**The Efficacy and safety of an autologous fractionated plasma extract(PDSC-PRP) on skin rejuvenation: An open-label clinical trial**

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## Introduction and Background

Aging is an inevitable fact of life. Two types of aging have been described – intrinsic and extrinsic aging. Intrinsic aging is the natural aging process. It begins in the mid to late 20's and continues as we age chronologically. As intrinsic aging progresses, collagen production slows, and dermal elastin becomes less elastic. In addition to these changes, desquamation of the epidermis occurs at a slower rate. These microscopic changes of aging are seen grossly as fine wrinkling, thin and uneven skin tone, loss of underlying fat leading to hollowed cheeks and eye sockets, noticeable loss of firmness on the hands and neck, xerotic and pruritic skin, among others.(1,2)

Extrinsic aging on the other hand is caused by external factors such as sun exposure, repetitive facial expressions, gravity and smoking, among others. Majority of premature aging is mediated by prolonged and repeated sun exposure. Photoaging occurs over time, leading to impaired regenerative capacity of the skin, breakdown of dermal collagen and elastin. In addition to these effects, photoaging also impairs the synthesis of new collagen. These effects are perceived as loose, wrinkled and leathery skin. (1,2,3)

The effects of both intrinsic and extrinsic aging have become a source of multiple inquiries and studies to formulate new products in the field of dermatology and plastic surgery. Statistics confirm that there is a trend toward nonsurgical procedures aimed to address skin rejuvenation. Patients currently prefer minimally invasive procedures that can provide maximum results with a quick recovery period. (4)

A fractionated plasma extract, a product prepared in clinic from the patient's own blood, is a combination of small peripheral blood derived pluripotent/multipotent cells and platelet rich plasma (PRP) lysate (PDSC-PRP) delivered by mesotherapy to enhance production of collagen and elastic fibers in the dermis thereby giving the skin a more youthful and fuller look.

The medical uses and dermatological benefits of platelet rich plasma have been reported since … The pharmacological mechanism of action of PRP ranges from being an anti-inflammatory to wound healing and regenerative properties, an the effects have been attributed to growth factors it contains, such as fibroblast growth factor (FGF), hepatocyte growth factor (HGF), a fibronectin like peptide, keratinocyte growth factor, and epidermal growth factor…..

## Statement of the Problem

Aging skin is produced by a decrease in production, regeneration and compromised functions of dermal collagen and elastin. Reversal of these processes may give the skin a more youthful look.

This study aims to address the problem of aging skin, using autologous PDSC-PRP preparation as an agent to counter premature skin aging by increasing dermal collagen and elastin.

Objectives of study

**General Objective**

This study aimed to assess the effectivity and safety of a fractionated protein complex in increasing collagen and elastin in the dermis.

**Specific Objectives**

1. Subjective assessment of PDSC-PRP in addressing the following signs of aging:
    a. Fine wrinkles
    b. Loss of radiance or glow of skin
    c. Loss of underlying fat, leading to hollowed cheeks and eye sockets as well as noticeable loss of firmness of facial skin

2. Objective assessment of density of collagen and elastic fibers, by histopathology, before and after administration of PDSC-PRP.

3. Objective assessment of safety was determined by complete blood count and blood chemistry (fasting blood sugar, creatinine, blood urea nitrogen, uric acid, total cholesterol, alanine phospatase and alanine aminotransferase) before and after administration of PDSC-PRP.

Experimental Design

This study was a non-randomized, open-label trial using PDSC-PRP administered intra-dermal on the face and upper neck, through mesotherapy.

Subjects

**Inclusion Criteria**

Healthy males and females between the ages of 40 and 65 years.

**Exclusion Criteria**

Patients with illnesses requiring maintenance therapy (diabetes, hypertension, thyroid disease, bronchial asthma, cardiovascular disease, cancer, HIV)
Patients with existing skin allergies, dermatologic lesions, eczema, psoriasis and/or skin eruptions
Presence of infection at the proposed injection site
Patients with allergic diatheses
Patients with known hypersensitivity to any component of the product to be used
Pregnant or lactating women
Patients with abnormal complete blood count and blood chemistry results
Incapacitated patients or patients with mental illness

Methods

**Initial Evaluation**

A total of 30 patients were included in this study with no dropout during the treatment period. All patients were asked to sign an informed consent for the trial as well as consent for skin biopsy and photograph taking. (Figures 1 and 2)

A one-week washout period prior to initiation of treatment was done. All patients were made to use one specific brand of hypoallergenic facial cleanser twice daily and a sunscreen in the morning. Instruction for cleansing with the specific brand of facial wash once in the morning and once in the evening was given to standardize facial cleansing.

