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Research

Morphometric study of facial wrinkles and aesthetic skin as dermaroller treatment combined with platelet rich plasma (PRP)

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Abstract

Background: Facial wrinkles are a multifactorial, complex process that negatively affects individuals' appearance, consequently their quality of life. The treatment for these wrinkles varies with the degree of severity. This prospective study aimed to evaluate the clinical effect of pure dermaroller and dermaroller combined with platelet rich plasma therapy (PRP dermaroller), to treat facial wrinkles, and to quantitatively evaluate histological changes of the skin that occur in the two different cohorts.

Methods and materials: Twenty healthy women aged 43-48 years with scores ranging between 2 and 4 on the baseline facial fine wrinkle grading scale were enrolled in the this clinical study. Nineteen of the patients were treated with a pure dermaroller on the one half of face, and with a dermaroller that included a platelet rich plasma on the other half of the face. Three treatments, each in a 4 weeks interval were performed. Standard photographs and skin biopsies were obtained from the treatment area at baseline and 8 weeks after the final session. Comparisons of the treatments were analyzed using clinical and histological findings.

Results: The degree of baseline facial fine wrinkle grading scale after treatment revealed statistically significant effects of the PRP dermaroller treatment side compared to the side of pure dermaroller treatment. At 8 weeks after the final session, the wrinkling grade on the PRP dermaroller side and the pure dermaroller side showed significant differences ($p<0.05$). Microscopic evaluation of haematoxylin eosin and Masson's trichrome stained sections revealed significant differences in dermal fibers, epidermal thickness, papillae and skin glands.

Conclusion: Significant changes were noted between treatments of facial wrinkles with pure dermaroller and PRP dermaroller. Dermaroller combined with platelet rich plasma is a promising novel method of facial rejuvenation.

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Keywords: dermaroller, platlet rich plasma, wrinkle, skin aging.

Background

Facial wrinkles are a multifactorial, complex process that negatively affects individuals' appearance, consequently their quality of life [1]. Aged skin displays with reduced number of dermal glands, fibroblasts, dermal fibers, and diminished ability of recovery. These findings are consistent with an atrophy of the dermal extracellular matrix (ECM) [2,3]. The treatment for these wrinkles varies with the degree of severity. Several therapeutic options are available today: muscle-relaxing injections, topical medications, dermabrasion, chemical peels, laser resurfacing, cosmetic filler injections and collagen induction therapy. Microneedling with dermarollers is a cheap and simple procedure for facial skin wrinkle. It is a technique that uses a sterile dermaroller to puncture the skin multiple times with a series of fine sharp needles. The skin develops multiple microbruises in the dermis that initiate the complex cascade of wound healing and growth factor release, and finally results in collagen production [4,5]. Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma that may be beneficial in the treatment of wrinkles by promoting collagen deposition [6]. Platelet rich plasma contains multiple autologous growth factors, including epidermal growth factor, fibroblast growth factor, transforming growth factor-b, macrophage inflammatory protein-1α, platelet derived growth factor and vascular endothelial growth factor [6,7,8]. The combination of skin needling and topical PRP could enhance the efficacy of both modalities. In cosmetics, PRP has been used for facelift, neck lift, breast augmentation, breast reduction, autologous fat transfer, and lip augmentation [9]. This prospective study aimed to evaluate the clinical effect of a therapy applied to facial wrinkles by treatment with pure dermaroller and of a PRP dermaroller. The morphological changes in the skin were quantitatively evaluated between the two methods of treatment.

Methods

Patients

From October 2015 to February 2016, a total of twenty healthy women with facial wrinkles with scores ranging from 2 to 4 on the baseline facial fine wrinkle grading scale [8] <Table1> were enrolled in the this clinical study. All patients were informed and agreed to a written consent. The local ethics committee approved the protocol in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

The average patients' age was computed to 45.47±1.92 years (range, 43 to 48 years). Our study's exclusion criteria included: facial skin rejuvenation done in the previous 1 year; age below 43 or above 48; severe skin allergies; use of any drugs which affect platelet function; herpes labialis; bacterial infection; actinic keratosis; skin cancer; systemic retinoids intake in the previous 6 months; diabetes; hematologic disorders; severe mental illness; pregnancy or lactation. A single-

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blinded prospective trial was implemented for avoiding bias. The sides of the patients' face were randomly selected for treatment with pure dermaroller and PRP dermaroller.

