Micro-Needling Depth Penetration, Presence of Pigment Particles, and Fluorescein-Stained Platelets: Clinical Usage for Aesthetic Concerns

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Abstract

Background: Aesthetic micro-needling (MN) has demonstrated skin permeability to cosmeceutical ingredients and platelet-rich plasma by creating reversible micro-channels in the skin.

Objectives: The purposes of this study were to determine: (1) actual needle depth-penetrations by adjusting needle lengths in a disposable tip of an electric MN device; (2) time-dependent passage of pigment and platelets; and (3) safety and efficacy profiles in patients.

Methods: Excised micro-needled pre-auricular skin was used to determine actual depths of tissue penetration with six needle lengths, and the presence of massaged pigment particles (histological examination) and fluorescein-labeled platelets (confocal laser microscopy) in 1 mm depth micro-channels over an hour. Patients were treated for wrinkles and skin laxity, scars, and alopecia with cosmeceuticals and plasma-rich platelets.

Results: Actual needle penetrations closely matched settings up to 1.0 mm, but were less consistent at settings from 1.5 to 2.5 mm. The optimal time for massaging pigment particles and labeled platelet-rich plasma (PRP) down 1.0 mm micro-channels was between 5 to 30 minutes after MN. Patients treated in the Skin Care Center (cosmeceuticals, 0.25-1 mm depth) and Surgical Center (PRP, 0.25-2.5 mm) demonstrated statistically significant improvements ($P \leq .05$) in wrinkle effacement, skin laxity, scar softening, and hair growth by Patient and Observer Satisfaction Scores at 12 months. The average hair count in a 10 mm spot size at baseline (88.3 ± 22.5) increased at the 12 month evaluation period (133.6 ± 13.8). All patients experienced minimal side-effects.

Conclusions: MN alone or in combination therapy resulted in safe and effective treatments from implemented guidelines.

Level of Evidence: 3

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Micro-needling (MN) of skin, a hybrid approach between transdermal patches and hypodermic needling, temporarily disrupts and bypasses the formidable bilayered lipid barrier of the stratum corneum. The production of numerous micro-channels in the dermis activates normal wound healing in both skin layers and concomitantly serves as an effective delivery system for macromolecules and cell fragments such as cosmeceuticals,[1] pharmaceuticals,[2-4] peptides,[5-9] and gene therapy and vaccines[10-14] for therapeutic[15] and cosmetic utility.[16,17]

The purposes of this study were to determine: (1) actual depth-penetrations by adjustable needle lengths of a uniform array of standardized needles in a disposable tip of an electric MN device; (2) time-dependent dissemination of pigment particles and fluorescein-stained platelets down reversible micro-channels; and (3) safety and efficacy profiles in patients treated with MN alone or in combination with topical cytokine-growth factors in cosmeceuticals or in platelet-rich plasma (PRP).

METHODS

Validation Study Procedures

Three healthy patients in the author's private practice consented to undergo a superficial muscular aponeurotic-face/

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necklift procedure and utilization of their excised pre-auricular skin for one of three Micro-needling Validation Studies. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki. The three patients included in the study provided written informed consent. Patients enrolled in the ex vivo verification investigations did not receive compensation for participation and were not financially responsible for any special studies in the validation research.

Study I: Depth-Penetration Validation

Patient #1, a 72-year-old Caucasian woman who presented in January 2016, underwent a facelift procedure with excision of pre-auricular skin strips. Four 1 × 1 cm^2 segments were marked on each strip that included a control section. Each tissue strip was stretched immediately on a cork board to prevent skin contraction. Within five minutes of pinning, micro-needling (Level 7 stroke frequency rate: 90 Hz) was performed in six of eight segments from both skin specimens randomized at either 0.25 mm, 0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, or 2.5 mm needle lengths. Each segment received 20 passes in the vertical, horizontal, and oblique directions. Immediately thereafter, each treated segment was sectioned, re-pinned on a plastic platform, and immersed in 10% formalin solution. The fixed specimens were transported in separate vials to an outsourced laboratory for sectioning (5 µm), staining [Hematoxylin and Eosin (H&E)] and interpretation. Measurements (mean µm ± SD) of maximum micro-depths from five microscopic sections (25 ×), selected at 2 mm distances within each 1 × 1 cm^2 specimen, determined the actual averaged penetration depths of differing needle lengths.

Study II: Validation of a Time-Dependent Presence of Pigment Particles

Patient #2, a 73-year-old Caucasian man who presented in February 2016, underwent a facelift procedure with excision of pre-auricular skin strips. Four 1 × 1 cm^2 segments were marked on each strip that included a control section. Each tissue strip was stretched immediately on a cork board to prevent skin contraction. Within five minutes of pinning, micro-needling (1 mm needle length, Level 7 stroke frequency rate: 90 Hz) was performed in six of eight segments from both skin specimens. Each segment received 20 passes in the vertical, horizontal and oblique directions. Immediately thereafter, a commercially available charcoal pigment particle suspension for cosmetic micropigmentation (SofTap Cosmetic Tattooing Supplies, Livermore, CA) was massaged into six 1 × 1 cm^2 segments from both strips randomized at 0, 5, 15, 30, 45, and 60 minutes after MN. The pattern and number of MN and massaging passes of the pigment suspension into the treated skin replicated passes used in Study l. The pigment suspension was removed from the epidermal surface five minutes after application with normal saline solution. Immediately thereafter, each treated segment was sectioned, re-pinned on a plastic platform, and immersed in 10% formalin solution. The fixed specimens were transported in separate vials to an outsourced laboratory for sectioning (5 µm), staining (H&E) and interpretation. Measurements (mean µm ± SD) of maximum depths of pigment location within each micro-channel from five microscopic sections (25 ×), selected at 2 mm distances within the 1 × 1 cm^2 specimen, determined whether: (1) massaging advances variable-sized particles down micro-channels produced by an array of 1 mm length needles; and (2) an optimal interval exists to facilitate particle entry through patent micro-channels by massaging.