Baseline laboratory determinations of complete blood count and blood chemistry were determined (fasting blood sugar, serum uric acid, cholesterol, blood urea nitrogen, creatinine, serum alanine phospatase and serum alanine aminotransferase). Subjective assessments of fine lines, skin texture and overall appearance were made by both the investigators and the patients. Appearance of fine lines, skin texture, radiance and tightness of skin before treatment were used as the baseline reference point. Subsequent improvements in these parameters were rated using a four-point scale (0= no improvement, 1 = slight improvement, 2 = moderate improvement, 3 = great improvement) during the third and sixth week of the study. Pictures of each patient were also taken. Standard lighting, background and camera were used for photography. After which, a 3mm skin punch biopsy was taken at the inferior aspect of the right side of the mandibular area as baseline.

An intradermal skin test containing a diluted from of the medication (1:100) was performed on the dorsal aspect of the right forearm of each patient prior to the procedure. The test was read after 30 minutes. The intradermal test was read as positive if there was note of a 5-10 mm radius of erythema and induration beyond the site of intradermal injection of the dilute test drug. Any patient who developed a positive reaction did not receive treatment. All patients with a negative skin test proceeded with treatment.

**Experimental Design**

After the above-mentioned procedures, the each patient received 1 course of PDSC-PRP administered by the investigator via mesotherapy using gauge 31 needle with the mesogun set on continuous 0.5mm mode, to the entire face (forehead, cheeks, chin and infero-mandibular area). (Fig. 3)

An observation period of 30 minutes was instituted after the procedure to observe for any immediate adverse effects. If the procedure was tolerated well, patient was then sent home. Strict compliance to daily cleansing with the provided cleanser was reiterated.

**Follow-up**

Patients were independently assessed by the investigators at the start of treatment (baseline), during each follow up (day 21) and at the end of treatment (day 42). Questionnaires similar to those used at baseline were accomplished by the investigators and the patients at each follow up. During each follow-up, standardized photographs of each patient were taken, and adverse reactions noted.

**Final Assessment**

At the end of six weeks, patients and investigators again accomplished the survey form similar to that accomplished at baseline. Photographs were again taken, and adverse reactions noted. A repeat skin biopsy immediately adjacent to the previous biopsy site was obtained. A 3mm punch was utilized for this procedure. All biopsies taken before and after treatment were performed by the investigator. Laboratory examinations were repeated at the end of the study.

All biopsies performed were read by a pathologist. Assessment was made with regards to changes in density collagen and elastin fibers from baseline and at the end of treatment.

**Criteria for discontinuation of treatment**

Any patient who developed a severe adverse reaction which would entail discontinuation of treatment (e.g. severe allergy requiring hospitalization) was excluded from the study. Any patient, who, for the duration of the study, developed an illness requiring hospitalization or maintenance therapy, was likewise excluded. Any patient who retracted consent was excluded from the study.

**Adverse Events**

All documented adverse events (local and systemic reactions) as a result of treatment were properly documented and reported. Any patient who developed an adverse reaction (local or systemic) as a result of this trial will be given appropriate treatment by the investigators, free of charge.

**GRADING METHODS AND STATISTICAL ANALYSIS**

A 4-point grading scale was used for both the subjective and objective evaluations. The patients and investigators assessed the efficacy of the test drug. Two determinations were taken, one during the first follow up (day 21) and another at the end of the study (day 42), in reference to baseline appearances of the patient. Injected sites were graded between 0 and 3. Subjective assessment of fine wrinkles, tightness of skin, radiance and glow, were made. The numerical grades corresponded to the following subjective responses: 0- no improvement, 1- very slight improvement (10-30% improvement), 2-moderate improvement (31-70% improvement), 3-marked improvement (71-100% improvement). (Figure 4-A and 4-B)

The patients were instructed to do self-assessment of the perceived improvement, if any, from baseline in comparison to their appearance during the third and sixth weeks of the study. For fine lines, subjective assessment was based on the depth of fine lines or wrinkling at baseline and during the third and sixth weeks of the study. Patients were instructed to give a grade of 0, if no decrease in fine lines was noticed, a grade of 1, if a slight but not highly perceptible decrease in fine lines was noted or if 10-30% of fine lines had a slightly become more shallow in comparison to baseline. A grade of 2 was given if there was 31-70% decrease in wrinkles in comparison to baseline, or if there was a more perceptible decrease in depth of wrinkles on the face, and a grade of 3 was given if there was a 71-100% reduction of fine lines from baseline or if there was a definite perceptible decrease in depth of wrinkles.