Treatment protocols

Three treatment sessions each at 4 weeks interval were carried out of microneedling on the one half of the face, and contemporary skin needling combined with platelet rich plasma on the other half of the face.

Control group

Three treatment sessions each at 4 weeks interval were carried out of the pure dermaroller on one half of the face and contemporary of the PRP dermaroller on the other half of the face. Local anesthetic cream (lidocaine) was applied to the face for approximately one hour before the procedure. After sterilization of the face with povidone iodine, 1.5-2 ml of PRP was applied topically to the treated area, followed by needling on the one half of face using the sterile dermaroller (Shanghai Astiland Technology Co., Ltd, Shanghai, China). It consists of 540 stainless steel needles; needle length is 1.5 mm long in a cylindrical assembly. The treatment was performed by rolling the dermaroller on the one half of face 10 times in 4 directions (vertically, horizontally, and diagonally right and left) with soft pressure.

Experimental group

Skin needling combined with platelet-rich plasma was carried out on the other half of the face. Platelet-rich plasma was obtained by double-spin method, followed by the collection of 10 ml of autologous whole-blood into tubes containing anticoagulant, finally produce 1.5～2.0 ml of PRP. The collected blood was first centrifuged at 215 g for 10 minutes to separate the red blood cells at the bottom of the tube, the buffy coat (containing the white blood cells) in the middle and the plasma above. The upper plasma was pipetted from the buffy coat and underwent an additional centrifugation at 863 g for 10 minutes in order to obtain a platelet pellet in the bottom of the tube, and a platelet-poor plasma (PPP) in the upper part. The PPP is partly removed and partly used to re-suspend the platelets to finally produce 1.5-2.0 ml of PRP. Platelet-rich plasma was activated by adding 10% calcium chloride 0.1 ml per 0.9 ml plasma. 1.5-2 ml of PRP was applied topically to the treated area, followed by needling the skin.

Histological analysis

Histology was performed using a 3 mm punch biopsies from the skin (treatment site) at baseline and 8 weeks after the final session. Specimens were fixed in 10% buffered formalin, embedded in paraffin, sliced into sections 5 µm thick and stained with Haematoxyline Eosin (HE) and Masson's Trichrome (MT). The HE stained skin specimens were reviewed for epidermal thickness, papillae, pigmented epithelial cells and glands. The MT stained skin specimens were measured for dermal fibers. Measurements of the epidermal thickness and dermal fibers were carried out with Diskus® Software and a light microscope (Olympus BX51, Japan). 2 to 3 randomly selected zones per histological slide of each individual patient were measured

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rectangular from the basal layer to the top of the stratum corneum. All microphotographs shared the same power of 10X objective. Data of the cutaneous casts were analyzed by computerized image analysis.

Outcome assessments

Standard photographs and skin biopsies were obtained from the treatment area at baseline, at 8 weeks after the final treatment. Photographic documentation was performed using identical camera settings and lighting and the same positioning with the same camera (Canon 6D, Canon Corp., Tokyo, Japan). Grading of wrinkle photographs using the baseline facial fine wrinkle grading scale <Table1> [10] was carried out by three dermatologists who did not participate in the treatment and were not informed of the treatment modalities on each side.

Grade	
Grade 0	No evidence of line or wrinkle.
Grade 1	A few short barely perceptible wrinkles.
Grade 2	A few shallow wrinkles discreetly visible with no deep wrinkles in the face.
Grade 3	Shallow easily visible wrinkles with 1 or 2 shorter deeper wrinkles in the face.
Grade 4	Moderate number of fine wrinkles nearly covering the entire area, with wrinkles deeper and longer in the face.
Grade 5	Many deep lines with several coarse wrinkles.

Table1: Baseline Facial Fine Wrinkle Grading Scale.