Study III: Validation of Time-Dependent Presence of Fluorescein-Labeled Platelets

Patient #3, a 62-year-old Caucasian woman who presented in February 2015, provided 60 mL of whole blood from which a concentrate of 7 mL of PRP (Harvest Technologies Corp., Plymouth, MA)[18] was derived and transported overnight to Cytonics Corporation (West Palm Beach., Fl. 33411) for platelet labeling with CMFDA (5-Chloromethylfluorescein diacetate from Molecular Probes, Eugene, OR[19]). The labeled platelets were returned overnight to our facility. The patient #3 underwent a facelift procedure the next day with excision of pre-auricular skin strips. Four 1 × 1 cm^2 segments were marked on each strip that included a control section. Each tissue strip was stretched immediately on a cork board to prevent skin contraction. Within five minutes of pinning, micro-needling (1 mm needle length, Level 7 stroke frequency rate: 90 Hz) was performed in six of eight segments from both skin specimens. Each segment received 20 passes in the vertical, horizontal and oblique directions. Immediately thereafter, the labeled platelet concentrate was massaged into six 1 × 1 cm^2 segments from both strips randomized at 0, 5, 15, 30, 45, and 60 minutes after MN. The pattern and number of MN and massaging passes of the labeled platelets into the treated skin replicated passes used in Studies l and II. The PRP remnant was removed from the epidermal surface five minutes after application with normal saline solution. Each 1 × 1 cm^2 segment was immediately snap frozen, sectioned (5 µm) and mounted on slides for laser scanning microscopy. Measurements (µm ± SD) of maximum depths of fluorescence-stained platelets within each micro-channel from five microscopic sections (25 ×), selected at 2 mm distances within the 1 × 1 cm^2 specimen determined whether: (1) massaging advances platelets fragments down micro-conduits produced by an array of 1 mm length needles; and (2) an optimal interval exists to facilitate platelet entry through patent micro-channels by massaging.

Validation Study Device

Micro-Needling

The Dermapen device (Dermapen LLC, Salt Lake City, UT; and Dermapen USA, Peachtree City, GA) consisted of a disposable, spring-loaded piston with an array of eleven 33-gauge needles protruding from the end that is attached to the motorized hand-piece. Each needle was about 0.02 mm (200 microns) in diameter and arranged in a fractional pattern design. The piston stroke speed frequency was selected at the non-working end of the hand-piece from Level 1 (10 Hz) to as high as Level 7 (90 Hz), that corresponded to an estimated 1000 passes per second. The needle penetration-depth was adjusted on a dial located at the top of the hand-piece at 0.25 mm increments from 0.25 mm up to 2.5 mm needle lengths.

Platelet-Rich Plasma

An automated FDA-cleared PRP device (SmartPreP2 System, Harvest Technologies Corp., Plymouth, MA) was used to separate and concentrate a buffy-coat containing high yields of platelets.[20] An aliquot of the subject's anticoagulant citrate dextrose-treated whole blood sample and 4 mL PRP Harvest concentrate were transported overnight to Harvest Technologies Corporation to obtain concentrated platelet counts in the two samples from the single patient in the Fluorescein Verification Study III and also in all patients who selected combined MN and PRP for skin rejuvenation, scar modification and hair stimulation.

Laser Scanning Microscopy

A confocal laser scanning microscope (LSM 510 Meta Confocal Microscope, Carl Zeiss Micro-imaging, Inc., Thornwood, NY) was used for visualization of the fluorescent dye (5-chloromethylfluorescein diacetate [CMFDA]) as a probe that passed through cell membranes of human platelets within PRP preparation.[19] Once inside the cell particle, the chloro-methyl group reacted with intracellular thiols, transforming the probe into a form that cytosolic esterases cleaved off the acetate groups of CMFDA. Under laser scanning microscopy at a 530 nm band-filter, the total number of each brightly labeled fluorescein (mean number ± SD) and their maximum depth locations (mean μm ± SD) in microchannels were enumerated from five separated sections (25 × and 100 ×).

Clinical Review

The safety and effectiveness of MN treatments on patients in the author's Skin Care and Surgical Center were retrospectively analyzed from December 2012 to December 2015. All enrolled patients were concerned with a variety of aged findings that included mild to moderate wrinkle lines, skin laxity, striae, acne scars, hypertrophic scars, or alopecia. Patients with either mild or moderate findings were treated in either the Skin Care Center or Surgical Center and were included. Candidates who exhibited any contraindications were excluded from treatments. Micro-needling depths in the Skin Care Center ranged from 0.25 mm to a maximum of 1.0 mm needle setting and indicated for fine wrinkles, mild skin laxity, and acne scars. Micro-needling depths with PRP in the Surgical Center ranged from 0.5 mm to 2.5 mm and indicated for moderate wrinkles, moderate lax skin, moderate striae distensae and hypertrophic/acne scars, and early stages of alopecia. Contraindications included, but not limited to, patients with active systemic (uncontrolled diabetes mellitus) or local skin diseases (suppurative acne, herpes zoster, herpes simplex), keloidal history, skin cancers, anti-coagulant/aspirin therapy, chemotherapy/radiation therapy, and pregnancy. Treatments were delayed at least six months for patients who received ablative skin procedures.

Clinical Protocols

The skin was prepared for at least three months with customized treatments that included combinations of topical vitamins A and C, antioxidants, growth factors, pigment inhibitors, and a sunscreen blocker. After completion of a detailed consultation and consent forms, standard photography in a dedicated photographic center (Canfield Imaging Systems, Parsippany, NJ) were obtained at baseline and 12 months. Anti-viral medication was prophylactically begun two days before and continued for six days after treatment. In the Skin Care Center, the number of MN sessions recommended was generally six sessions at six weeks intervals and then every other month for a year. In the Surgical Center, the number of MN and PRP treatments was recommended at least once per year.

Photographic assessment[21] by the patient themselves and three independent observers using a Visual Analogue Scale (0 = absolutely dissatisfied and 10 = completely satisfied) was evaluated at baseline and 12 months in the Group 1 (MN) and in Group 2 (MN and PRP). Photographic assessment and computerized hair counting of hair (MN and PRP) was similarly evaluated by patients and observers at baseline and at 12 months, using a Visual Analogue Scale (0 = baseline; 1 = not improved, 2 = somewhat improved, and 3 = very improved). The hair count was performed with the EpiFlash Camera System (Canfield Imaging Systems, Parsippany, NJ) within a 10 mm spot size at several tattooed sites.

Statistical Analysis

Statistical analysis was performed using the standard deviation. Significance was accepted at a level of $P \leq .05$.

