clinical improvement in the mean number of hairs, with a mean increase of 33.6 hairs in the target area and a mean increase in total hair density of 45.9 hairs per cm<sup>2</sup> compared with baseline values. No side effects were noted during treatment. The data clearly highlight the positive effects of PRP injections on male pattern hair loss and absence of major side effects. PRP may serve as a safe and effective treatment option against hair loss; more extensive controlled studies are needed.

15. Ibrahim, Z. A., et al. (2015). "Comparison between the efficacy and safety of platelet-rich plasma vs. microdermabrasion in the treatment of striae distensae: clinical and histopathological study." J Cosmet Dermatol **14**(4): 336-346.

Striae distensae is a challenging cosmetic problem for which various treatment modalities have been applied. To compare between the efficacy and tolerability of intradermal injection of autologous platelet-rich plasma (PRP) vs. microdermabrasion in the treatment of striae distensae. Sixty-eight patients with striae distensae were randomly assigned to three groups according to therapeutic modalities. Patients of group I were treated by intradermal injection of PRP alone, patients of group II were treated with microdermabrasion alone, and patients of group III were treated with combination of intradermal PRP and microdermabrasion in the same session. Each patient underwent maximum of six sessions at 2-week interval. Skin biopsies were taken from some patients at baseline, and 3 months after the last sessions stained with hematoxylin and eosin stain, Masson trichrome, orceun, and Van Gieson stains to study of histopathological changes and efficacy of treatment. There was significant clinical improvement of striae distensae in patients treated with PRP injection and patients treated with combination of PRP and microdermabrasion when compared with patients treated with microdermabrasion. However, combination of PRP and microdermabrasion in the same session showed better results in short duration. Collagen and elastic fibers were markedly increased in the dermis at the end of treatment sessions. Platelet-rich plasma alone is more effective than microdermabrasion alone in the treatment of striae distensae, but it is better to use the combination of both for more and rapid efficacy. However, each one of them is well tolerated by the patients, safe and cost effective.

16. Kawazoe, T. and H. H. Kim (2012). "Tissue augmentation by white blood cell-containing platelet-rich plasma." Cell Transplant **21**(2-3): 601-607.

Platelet-rich plasma (PRP) is a matrix of fibrin and platelets that releases cytokines that are important in wound healing. PRP is produced from the patient's blood and therefore has less risk of allergic reaction and infection. We have obtained PRP with an enhanced white blood cell component (W-PRP) by optimizing the centrifugal separation of PRP from plasma. Here we show that injection of W-PRP into the auricle of nude mice gave greater tissue augmentation compared to PRP. Further augmentation occurred when bFGF was added to W-PRP, and there was a significant increase in the number of alpha-smooth muscle actin-positive cells in mice treated with W-PRP+bFGF. Our results suggest that W-PRP may have value in cosmetic surgery aimed at rejuvenation of wrinkled and sagging skin. W-PRP injection constitutes a new concept in cell transplantation, in which cells required for tissue regeneration are induced by cytokines released from the transplanted cells.

17. Kim, D. H., et al. (2011). "Can Platelet-rich Plasma Be Used for Skin Rejuvenation? Evaluation of Effects of Platelet-rich Plasma on Human Dermal Fibroblast." Ann Dermatol **23**(4): 424-431.

**BACKGROUND:** Autologous platelet-rich plasma has attracted attention in various medical fields recently, including orthopedic, plastic, and dental surgeries and dermatology for its wound healing ability. Further, it has been used clinically in mesotherapy for skin rejuvenation. **OBJECTIVE:** In this study, the effects of activated platelet-rich plasma (aPRP) and activated platelet-poor plasma (aPPP) have been investigated on the remodelling of the extracellular matrix, a process that requires activation of dermal fibroblasts, which is essential for rejuvenation of aged skin. **METHODS:** Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared using a double-spin method and then activated with thrombin and calcium chloride. The proliferative effects of aPRP and aPPP were measured by [(3)H]thymidine incorporation assay, and their effects on matrix protein synthesis were assessed by quantifying levels of procollagen type I carboxy-terminal peptide (PIP) by enzyme-linked immunosorbent assay (ELISA). The production of collagen and matrix metalloproteinases (MMP) was studied by Western blotting and reverse transcriptase-polymerase chain reaction. **RESULTS:** Platelet numbers in PRP increased to 9.4-fold over baseline values. aPRP and aPPP both stimulated cell proliferation, with peak proliferation occurring in cells grown in 5% aPRP. Levels of PIP were highest in cells grown in the presence of 5% aPRP. Additionally, aPRP and aPPP increased the expression of type I collagen, MMP-1 protein, and mRNA in human dermal fibroblasts. **CONCLUSION:** aPRP and aPPP promote tissue remodelling in aged skin and may be used as adjuvant treatment to lasers for skin rejuvenation in cosmetic dermatology.