Statistical Analysis

Data were checked, entered, and analyzed using RStudio (Version 1.0.44) and SPSS (Version 19.0). Data were represented as mean ± SD for quantitative variables. Numbers and percentages were used for categorical variables. Before-and-after treatment comparisons were performed using the parametric t-test for paired samples. The statistical tests were two-sided, and a probability value of less than 5% was considered statistically significant. The differences in the degree of improvement and percentage improvement between groups were compared using an independent sample t-test. Statistical analysis was performed using the chi-square test. Significance was accepted at a level of $p<0.05$.

Results

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Demographics

Twenty patients were enrolled in the study, nineteen patients completed the three sessions treatment. One patient withdrew due to pain during dermaroller treatment. The mean age was 45.47±1.92 years with a range of 43 to 48 years <Table 2>.

Clinical variables		
Total cases	19	
Age, years		
Mean ± SD	45.47±1.92	
Range	43 – 48	
Gender		
Male	0 (0%)	
Female	19 (100%)	
At baseline wrinkle grade	**Experimental group**	**Control group**
Grade 0	0 (0%)	0 (0%)
Grade 1	0 (0%)	0 (0%)
Grade 2	1 (5.3%)	2 (10.5%)
Grade 3	12(63.2%)	11 (57.9%)
Grade 4	6 (31.6%)	6 (31.6%)
Grade 5	0 (0%)	0 (0%)
P value	P=0.331>0.05	

Table 2: Demographics of the 19 patients who completed the study.

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Figure 1: Clinical improvement of facial wrinkles on both sides. ***a*** *baseline,* ***b*** *at 8 weeks after the final session on the dermaroller combined with PRP treatment,* ***c*** *baseline,* ***d*** *at 8 weeks after the final session on the dermaroller treatment.*

Clinical improvement

The degree of facial wrinkling was compared between the experimental group or the control group at before treatment and at 8 weeks after the final session <Figure 1>. At baseline, the wrinkling grade on the dermaroller combined with PRP side (3.26 ± 0.56) and the pure dermaroller side (3.21 ± 0.63) showed no significant differences (p>0.05). At 8 weeks after the final session, the wrinkling grade on the dermaroller combined with PRP side (2.32 ± 0.82) and the pure dermaroller side (2.89 ± 0.66) showed high significant differences (p<0.05).

	Experimental group		**Control group**	
Group	**Baseline**	**after treatment**	**Baseline**	**after treatment**
N	19	19	19	19
Average	3.26±0.56	2.32±0.82	3.21±0.63	2.89±0.66
p value	P=0.000<0.01		P=0.011<0.05	
p value (after treatment)	P=0.001<0.01			

Table 3: Clinical improvement of facial wrinkles on both groups.

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Histological results

The microscopic evaluation of the HE stained sections revealed significant difference in epidermal thickness, papillae, pigmented epithelial cells and skin glands. An increase in epidermal thickness between the experimental and the control group was noted <Figure 2>.

The epidermal thickness measured 163.44±5.99 µm at baseline, 235.8±31.94 µm at 8 weeks after the final session on the PRP dermaroller (P<0.05); 200.61±14.09 µm at 8 weeks after the final session on the pure dermaroller (P<0.05) <Table 4>.

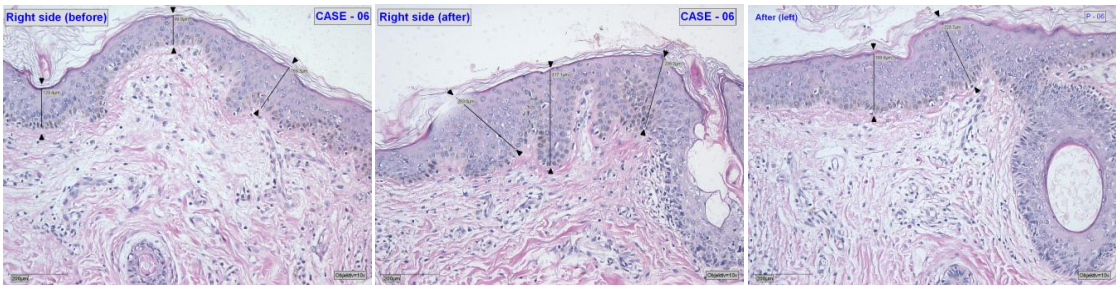

*Figure 2: Microphotographs taken of representative skin samples stained with hematoxylin-eosin presenting the epidermal thickness. **a** baseline, **b** at 8 weeks after the final session of the dermaroller combined with PRP treatment, **c** at 8 weeks after the final session of the dermaroller treatment. The increase in thickness of the epidermis with repetitive treatments in addition to the PRP dermaroller and the dermaroller demonstrates how effective these combined techniques are. Furthermore, it can be seen, that the stratum corneum is more compact in the experimental (b) and control (c).*