Treatment Protocols

In the Skin Care Center, the recommended protocol for percutaneous collagen induction (PCI) skin rejuvenation consisted of the following:

(1) Clean skin with a gentle antiseptic soap after removal of all makeup and sunscreen creams, complying with OSHA blood-borne pathogen certification criteria with protective goggles, sterile gloves and mask.
(2) Apply topical Lidocaine and Prilocaine cream (2.5% lidocaine; 2.5% prilocaine) with saran wrap between 10 and 15 minutes one area at a time. Topical anesthesia was applied in all patients because of variable responses to MN in sensitive areas of the forehead and perioral and periorbital areas.
(3) Remove Prilocaine cream with sterile gauzes, cleanse with HIBICLINS (chlorhexidine gluconate 4% w/v), and final wash with sterile saline wash.
(4) Select depths for MN: 0.25 mm-1.0 mm for forehead/face/eyelids/lip skin and chin/neck/and decollatage. Select depths of 1.0 mm to skin of the upper extremities, mild-moderate wrinkles, lax crepey skin, hypo/hyperpigmentations, and hypertrophic scars.
(5) Using another pair of sterile gloves, apply gentle steady gliding passes at least 10 to 20 times each in vertical, horizontal, and oblique directions on a hyaluronic acid wetting agent to each section of the face and neck.
(6) Use a rocking stamp method over severe wrinkle lines, deeper pigmentations, and deeper acne scars.
(7) Cleanse treatment sites with sterile saline and observe end point of uniform pinkness or punctate bleeding. Uneven or missed areas require additional passes.
(8) Massage cosmeceuticals containing cytokine growth factors in the treated skin within 5 to 15 minutes in the same pattern and frequency as performed during MN.
(9) Apply a hyaluronic acid moisturizer.
(10) After 2 days of twice daily applications of cytokine growth factors, regular skin care regimen of sunscreen, pigment inhibitors, anti-oxidants, vitamins A and C, and mineral-based makeup was added.

In the Surgical Center, the recommended protocol was similar to that of the Skin Care Center with the following exceptions: (1) treatments were considered a surgical procedure when employing PRP application with plasma-poor plasma (PPP) used as the wetting agent. After MN was completed in each facial or body region, PRP was massaged into the treated skin within 3 to 5 minutes in the same pattern and frequency passes as performed during MN; and (2) micro-needling depths between 0.5 to 2.5 mm were recommended for prominent scars, deep rhytids, and hair-stimulation. There were no major technical differences in treating the head and neck compared to the body, in all patients in both the Skin Care Center and Surgical Center.

RESULTS

Validation Studies

Actual maximum depths of tissue penetration at 0.25 to 2.5 mm needle length settings were evaluated by microscopy and expressed as measurements (mean µm ± SD) as shown in Table 1. The 0.25, 0.5, and 1.0 mm needle length settings correlated closely with measured depths from the epidermis down to the lowest level of the micro-channels within the dermis on H&E microscopy. Although actual penetrations corresponding to the 1.5, 2.0, and 2.5 mm needle length settings were achieved, the number of expected micro-channels were observed less at these longer needle length settings.

At 1.0 mm needle length, tattoo pigment particles of different diameters were detected at variable depths within the 1 mm length micro-channel conduits at 0, 5, 15, 30, 45, and 60 minutes after MN (Table 2). The maximum depth penetration of dye particles five minutes after MN (1 mm length needle) is depicted at 650 to 700 µm in H&E staining (25×) (Figure 1A). In control specimens (not listed), pigment was confined on the surface of the stratum corneum without penetration into the normal conduits associated with hair follicles, sebaceous glands, and sweat glands. The presence of intradermal pigment was less observed immediately after MN at a depth of 200 µm ± 15 SD, more consistently detected after 5 to 30 minutes at depths between 600 µm ± 75 SD to 725 µm ± 100 SD, and less seen after 45 to 60 minutes at depths between 50 µm ± 15 to 210 µm ± 50 SD.

At 1.0 mm needle length, fluorescence dye intensity within platelet fragments was detected by laser scanning microscopy in conduits 0, 5, 15, 30, 45, and 60 minutes after MN (Table 2). The maximum depth of fluorescein-stained

Table 1. Comparison of Variable Needle Lengths to Actual Tissue Penetrations

Needle Length (µm, mm)	Methodology	Mean Depth of Micro-channels (µm ± SD)
250, 0.25	Micro-needling	235 ± 50
500, 0.50	Micro-needling	475 ± 35
1000, 1.0	Micro-needling	920 ± 75
1500, 1.5	Micro-needling	900 ± 500
2000, 2.0	Micro-needling	1275 ± 600
2500, 2.5	Micro-needling	1100 ± 650

Table 2. Comparison of Presence and Depth of Pigment Particles and Fluorescein-Labeled Platelets over Time (1 mm needle length)

Time (min)	Methodology	Mean Depth of Pigment (μm ± SD)	Mean Depth of Fluorescein Spots (μm ± SD)	Mean Number of Fluorescein Spots (n ± SD)
0	Micro-needling: Massage	200 ± 15	825 ± 25	14 ± 7
5	Micro-needling: Massage	650 ± 55	975 ± 30	120 ± 15
15	Micro-needling: Massage	725 ± 100	950 ± 15	55 ± 12
30	Micro-needling: Massage	600 ± 75	975 ± 20	37 ± 5
45	Micro-needling: Massage	210 ± 50	925 ± 25	15 ± 5
60	Micro-needling: Massage	50 ± 15	900 ± 15	10 ± 3

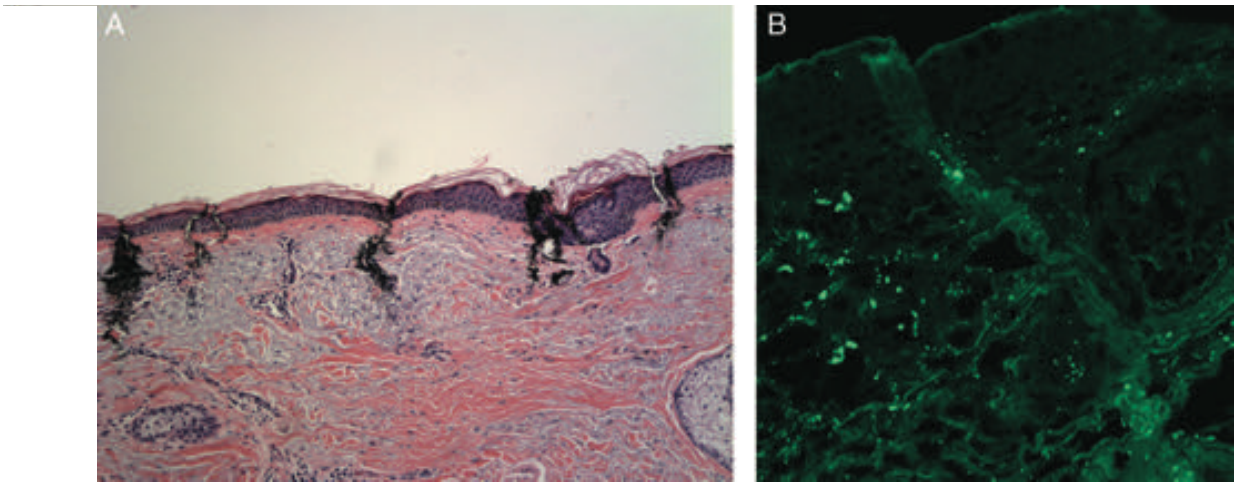

Figure 1. (A) The maximum depth penetration of dye particles five minutes after micro-needling (1 mm length needle) is depicted at 650 to 700 μm in H&E staining (25 ×). (B) The maximum depth of fluorescein-stained platelets five minutes after micro-needling (1 mm length needle) is shown at 975 to 1000 μm in snapped frozen section with laser scanning microscopy (25 ×).

platelets five minutes after MN (1 mm length needle) is shown at 975 to 1000 μm in snapped frozen section with laser scanning microscopy (25 ×) (Figure 1B). Platelet fragments (5 μm diameter) were able to be massaged down to almost the entire extent of the micro-channels produced by the 1 mm needle lengths. In control specimens (not listed), fluorescence was observed only on the surface of the stratum corneum. The number of counted fluorescent spots was low (14 ± 7) immediately after MN, increased to the highest numbers (120 ± 15, 55 ± 12, and 37 ± 5) at 5, 15, and 30 minutes, and diminished (15 ± 5 and 10 ± 3) at 45 and 60 minutes, respectively.