18. Kushida, S., et al. (2013). "Effects of platelet-rich plasma on proliferation and myofibroblastic differentiation in human dermal fibroblasts." *Ann Plast Surg* **71**(2): 219-224.

Platelet-rich plasma (PRP) is plasma that is produced from autologous blood, and its usefulness in plastic surgery and dermal wound healing has garnered attention in recent years. The aim of this study was to investigate the effects of PRP and platelet-poor plasma on the proliferation and differentiation of skin fibroblasts into myofibroblasts and on wound contraction using Western blotting, immunofluorescence staining, and collagen gels containing an embedded fibroblast model. PRP promotes proliferation of human dermal fibroblasts. PRP addition enhanced the expression of alpha-smooth muscle actin protein, a myofibroblast marker, as shown by immunofluorescence staining and Western blotting. PRP-treated groups demonstrated more marked contraction in the collagen gel model than the platelet-poor plasma and vehicle groups. PRP promotes proliferation, causes the differentiation of human dermal fibroblasts into myofibroblasts and promotes wound contraction, thus providing a potential therapeutic agent for skin wound healing.

19. Lee, S. H., et al. (2015). "Therapeutic efficacy of autologous platelet-rich plasma and polydeoxyribonucleotide on female pattern hair loss." *Wound Repair Regen* **23**(1): 30-36.

Autologous platelet-rich plasma (PRP) exerts positive therapeutic effects on hair thickness and density in patients with pattern hair loss. The aim of our study was to evaluate the efficacy of intra-perifollicular autologous PRP and polydeoxyribonucleotide (PDRN) injections in treating female pattern hair loss (FPHL). Twenty FPHL patients were treated with a single session of PRP injection, followed by 12 sessions of PDRN intra-perifollicular injection, along the scalp at weekly intervals. Additionally, another 20 FPHL patients were treated with 12 sessions of PDRN injection only. Meanwhile, one half of the backs of two rabbits was injected with the PRP preparation, while the other half was injected with phosphate buffered saline as a control. Tissue samples from the rabbits were analyzed by real-time polymerase chain reaction and Western blotting. Compared with baseline values, patients treated with PRP and PDRN injections exhibited clinical improvement in mean hair counts (23.2 +/- 15.5%; p < 0.001) and mean hair thickness (16.8 +/- 10.8%; p < 0.001). In addition, patients treated with

the 12 sessions of intra-perifollicular PDRN injection alone also showed clinical improvement in mean hair counts (17.9 +/- 13.2%;  $p < 0.001$ ) and mean hair thickness (13.5 +/- 10.7%;  $p < 0.001$ ). Comparison analyses between the two groups revealed that combined therapy with PRP and PDRN induces greater improvement in hair thickness than treatment with PDRN therapy alone ( $p = 0.031$ ), but not in hair counts ( $p > 0.05$ ). The pilot animal study revealed significant up-regulation of WNT, platelet-derived growth factor, and fibroblast growth factor expression in rabbit skin treated with the PRP preparation, compared with control skin. In conclusion, intra-perifollicular injections of autologous PRP and/or PDRN generate improvements in hair thickness and density in FPHL patients.

20. Leo, M. S., et al. (2015). "Systematic review of the use of platelet-rich plasma in aesthetic dermatology." *J Cosmet Dermatol* **14**(4): 315-323.