Group	Dermaroller combined with PRP			Pure Dermaroller		
	Baseline	**After**	**Change(%)**	**Baseline**	**After**	**Change(%)**
Epidermal thickness (µm)*	163.44±5.99	235.8±31.94	44.23*	163.44±5.99	200.61±14.09	22,74*
Number of epidermal papillae *	0.37±0.49	2.84±1.21	667.57*	0.37±0.49	1.42±0.96	283,78*
Number of dermal glands*	0.58±0.51	5.00±1.37	762.06*	0.58±0.51	3.84±2.06	562.07*
Density of dermal fiber(mm^2)*	0.348±0.003	0.502±0.025	44.25*	0.348±0.003	0.409±0.016	17.53*

Table 4: The main histological findings of the two groups. Quantity of Epidermal thickness, dermal glands and epidermal papillary count, and number, and dermal fiber. Statistically significant difference (p < .05) comparing before and after treatment. *Statistically significant difference between combination treatment with platelet-rich plasma (PRP) and pure dermaroller treatment.*

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A significant increase in the number of dermal glands and epidermal papillary <Table 4> between the experimental and control group could be noted. The glands and epidermal papillae in the experimental group were increased compared to the control group (P<0.05) <Table 4>.

MT stained slides displayed with a considerable increase in dermal fiber deposition at 8 weeks after the final session. Their microscopic evaluation <Figure 3> revealed a significant difference in dermal fibers of the PRP dermaroller cohorts (baseline and at 8 weeks after the final session; <0.05), and between the PRP dermaroller and pure dermaroller cohort at 8 weeks after the final session (P <0.05) <Table 4>.

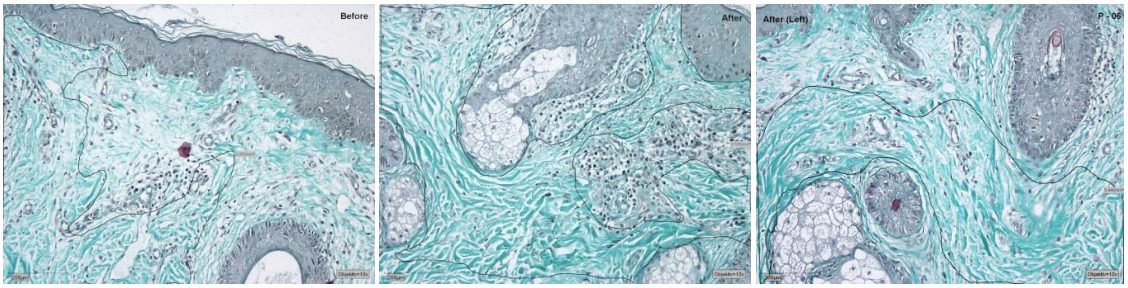
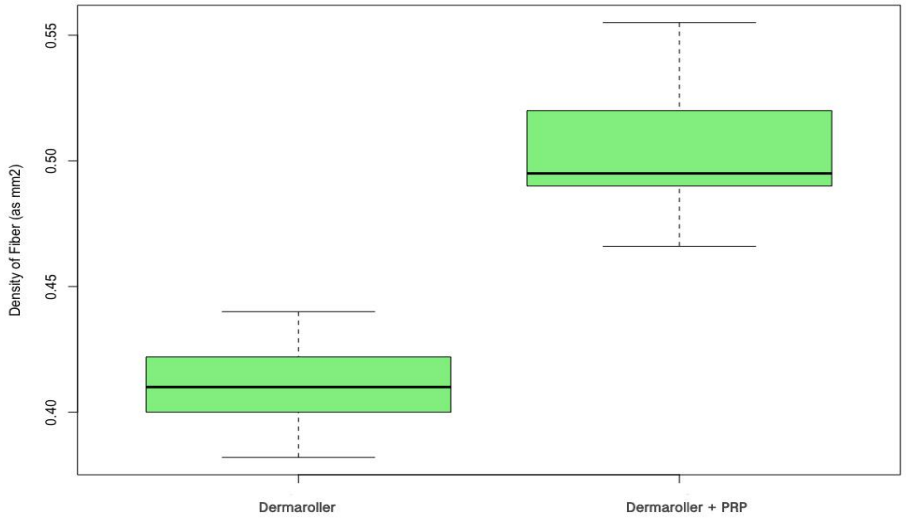