Clinical Experience

In the Skin Care Center, 447 patients underwent MN over the last three years (Table 3). The demographic data consisted of 443 females and 4 males (average age, 48.3 years; range, 22-81 years) primarily in Caucasians (342 patients) and to a lesser number of patients within four pigment-prone ethnicities. Each patient received about 8 sessions per year. As listed in Table 4, a total of 2402 treatment sessions were distributed to the face (1,518; 63.2%) with lesser number of treatments to the neck (454; 18.9%), decollatage (373; 15.5%), scars (53; 2.2%), and upper extremities (4; 0.17%). The average speed of needle frequency was at Level 6 (range, Levels 5-7) with an average depth of needle penetration at 0.75 mm (range, 0.25-1.0 mm).

In the Surgical Center, 113 patients underwent MN + PRP over the last three years (Table 3). The demographic data consisted of 105 females and 8 males (average age, 60.8 years; age range, 29-79 years), primarily in Caucasians (89 patients) and a lesser number of patients within four pigment-prone ethnicities. Each patient received about 1.5 sessions per year. As listed in Table 4, a total of 915 treatment sessions were distributed to the face (615; 67.2%), upper extremities (120; 13.1%), neck (80; 8.7%), decollatage (58; 6.3%), scalp alopecia (10; 1.1%), lower extremities (7; 0.8%), breasts (4; 0.4%), and abdominal striae (3; 0.3%). The average speed of needle frequency was a Level 6 (range, Levels 5-7) with an average depth of needle penetration at 1.25 mm (range, 0.25-2.5 mm). The

Table 3. Micro-needling Demographics

	Patients (n)	Average Age, Years (range)	Female/male	Number of Patients (n)				
				Caucasian	Asian	Hispanic	Middle Eastern	African American
Skin Care	447	48.3 (22-81)	443:4	342	45	50	4	6
Surgery Center	113	60.8 (29-79)	105:8	89	10	8	4	2

Table 4. Distribution of Sites, Sessions, Speed, Depth, and PRP

	Areas (%)	Face (%)	Neck (%)	Décolletage (%)	Scars (%)	Breast (%)	Upper Extremities (%)	Lower Extremities (%)	Abdomen Striae (%)	Alopecia (%)
Skin Care Center	Sessions (%)	1518 (63.2%)	454 (18.9%)	373 (15.5%)	53 (2.2%)	0 (0%)	4 (0.17%)	0 (0%)	0 (0%)	0 (0%)
Surgery Center	Sessions (%)	615 (67.2%)	80 (8.7%)	58 (6.3%)	18 (2.0%)	4 (0.4%)	120 (13.1%)	7 (0.8%)	3 (0.3)	10 (1.1%)
	Average Speed					Average Depth				
Skin Care Center	Level 6 (range, 5-7)					0.75 mm (range, 0.25-1.0 mm)				
Surgery Center	Level 6 (range, 5-7)					1.5 mm (range, 0.25-2.5 mm)				
	Average PRP (cc)				PRP/PPP Ratio	Average Platelet (fragments/μL)				
Surgery Center	5 (range, 1.5-11.0)				3:1	236,000 (range, 139,000-381,000)				
Skin Care Center	PRP's are not used in the Skin Care Center									

average PRP volume used was 5 cc (range, 1.5-11.0 cc) with a PRP:PPP ratio of 3:1 dilution. The average platelet count was 236,000 fragments/μL (range, 139,000-381,000 fragments/μL).

Patient and observer satisfaction scores, based on the Visual Analogue Scale, assessed patients, who returned for follow up photographs 12 months after MN in (Group 1) and MN + PRP (Group 2), as listed in Table 5. In Group 1 the follow up time average is 9 months post-treatment (range, 6-36 months) (average age, 46.3 years; range, 25-80 years), the mean baseline scores by patients and observers for wrinkles (5.9 ± 2.7; 6.5 ± 1.6), lax skin (5.4 ± 2.7; 6.5 ± 1.7), and hypertrophic scars (3.2 ± 1.7; 4.5 ± 0.1), respectively, improved at 12 months for wrinkles (7.1 ± 2.7; 7.7 ± 1.2), lax skin (6.4 ± 3.0; 7.9 ± 1.1), and hypertrophic scars (6.4 ± 1.3; 7.0 ± 0.0), respectively, by MN alone (Figures 2 and 3). In Group 2 the follow up time average is 17 months post-treatment (range, 6-36 months) (average age, 58.3 years; range, 30-77 years), the mean baseline scores by patients and observers for wrinkles (5.1 ± 2.0; 4.5 ± 2.2), lax skin (5.1 ± 2.0; 4.7 ± 2.3), hypertrophic scars (6.2 ± 2.3; 3.0 ± 0.3), acne scars (6.3 ± 3.0; 3.0 ± 0.7), crepey skin (1.0 ± 0.1; 4.9 ± 2.0), and striae (7.1 ± 2.0; 4.1 ± 0.9), respectively, improved at 12 months for wrinkles (7.3 ± 2.1; 6.3 ± 2.6), lax skin (7.3 ± 2.3; 5.9 ± 3.4), and hypertrophic scars (8.6 ± 0.6; 5.3 ± 0.1), acne scars (8.4 ± 2.4; 5.3 ± 1.0), crepey skin (2.0 ± 00; 7.3 ± 0.5), and striae (8.6 ± 0.8; 8.0 ± 0.3), respectively by MN and PRP (Figures 4 and 5).

Patient and observer assessment scores, based on the Visual Analogue Scale, assessed hair growth after a single treatment session with MN and PRP, listed in Table 6. The mean baseline score (0.0) by patients and observers improved at 12 months (2.0 ± 1.1; 2.5 ± 0.3) respectively. The average hair count in a 10 mm spot size at baseline (88.3 ± 22.5) was increased at the 12 month evaluation period (133.6 ± 13.8) (Figure 6).