Platelet-rich plasma (PRP) is a highly concentrated autologous solution of plasma prepared from a patient's own blood. PRP contains platelets that are purported to release numerous growth factors that may be valuable in numerous dermatologic applications. Here, we review systematically the clinical cosmetic applications of PRP including: androgenetic alopecia, scar revision, acne scars, skin rejuvenation, dermal augmentation, and striae distensae to understand the potential and best practices for PRP use. A systematic search was conducted on three databases: Pubmed, Embase, and Web of Science. Publications were included if they were in English, investigated the clinical applications of PRP in aesthetic dermatology and reported clinical results either as case reports or clinical studies. There were a total of 22 manuscripts that fulfilled these criteria. Four evaluated hair-related applications, eight evaluated the treatment of scars and postprocedure recovery, eight evaluated skin rejuvenation and dermal augmentation, and two evaluated treatment of striae distensae. PRP is a relatively new treatment modality with studies suggesting its utility in aesthetic dermatology. The combination of PRP with other therapies is particularly interesting. Future studies should include controls, including incorporation of split-face comparisons, to reduce intersubject variability.

21. Li, Z. J., et al. (2012). "Autologous platelet-rich plasma: a potential therapeutic tool for promoting hair growth." *Dermatol Surg* **38**(7 Pt 1): 1040-1046.

**BACKGROUND:** Recently, autologous platelet-rich plasma (PRP) has attracted attention in various medical fields, including plastic and orthopedic surgery and dermatology, for its ability to promote wound healing. PRP has been tested during facelift and hair transplantation to reduce swelling and pain and to increase hair density. **OBJECTIVE:** To investigate the effects of PRP on hair growth using in vivo and in vitro models. **METHODS:** PRP was prepared using the double-spin method and applied to dermal papilla (DP) cells. The proliferative effect of activated PRP on DP cells was measured. To understand the mechanisms of activated PRP on hair growth, we evaluated signaling pathways. In an in vivo study, mice received subcutaneous injections of activated PRP, and their results were compared with control mice. **RESULTS:** Activated PRP increased the proliferation of DP cells and stimulated extracellular signal-regulated kinase (ERK) and Akt signaling. Fibroblast growth factor 7 (FGF-7) and beta-catenin, which are potent stimuli for hair growth, were upregulated in DP cells. The injection of mice with activated PRP induced faster telogen-to-anagen transition than was seen on control mice. **CONCLUSIONS:** Although few studies tested the effects of activated PRP on hair growth, this research provides support for possible clinical application of autologous PRP and its secretory factors for promotion of hair growth.

22. Lubkowska, A., et al. (2012). "Growth factor content in PRP and their applicability in medicine." J Biol Regul Homeost Agents **26**(2 Suppl 1): 3S-22S.

This paper reviews available reports on the advantages and possibilities of clinical use of platelet-rich plasma preparations, with particular emphasis on platelet growth factors. Platelets, an important reservoir of growth factors in the body, play an important role in many processes such as coagulation, immune response, angiogenesis and the healing of damaged tissues. Numerous proteins are contained in the alpha-granules of platelets: platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet factor interleukin (IL), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor IGF and fibronectin. The development of methods and systems for blood and cell sorting (e.g. CAPSS - compact advanced platelet sequestration system Elektromedics 500, PCCS - platelet concentrate collection system Curasan) have made it possible to obtain significant concentrations of platelets (even by 338 percent) and high concentrations of growth factors, in a form of sterile mass that can be used immediately for clinical purposes. Platelet-rich plasma (PRP; autologous platelet-rich plasma - APRP) are platelet concentrates made of autogenous blood with a high number of platelets in a small volume of plasma. The clinical efficacy of platelet concentrates depends mainly on the number of platelets and the concentration of their growth factors, which act as transmitters in most processes in tissues, particularly in healing where they are responsible for proliferation, differentiation, chemotaxis and tissue morphogenesis. They operate as part of autocrine, paracrine and endocrine mechanisms. Growth factors derived from centrifuged blood were first used in patients with chronic skin ulcers. The clinical use of PRP for a wide variety of applications has been reported mostly in oral and maxillo-facial surgery, orthopedic surgery, treatment of soft tissue diseases and injuries, treatment of burns, hard-to-heal wounds, tissue engineering and implantology.