Figure 3: Microphotographs taken of representative skin samples stained with Masson's trichrome presenting the dermal fiber. ***a*** *baseline,* ***b*** *at 8 weeks after the final session of the dermaroller combined with PRP treatment,* ***c*** *at 8 weeks after the final session of the dermaroller treatment.*



Graph 1: Quantitative analysis of the elastic fibers. It demonstrates significant different distributions of dermal fibers between the areas of PRP dermaroller and pure dermaroller treatment.

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Discussion

We observed remarkable differences of clinical and histological findings between the two cohorts in this study. The PRP dermaroller group displayed with an objective improvement of facial wrinkle grade, an increase in the epidermal thickness, epidermal papillae, dermal glands and dermal fiber density.

The clinical changes associated with aging of the skin include pigment alterations, thinning, dryness, fine wrinkling, and coarser textural changes such as sagging and wrinkling [2, 11]. Microscopically, aged skin possesses a decreased dermal thickness and vascular density, a reduced number of dermal fibroblasts, glands and levels of collagen and elastin [12]. The regeneration of human skin in terms of cellular replacement and wound healing decreases with age [13]. In the present study, the treatment with PRP dermaroller increased the epidermal thickness, the number of epidermal papillae and dermal gland, as well as the thickness of collagen fibers. As a result, we believe that PRP dermaroller treatment might pose a positive effect to reverse skin damages, for example, in patients with poor wound healing capacity due to aging.

In a study from Korea, twenty-two participants (age range: 30–56) underwent three sessions of fractional laser; 11 were treated with topical application of PRP combined with fractional laser. One month after the final treatment, PRP combined with fractional laser increased the subjects' satisfaction and skin elasticity, and decreased the erythema index. PRP increased the thickness of the dermo-epidermal junction, the amount of collagen, and the number of fibroblasts [13].

In a study from Germany, 480 patients with fine wrinkles, lax skin, scarring, and stretch marks were treated with percutaneous collagen induction using the dermaroller (length 3mm) combined with topical vitamin A and C cosmetic creams to produce tighter, smoother skin. Most patients underwent only one treatment, some patients underwent until to four treatments. On average, the patients in Germany rated their improvement 60 % to 80 % after their treatment. The epidermis demonstrated 40% thickening of the stratum spinosum and normal rete ridges after 1 year [4].

The microscopic skin examination of our 19 patients revealed a considerable increase in collagen and elastin deposition after 6 months. In this study, the increase of epidermal thickness was measured to 44.23% in the PRP dermaroller cohort.

Ryan et al. have shown that microneedle-puncture resulted in significantly less microbial penetration compared to a hypodermic needle puncture. In addition, no microorganisms crossed the viable epidermis in microneedle-punctured skin, in contrast to needle-punctured skin [14].

Zeitter et al. have shown that in an animal model needling for four times in addition to skin care induces an increased number of connective tissue fibers in the subepidermal layers [15].

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In our study, 19 patients were treated with PRP dermaroller in three sessions. The microscopic examination displayed with an increase of dermal fiber deposition of 44.25% after 8 weeks. In addition, a significant increase in the number of dermal glands, epidermal papillae and epidermal thickness was noted.

PRP is obtained from patient's blood. Disease transmissions and immunogenic reactions are not expected [16]. The procedure has been applied in different medical disciplines. It has been described to be safe [17]. We also did not observe any side effects.

In conclusion, we report of significant skin changes that are noted in dermaroller treatment of facial wrinkles. Especially PRP dermaroller treatment is a safe and effective cosmetic procedure for facial skin rejuvenation.

The face of the patient has been published in accordance with her permission.

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