Micro-needling with or without PRP to the face, neck, decollatage, extremities, scars, and alopecic areas averaged between 45 to 90 minutes. All patients returned to normal daily activities within twenty-four hours. No patient required analgesias or oral antibiotics. No patient developed herpes zoster or simplex infections. Minimal bruising, swelling and erythema occurred for up to seven to ten days and required no treatment. No patient reported scarring or hypopigmentation. One patient developed mild hyperpigmentation that responded within three months to combined treatments with Vitamin A and tyrosinase inhibitors (Table 7).

DISCUSSION

Since the development of prototypical microneedles over 15 years ago, the microelectronics industry adapted microfabrication technology to manufacture hollow or solid needles from glass,[20] silicon,[22] biodegradable polymers,[23] and metals.[24] Historically, different instruments have been used for controlled MN that included a single subcision

Table 5. Patient and Observer Satisfaction Visual Analogue Scale

	Group 1 (micro-needling)						Group 2 (micro-needling and PRP)											
	Wrinkle		Lax Skin		Hypertrophic Scars		Wrinkle		Lax Skin		Hypertrophic Scars		Acne Scars		Crepy Skin		Striae	
	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob	Pt	Ob
Pre-treatment (mean ± SD)	5.9 ± 2.7	6.5 ± 1.6	5.4 ± 2.7	6.5 ± 1.7	3.2 ± 1.7	4.5 ± 0.1	4.6 ± 3.7	4.5 ± 2.2	4.6 ± 3.7	4.7 ± 2.3	6.2 ± 2.3	3.5 ± 2.1	6.3 ± 3.0	3.4 ± 0.7	1.0 ± 0.1	4.9 ± 2.0	7.1 ± 2.0	4.4 ± 1.0
Post-treatment (12 months) (mean ± SD)	7.1 ± 2.7	7.7 ± 1.2	6.4 ± 3.0	7.9 ± 1.1	6.4 ± 1.3	7.0 ± 0.0	7.4 ± 3.4	6.3 ± 2.6	7.5 ± 3.4	5.9 ± 3.4	8.6 ± 0.6	6.0 ± 1.2	8.4 ± 2.4	5.7 ± 1.0	2.0 ± 0.0	7.3 ± 0.5	8.6 ± 0.8	8.0 ± 0.3
No. of patients	16	35	16	35	3	3	21	53	21	53	3	9	3	3	1	5	2	2

P value, 0.05. Ob, observer; Pt, patient; SD, standard deviation.

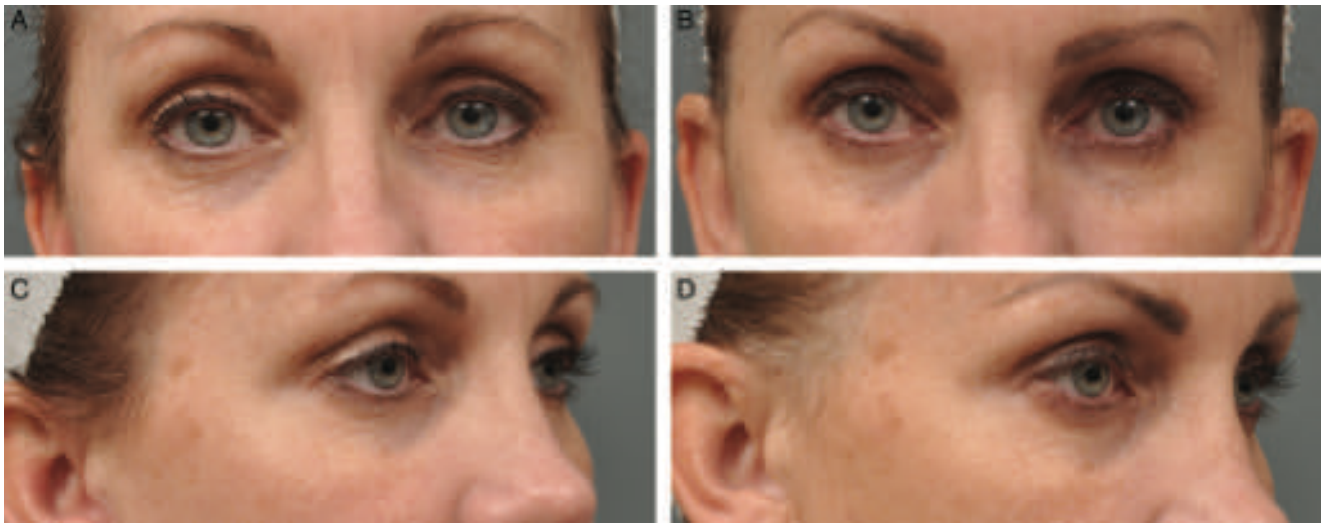

Figure 2. This 48-year-old Caucasian woman underwent four sessions of micro-needling six weeks apart (0.5-1.0 mm depth; speed frequency Level 6) for periorbital wrinkles and laxity. Both patient and observer scores from (A, C) baseline to (B, D) 12 months improved for wrinkles (3 to 4) (6 to 8) and laxity (2 to 3) (7 to 8), respectively.

needle[25] or diverse needle arrangements within tattoo devices,[26] rollers,[27] or electronic devices.[28] In clinical aesthetic or reconstructive practice today, the most accepted appliances are rollers and electronic devices that vary needle material, length, diameter, number, and arrays, and are designed for multiple personal usage or equipped with a disposable sterile needle cartridge[28,29] for a single procedure. Electronic devices are powered by batteries or electrical sources, have adjustable needle speeds and depths, and operated by gliding or stamping maneuvers across the skin.

Over the past decade, aesthetic MN has been shown to dramatically increase PCI,[1,16-17] as well as skin permeability[27,28] to a board range of compounds by creating reversible micro-channels in the skin. In general, these solid micron-dimensional solid needles have been designed to have differing geometries (lengths, 250-2500 µm; widths, 200-500 µm; thicknesses, 75-125 µm; and varying array 10-50 needles) to breach the skin's barrier, yet avoid stimulating pain fibers. From an efficiency standpoint, the channels must be large enough to permit cosmeceuticals to passively enter by diffusion, to remain patent during their application periods, and to desirably reseal quickly to prevent further exposure to toxic or irritating substances and pathogenic microbes.