23. Mehryan, P., et al. (2014). "Assessment of efficacy of platelet-rich plasma (PRP) on infraorbital dark circles and crow's feet wrinkles." J Cosmet Dermatol **13**(1): 72-78.

BACKGROUND: Infraorbital skin hyperpigmentation, commonly called dark circles, and crow's feet wrinkles are common cosmetic concerns. Various methods of treatment have been evaluated with variable outcomes. OBJECTIVE: This study was performed to assess the efficacy of platelet-rich plasma (PRP) injection for treating periorbital dark circles and crow's feet. METHODS: Ten participants with a mean age of 41.2 years were treated in a single session with intradermal injections of 1.5 mL PRP into tear trough area and crow's feet wrinkles on each side. The effects on melanin content, color homogeneity of the treated area, epidermal stratum corneum hydration, and wrinkle volume and visibility index were compared 3 months after treatment with baseline. Physician's global assessment and participants' satisfaction and any potential side effects were also assessed. RESULTS: The improvement in infraorbital color homogeneity was statistically significant ( $P = 0.010$ ), but no statistically significant changes were observed in melanin content, stratum corneum hydration, wrinkle volume, and visibility index. Participant's satisfaction score and physician's global assessment score were 2.2 and 1.7, respectively, on a 0-3 scale. CONCLUSION: Platelet-rich plasma may have the potential to improve infraorbital dark circle in terms of color homogeneity of the region, though this remains to be proven using larger, controlled studies using multiple injections.

24. Miao, Y., et al. (2013). "[Effect of PRP on the proliferation of dermal papilla cells and hair follicle regeneration in mice]." Zhonghua Zheng Xing Wai Ke Za Zhi **29**(2): 131-135.

OBJECTIVE: To investigate the effects of platelet-rich plasma (PRP) on the proliferation of dermal papilla cells (DPCs) and hair follicle regeneration. METHODS: PRP was prepared using

the double-spin method and applied to DPCs. The proliferative effect of activated PRP on DPCs was measured using MTT assay. To understand the influence of activated PRP on the hair-inductive capacity of DPCs, freshly isolated epidermal cells and DPCs of passage 4 were resuspended, mixed with various concentrations of a PRP (0%, 5% or 10%) and were then transferred to a grafting chamber, which was implanted onto the dorsal skin of nude mice. The chambers were removed 1 week after grafting and HF formation was monitored for 4 weeks; the graft site was harvested and processed for histological examination. RESULTS: Activated PRP increased the proliferation benefited the aggregative growth of DPCs. There are significant difference in the yield of hair follicles compared with 10% PRP (344 +/- 27) with 0% PRP (288 +/- 35) in the area of reconstituted skin ( $P < 0.05$ ). The areas treated with PRP demonstrated an increase in hair follicles density of 19.4%. Ten percent PRP (18 +/- 1) d also can significantly shorten the time of hair formation, compared with 0% PRP (20 +/- 1) d ( $P < 0.05$ ). CONCLUSIONS: There is a considerable effect of PRP on the time of hair formation and the yield of hair follicles reconstitution.

25. Miao, Y., et al. (2013). "Promotional effect of platelet-rich plasma on hair follicle reconstitution in vivo." *Dermatol Surg* **39**(12): 1868-1876.

BACKGROUND: Platelet-rich plasma (PRP) containing various growth factors has attracted attention in various medical fields. PRP has recently been used during hair transplantation to increase hair density. OBJECTIVE: To investigate the effects of PRP on hair follicle (HF) reconstitution. METHODS AND MATERIALS: Freshly isolated epidermal cells and cultured dermal papilla cells (DPCs) were mixed with various concentrations of activated PRP and transferred to a grafting chamber that was implanted onto the dorsal skin of nude mice. The chambers were removed 1 week after grafting, and HF formation was monitored for 4 weeks. RESULTS: We observed a significant difference ( $p < .05$ ) in the number of newly formed follicles in the area of reconstituted skin (344 +/- 27 with 10% PRP vs 288 +/- 35 without PRP). PRP also shortened the time of hair formation significantly; the first hairs were observed in 18 +/- 1 days using 10% PRP, versus 20 +/- 1 days without PRP. CONCLUSION: A considerable effect of PRP on the time of hair formation and the yield of HF reconstitution was observed in this study. Considering the limited evidence available to judge its efficacy, further studies are required to investigate the mechanism of action of PRP.

26. Pinto, J. M., et al. (2014). "Application of platelet-rich plasma in the treatment of chronic skin ulcer - case report." *An Bras Dermatol* **89**(4): 638-640.

The platelet-rich plasma (PRP) has proved promising regarding its applicability in dermatology, especially in the healing of chronic ulcers. The autologous platelet-rich plasma is obtained by centrifuging the blood, so that the components are separated by density gradient. The final product is a gel rich in growth factors that act in tissue repair by activating fibroblasts and inducing extracellular matrix remodeling.

27. Ramos-Torrecillas, J., et al. (2014). "Clinical utility of growth factors and platelet-rich plasma in tissue regeneration: a review." *Wounds* **26**(7): 207-213.

INTRODUCTION: Many elements are involved in the regenerative process of injured tissues, such as cytokines and growth factors. Growth factors contained in platelets represent an important physiological mixture of factors that may be involved in the healing process. METHOD: A literature review on the role played by certain biomolecules, such as growth factors and platelet-rich plasma (PRP), in the healing of different tissues and on their therapeutic use to promote this process was undertaken. RESULTS: In this review, the authors found a great number of studies that showed the effect of growth factors and PRP in the regeneration of different tissues. Platelet-rich plasma is a natural source of growth

factors and this has been successfully used in different clinical situations such as dentistry, plastic surgery, trauma and orthopedics, ocular surgery, gastroenterology, or skin ulcers. However, standardized protocol for its preparation and application has yet to be established. CONCLUSION: The authors recommend further research is required to establish the exact role of PRP in the treatment of tissue regeneration and to develop a standardized protocol for its use.

28. Redaelli, A., et al. (2010). "Face and neck revitalization with platelet-rich plasma (PRP): clinical outcome in a series of 23 consecutively treated patients." *J Drugs Dermatol* 9(5): 466-472.

BACKGROUND: Platelet-rich plasma (PRP) has long been known as an effective treatment in various surgical and medical fields. Face and neck revitalization with PRP is an application that is currently being explored. The aim of this paper is practical: to evaluate if there are real outcomes, benefits and side effects of a standardized injection protocol in a continuous series, without control groups. MATERIALS AND METHODS: In a three-month study, a consecutive series of 23 patients were treated with one session of injections with PRP every month from September 2008 to December 2008 (a total of three sessions). For blood management, a sterile Regen Lab Kit was used. Patients received 4 mL of PRP, activated with calcium chloride, at standard injection points into face and neck skin. The study was documented with imaging before and after each session using a dermoscope, a digital camera, as well as a comprehensive state-of-the-art imaging system and dedicated medical imaging software. RESULTS: The results were evaluated one month after the last session (January 2009) by a special spider improvement score, a photograph score, a patient's satisfaction score and a doctor's satisfaction score. Finally, a definitive graduated score was calculated for each patient. Overall, the results were satisfactory. No serious and persistent side effects were detected. CONCLUSION: Face and neck revitalization with PRP is a promising easy-to-perform technique in face and neck rejuvenation and scar attenuation. Further work needs to be carried out to investigate its exact mechanism of action.

29. Rigotti, G., et al. (2016). "Expanded Stem Cells, Stromal-Vascular Fraction, and Platelet-Rich Plasma Enriched Fat: Comparing Results of Different Facial Rejuvenation Approaches in a Clinical Trial." *Aesthet Surg J* 36(3): 261-270.