Bal et al[30] compared on the forearm of volunteers the effect of increasing solid micro-needle lengths (200, 300, and 400 µm) to barrier restoration, as evaluated by return of transepidermal water loss (TEWL) to control levels. Of interest, TEWL values did not change after production of short conduits with 200 µm length needles. In contrast, the pattern of TEWL values with 300 and 400 µm length needles increased immediately, then slowly declined after 15 minutes and reached baseline values after 30 minutes. In a subsequent study, Bal et al[31] visualized by laser scanning microscopy the dynamics of passive absorption of fluorescein dye into the deepest depths of micro-channels after MN (dermaroller, 300 µm length and 120 µm diameter). The greatest volume of dye in the conduits was

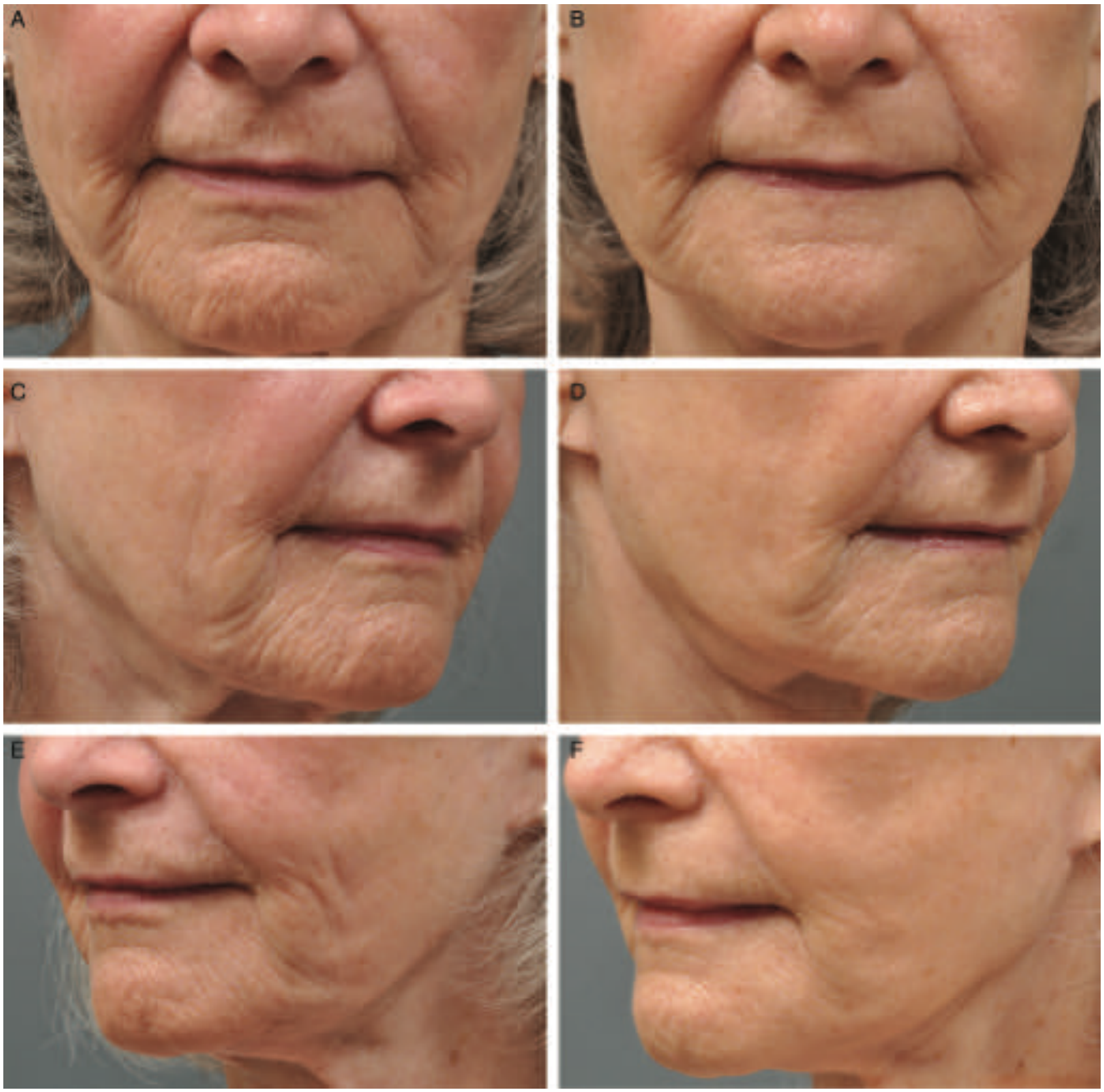

Figure 3. This 79-year-old Caucasian woman underwent one session of micro-needling (0.5-1.5 mm depth; speed frequency Level 6) and PRP (baseline, 180,000/μL) for wrinkles and skin laxity. Both patient and observer scores from (A, C, E) baseline to (B, D, F) 12 months improved for wrinkles (6.5 to 9) (3 to 7) and laxity (6.5 to 9) (3 to 7), respectively.

achieved after 5 minutes, slowly decreased by 10 minutes, and reached baseline values by 15 minutes.

Gupta et al[32] demonstrated in healthy adult subjects that, in the absence of skin occlusion, all sites resealed with restoration of barrier properties within 2 hours after MN by electrical impedance spectroscopy. These authors also found that with skin occlusion the use of longer micro-needles (500 vs 1500 μm) with wider cross-sectional areas (200 vs 500 μm) and with increased number of needles (10 vs 50) produced slower resealing times up to 3 to 40 hours for extended transdermal delivery. Their findings estimated that the micro-channel size was 1 to 2 orders of magnitude smaller than the cross-sectional diameter of the selected micro-needle. In their opinion, significant shrinking of conduits after MN was due largely to the skin's elasticity, as was suggested in this report and other studies.[6,33] Lima