BACKGROUND: In a previous study, the authors demonstrated that treatment with expanded adipose-derived stem cells or stromal vascular fraction (SVF)-enriched fat modify the pattern of the dermis in human beings, representing a skin rejuvenation effect. Considering that expanded stem cells require a cell factor, the authors wanted to assess similar results by replacing them with platelet-rich plasma (PRP), which is easier to obtain and for which an empirical regenerative effect has been already described. OBJECTIVES: To determine if PRP injection could replace the cutaneous regenerative effect of adipose-derived stem cells. METHODS: This study was performed in 13 patients who were candidates for facelift. The patients underwent sampling of fat by liposuction from the abdomen and submitted to one of three protocols: injection of SVF-enriched fat or expanded adipose-derived stem cells or fat plus PRP in the preauricular areas. Fragments of skin were removed before and 3 months after treatment and analyzed by optical and electron microscopy. RESULTS: The use of fat plus PRP led to the presence of more pronounced inflammatory infiltrates and a greater vascular reactivity, increasing in vascular permeability and a certain reactivity of the nervous component. The addition of PRP did not improve the regenerative effect. CONCLUSION: The use of PRP did not have significant advantages in skin rejuvenation over the use of expanded adipose-derived stem cells or SVF-enriched fat. The effect of increased vascular reactivity may be useful in pathological situations in which an intense angiogenesis is desirable, such as tissular ischemia. LEVEL OF EVIDENCE 4: Therapeutic.

30. Shin, M. K., et al. (2012). "Platelet-rich plasma combined with fractional laser therapy for skin rejuvenation." *Dermatol Surg* **38**(4): 623-630.

**BACKGROUND AND OBJECTIVES:** Platelet-rich plasma (PRP) is an autologous concentration of human platelets contained in a small volume of plasma and has recently been shown to accelerate wound healing and rejuvenate aging skin. The current study was conducted to determine whether there are additional effects of PRP combined with fractional laser therapy. **MATERIALS AND METHODS:** Twenty-two Korean women underwent three sessions of fractional laser; 11 were treated with topical application of PRP combined with fractional laser. Evaluations were done at baseline and 1 month after the final treatment. The outcome assessments included subjective satisfaction scale; blinded clinical assessment; and the biophysical parameters of roughness, elasticity, skin hydration, and the erythema and melanin index. Biopsies were analyzed using hematoxylin and eosin, Masson-trichrome, and immunohistochemistry for matrix metalloproteinase-1. **RESULTS:** PRP combined with fractional laser increased subject satisfaction and skin elasticity and decreased the erythema index. PRP increased the length of the dermoepidermal junction, the amount of collagen, and the number of fibroblasts. **CONCLUSION:** PRP with fractional laser treatment is a good combination therapy for skin rejuvenation. Keratinocyte and fibroblast proliferation and collagen production can explain the capacity of PRP to increase dermal elasticity.

31. Venter, N. G., et al. (2016). "Use of platelet-rich plasma in deep second- and third-degree burns." Burns.

Unfortunately burns are a common occurrence, leading to scarring or death. Platelet-rich plasma (PRP) contains many growth factors that can accelerate wound healing. We analyzed the use of PRP in deep second-degree (dSD), deep second-degree associated with diabetes mellitus (dSDD), and third-degree (TD) burns in rats. Sixty syngeneic rats divided into three groups (dSD, dSDD, and TD) were burned, half receiving topical PRP and half being used as control; 10 additional rats per group were used for PRP preparation. On day 21, the animals were sacrificed and skin biopsies were collected. dSD and dSDD wounds treated with PRP showed faster wound closure, reduction in CD31-, CD68-, CD163-, MPO-, and in TGF-beta-positive cells, and an increase in MMP2-positive cells. The neo-epidermis was thinner in the control of both the dSD and dSDD groups and granulation tissue was less reduced in the control of both the dSDD and TD groups. These results indicate that PRP can accelerate the healing process in dSD and dSDD, but not in TD burns.

32. Willemsen, J. C., et al. (2014). "The effects of platelet-rich plasma on recovery time and aesthetic outcome in facial rejuvenation: preliminary retrospective observations." Aesthetic Plast Surg **38**(5): 1057-1063.