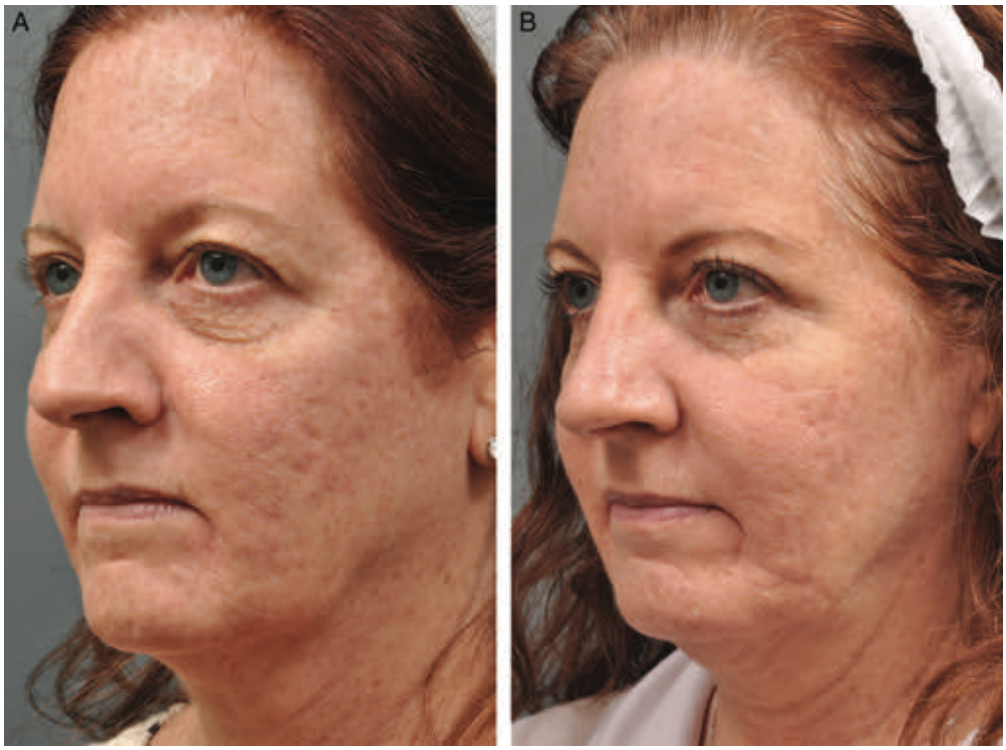

Figure 4. This 54-year-old Caucasian woman with rolling pits and hyperpigmentation from adult acne underwent one session of micro-needling (1.5-2.5 mm; speed frequency, Level 6) and PRP (baseline, 166,000/μL). Both patient and observer scores from (A) baseline to (B) 12 months improved (4 to 10) (3 to 6), respectively.

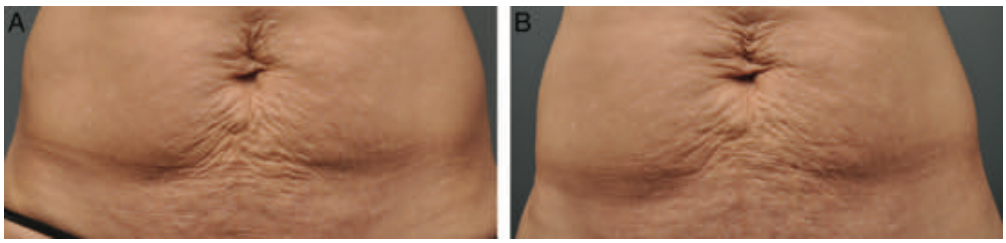

Figure 5. This 44-year-old Asian woman underwent one session of micro-needling (2.0-2.25 mm depth; speed frequency, Level 6) for abdominal striae. Both patient and observer scores from (A) baseline to (B) 12 months improved for striae (2 to 6) (3 to 8) and laxity (3 to 8) (4 to 8), respectively.

et al[34] estimated in a porcine model that 3 mm length needles on a roller device penetrated only 1.5 to 2.0 mm (50%-75% of its actual length).

The current report observed that the actual depths of generated dermal micro-channels, as performed in clinical practice, closely matched the selected array of needle lengths from 0.25 to 1 mm but were less frequently detected with longer needle lengths from 1.5 to 2.5 mm by histological measurements. In an earlier pilot study (Sasaki G, unpublished September 2013), the use of in situ MN to preauricular tissue prior to excision was performed in seven facelift patients to determine the most reliable method to replicate depth of needle penetrations from 0.25 to 2.5 mm in preparation for the present study. Each treated segment was sectioned, re-pinned on a plastic platform, and immersed in 10% formalin solution within 10 minutes. To our disappointment, histological findings of channels at deeper needle settings were inconsistent or not observed (Murakami S, Senior Pathologist, Huntington Medical Center, Pasadena, CA) as was found in Study I. The reasons for the discrepancy were unclear as the needle protrusion distances from the disposable tip clearly corresponded to the dial settings. The most likely explanations might be attributed to premature closure of deeper

Table 6. Patient and Observer Analogue Scale and Microscopic Hair Count

	Visual Analogue Scale	
	Patient	Observer
Baseline score	0 ± 0	0 ± 0
Post-treatment score	2.0 ± 1.1	2.5 ± 0.3
No.	4	8
Microscopic Hair Count		
Baseline count	88.3 ± 22.5	
Post-treatment count	133.6 ± 13.8	
No.	8	

channels by intact skin's visco-elastic properties and to tangential micro-sectioning that could disrupt the actual lengths of the created micro-channels. Since MN at different lengths in pre-auricular skin in situ prior to excision demonstrated limitations of histologic documentation, ex vivo tissue usage was selected in the current studies because of introduction of possible contaminants of micro-pigmented particles or fluorescein-stained platelets into patients. In a second pilot study (Sasaki G, unpublished March 2016) there was a direct correlation of needle length up to 2.5 mm to penetration depths into ballistic gel. Other weakness of Studies I, II, and III were the limited sampling of single subjects for each study and selection of only of facial skin. This deficiency was somewhat mitigated by sampling five sections 2 mm apart within each 1 × 1 cm^2 segment. It is unclear whether the addition of multiple subjects with a range of ages and skin conditions and skin from other areas of the body would significantly impact the interpretation of the current findings and clinical protocols. Future protocols should address these areas of interests for completeness of data entry.

The current study also reported that the optimal time to massage pigment particles and platelet fragments down the reversible micro-channels in ex vivo skin was most favorable between 5 and 30 minutes after MN. These findings would have significant influence on the clinical timing and maximum effect of topical agents applied after MN. CMFDA (5-Chloromethylfluorescein diacetate was selected to label platelet particles for ex vivo massaging into the needle conduits because: (1) it was nonisotopic; (2) fluorescence was bright and stable over time; and (3) labeling was intracellular with minimal manipulation of the required platelets. These findings were consistent with the data from the previously mentioned human volunteer studies for opening and closing of portals and conduits as interpreted by measurement changes in TEWL and electrical impedance spectroscopy. Upon disruption of the stratum corneum, lamellar body secretion is believed to be immediately initiated and then followed by synthesis of lipids, which were necessary to restore the lipid barrier.[35,36]

Recent evidence suggested that micro-trauma of MN stimulated PCI by promoting the body's normal phases of wound healing in fragile collagen-deficient actinic or chronological aged skin.[16,37] The orchestrated cascade of events are believed to begin with the initial release of TGF-β, platelet-derived growth factor, vascular endothelial growth factor, and other cytokines from activated neutrophils, platelets, endothelial cells, and monocytes.[38,39] Subsequently, recruited fibroblasts produced collagen I and III, elastin, glycosaminoglycans within the matrix. Focal adhesion complexes (integrins) on the fibroblast cell surfaces connected the matrix (type I collagen) to the cytoskeleton, which in turn, regulated the balance between production of collagen and its breakdown by matrix metalloproteinases.[40,41] Fernandes[1] and Aust et al[17] have reported on the vital contributions of vitamins A and C for PCI and emphasized the importance of released growth factors from all major cells in the epidermis and dermis that participate in the formation of a stable pattern of collagen in the matrix.