**BACKGROUND:** This study focused on the possible effect of platelet-rich plasma (PRP) on recovery time and aesthetic outcome after facial rejuvenation. We conducted a retrospective analysis with regard to recovery time and the aesthetic improvement after treatment among four groups of patients: those treated with fat grafting only (Group I), those treated with fat grafting and PRP (Group II), those treated with a minimal access cranial suspension (MACS)-lift and fat grafting (Group III), and those treated with a MACS-lift, fat grafting, and PRP (Group IV). **METHODS:** For the first part of this study, i.e., evaluation of recovery time after surgery, the following selection criteria were used: nonsmoking females, aged 35-65 years, with a complete documented follow-up. In total, 82 patients were included in the evaluation of patient-reported recovery time. For the second part of the study, i.e., evaluation of potential differences in aesthetic outcome, the records of these 82 patients were screened for the presence of pre- and postoperative standardized photographs in three views (AP,

lateral, and oblique), leaving 37 patients to evaluate. A questionnaire was developed to evaluate the aesthetic outcome in all four groups of patients. This questionnaire was given to an expert panel that consisted of ten plastic surgeons. RESULTS: The addition of PRP to a lipofilling procedure resulted in a significant drop in the number of days needed to recover before returning to work or to restart social activities [Group I (no PRP) took 18.9 days vs Group II (PRP) took 13.2 days,  $p = 0.019$ ]. There seemed to be no effect when PRP was added to a MACS-lift + lipofilling procedure. Also, the aesthetic outcome of the lipofilling and MACS-lift + lipofilling groups that received PRP (Groups II and IV) was significantly better than the groups without PRP (Groups I and III). CONCLUSIONS: Adding PRP to facial lipofilling reduces recovery time and improves the overall aesthetic outcome of a MACS-lift. LEVEL OF EVIDENCE V: This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors [www.springer.com/00266](http://www.springer.com/00266).

33. Yuksel, E. P., et al. (2014). "Evaluation of effects of platelet-rich plasma on human facial skin." J Cosmet Laser Ther **16**(5): 206-208.

INTRODUCTION: Platelet-rich plasma (PRP) has been used for rapid healing and tissue regeneration in many fields of medicine. This study was conducted to evaluate the effects of PRP application procedure on human facial skin. METHODS: PRP was applied thrice at 2-week intervals on the face of ten healthy volunteers. It was applied to individual's forehead, malar area, and jaw by a dermaroller, and injected using a 27-gauge injector into the wrinkles of crow's feet. Participants were asked to grade on a scale from 0 to 5 for general appearance, skin firmness-sagging, wrinkle state and pigmentation disorder of their own face before each PRP procedure and 3 months after the last PRP procedure. While volunteers were evaluating their own face, they were also assessed by three different dermatologists at the same time by the same five-point scale. RESULTS: There was statistically significant difference regarding the general appearance, skin firmness-sagging and wrinkle state according to the grading scale of the patients before and after three PRP applications. Whereas there was only statistically significant difference for the skin firmness-sagging according to the assessment of the dermatologists. CONCLUSION: PRP application could be considered as an effective procedure for facial skin rejuvenation.

34. Zhu, J. T., et al. (2013). "The efficacy of autologous platelet-rich plasma combined with erbium fractional laser therapy for facial acne scars or acne." Mol Med Rep **8**(1): 233-237.

The aim of this study was to evaluate the efficacy of autologous platelet-rich plasma (PRP) combined with erbium fractional laser therapy for facial acne or acne scars. PRP combined with erbium fractional laser therapy was used for the treatment of 22 patients, including 16 patients who suffered from facial acne scars and 6 patients who suffered from acne scars concomitant with acne. Whole blood (40 ml) was collected from each patient, and following differential centrifugation, PRP was harvested. After using an erbium fractional laser, we applied PRP to the entire face of every patient. Digital photos were taken before and after the treatment for evaluation by dermatologists and the patients rated the efficacy on a 5-point scale. The erythema was moderate or mild, while its total duration was <3 days; after receiving the treatment three times, 90.9% of the patients showed an improvement of >50%, and 91% of the patients were satisfied; no acne inflammation was observed after treatment. PRP combined with erbium fractional laser therapy is an effective and safe approach for treating acne scars or acne, with minimal side-effects, and it simultaneously enhanced the recovery of laser-damaged skin.