Our clinical experience, based on our investigative validation findings, suggested that the use of MN alone or with PRP improved a variety of skin and alopecia concerns when combined with customized skin care maintenance programs as assessed by Patient and Observer Assessment scores. The observation of the contrasting baseline satisfaction scores in Group 1 patients (more negative) vs their counterparts in Group 2 (less negative) than the observers' evaluation scores were difficult to explain. Of importance were the positive changes in both groups a year after their initial treatments. Previous investigators[1,16,17,26,28] have published promising results utilizing MN and aftercare protocols for improvement of aged skin, hypertrophic scars, and hair stimulation. Although the number of clinical publications[42-45] on the use of PRP alone has grown in recent years, our clinical experience with MN and PRP usage for skin rejuvenation, scar modification and hair stimulation represented an additional report utilizing combined therapies.[46-49] In the Skin Care Center, the regimen of MN, immediately followed by massaging of topical cytokine growth factors within 30 minutes, and post-op medical skin care programs smoothed out mild wrinkles and tightened lax skin. For optimal skin rejuvenation, micro-needling was repeated at six week intervals for six sessions and monthly maintenance. Patients were referred to the Surgical Center for combined MN and PRP treatments yearly for moderate wrinkles and lax skin, striae, hypertrophic scars, and hair stimulation. Micro-needling and PRP treatments were integrated with the MN sessions and maintenance protocols in the Skin Care Center. The occurrence of minimal and reversible side-effects suggested a safe profile for micro-needling, effective passive and active

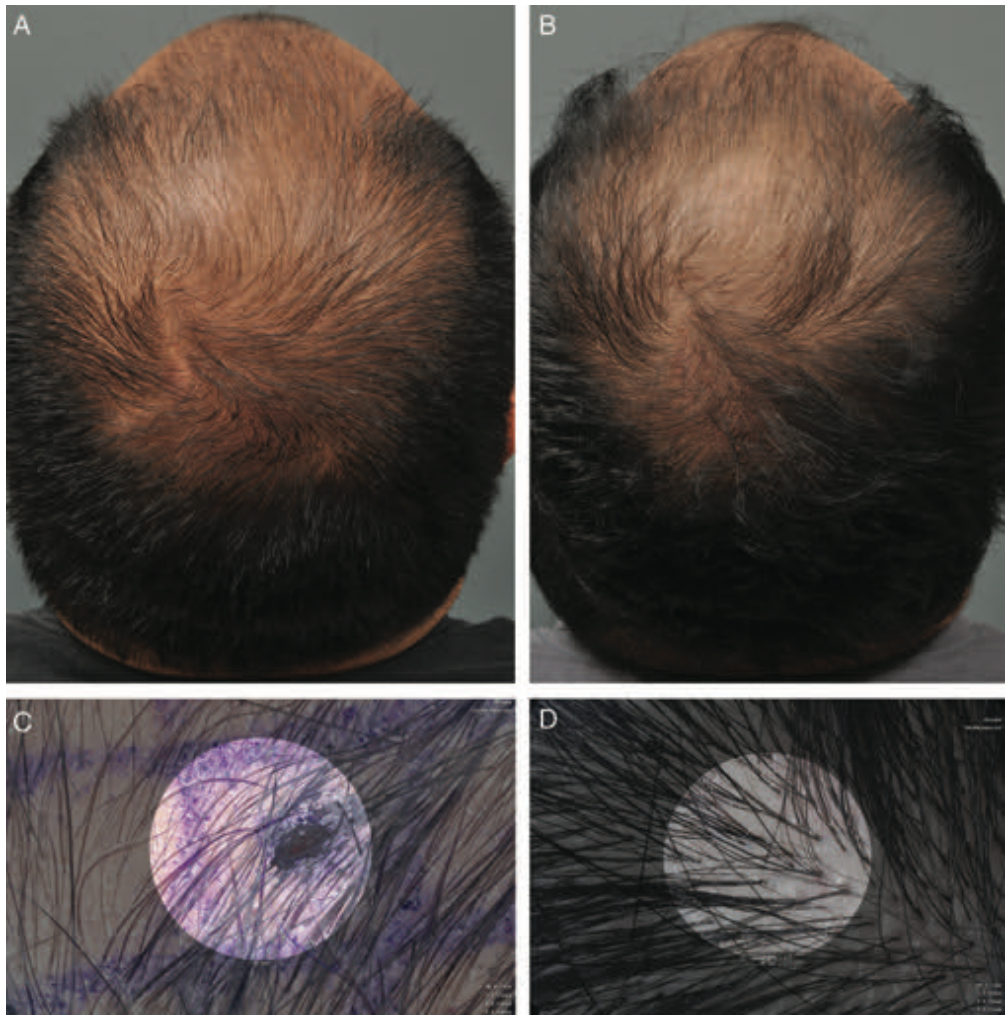

Figure 6. This 27-year-old Asian man exhibited early male-pattern baldness to the fronto-occipital scalp after minimal results from 5% minoxidil applications over a year. The patient's alopecic area was marked into 250 one centimeter squares. The Lipogems[50] (Lipogems International Spa, Milan, Italy) non-enzymatic device harvested and processed fat from the hip rolls. Under local anesthesia, 25 mL fat mixed with 3 mL of PRP (baseline, 214,000 platelets/μL), was injected in the subdermal space under each square (0.1-0.15 mL/square). Thereafter, micro-needling was performed (1.5-2.5 mm depth; stamping technique three times/square). Hair growth was stimulated at the 6 month evaluation period with increased terminal hair follicles and density. Hair count by The EpiFlash Canfield System (Canfield Imaging System, Parsippany, NJ) demonstrated an increased hair count from (A, C) baseline to (B, D) 6 months.

Table 7. Side-effects and Complications for the Micro-needling (MN) and MN/PRP Groups

	No. of Patients	Herpes Zoster or Simplex Infections	Minimal (bruising, swelling, and erythema)[a]	Hyperpigmentation
Skin Care	447	0/447 (0%)	447/447 (100%)	1/447 (0.01%)
Surgery Center	113	0/113 (0%)	113/113 (100%)	0/113 (0%)

[a]Lasting up to 7 to 10 days, no required treatment need.

passage of cosmeceuticals and PRP, improved clinical responses, and rapid sealing of openings as an inherent safety feature. The precise mechanisms of actions remain to be determined.

CONCLUSION

MN alone or in combination therapy, based on validation experimental studies, resulted in safe and effectivtreatment

of the aged skin, striae, scars, and alopecia by Patient and Observer Satisfaction Scores. Further investigations on mechanisms of action and prospective randomized studies are needed to confirm these initial findings. In the future, larger cohort of samplings along with increased number of patients in prospective randomized split-face studies should be conducted to confirm our initial impressions.

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Disclosures

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