In this study, radiance and glow of the face was the measure of texture – smoothness and evenness of facial skin. A grade of 0 was given if no improvement in texture was noted, and a grade of 1 was given if there was a 10-30% improvement of texture in comparison to baseline. A grade of 2 was given for a moderately perceptible improvement of skin texture or a 31-70% improvement of skin texture in comparison to baseline, and a grade of 3 was given if there was a definite perceptible improvement in smoothness and evenness of skin from baseline, or if there was a 71-100% improvement of skin texture compared to baseline.

Tightness of skin was described as the taut or firm sensation of skin, in contrast to sagging or loose aging skin. For this parameter, patients were instructed to give a grade of 0 if no improvement was noted and a grade of 1 if there

was a 10-30% increase in firmness of skin compared to baseline. A grade of 2 was given for a perceived increase of firmness by 31-70% from baseline, and a grade of 3 was given if there was note of a 71-100% improvement in firmness, or a definite loss of loose facial skin.

In addition to the 4-point grading scale assessment accomplished by the patient and investigator, photographs of each patient were taken before, during and upon completion of the study. These photographs were assessed by an independent investigator using the same 4-point grading scale. (Figure 5)

The von Giesson elastic stain was used to stain of elastic fibers and hematoxyllin and eosin stain for collagen fibers. Determination of an increase in collagen fibers was made by fibroblast cell count per high power field while density of elastic fibers were approximated. A 4-point grading scale was used in the assessment of both the collagen and elastic fibers. The numerical grades correspond to the following microscopic observations: 0-no improvement (0-9% increase), 1-slight increase in collagen/elastic fibers (10-30% increase), 2-moderate increase in collagen/elastic fibers (31-70% increase), 3-great increase in collagen/elastic fibers (71-100% increase). (Figure 6)

In addition to determination of complete blood count and blood chemistry at baseline, the investigators provided patient diaries for each subject for patients to detail any adverse reactions during the succeeding days after treatment. Patients were instructed to write if they experienced any adverse reactions (local or systemic) and to quantify the length of symptoms during the day. (Figure 8)

**DATA MANAGEMENT AND ANALYSIS**

Standard case record forms were used to record patient information and responses to treatment. (Figure 6)

Data was entered into a Microsoft Excel spreadsheet.

For continuous data, means, standard deviations, and ranges were presented. Mean differences between baseline scores and assessments made at week 3 and week 6 were computed. For categorical data (eg. responses to treatment, changes in collagen and elastic fibers), the number and proportion of patients were presented.

Wilcoxon's signed test using Vassarstats was performed on wrinkle scores of week 3 and week 6.

The criteria employed for the subjective assessment is as follows:

0 – no improvement
1 – very slight improvement (10-30% improvement)
2 – moderate improvement (31-70% improvement)
3 – marked improvement (71-100% improvement)

The numerical grades correspond to the following microscopic observations:

0 - no improvement (0-9% increase)
1 - slight increase in collagen/elastic fibers (10-30% increase)
2 - moderate increase in collagen/elastic fibers (31-70% increase)
3 - great increase in collagen/elastic fibers (71-100% increase)

<u>Human Subject Protection Plan</u>

Informed consent was acquired from each patient. Nature of therapy and possible adverse effects were carefully explained to each subject by the investigator. Consent was obtained from the participant only. Any participant was free to discontinue the study should they decide to withdraw from the trial without any liability.

Identities of all participants were kept in strict confidence.

Subject Reimbursement

Subjects in the study were provided all the materials for participation, free of charge. Contact numbers of the investigators were given to all participants in case of adverse reactions. Everyone was informed that should it occur, they will be treated free of any charge.

**RESULTS**

Thirty- two patients were screened initially but two patients were not included. One patient was excluded because of a co-existing diffuse non-toxic goiter. The other patient was excluded due to co-existing diabetes mellitus and hypertension. A total of thirty Filipino patients were included in this study with no drop-outs.

Of the 30 enrolled patients, 2 were males and 28 were females (Table IA). The mean age of the patients was 52 (SD 7) with ages ranging from 41-65 years. Seventy three percent of patients were housewives, while the 13% were self-employed. The two male patients were unemployed.

Baseline evaluation of Fitzpatrick skin type revealed that all patients were Fitzpatrick skin type IV. Glogau's classification of skin aging at baseline revealed that 12 (40%) of patients were classified under group 2, while 18 (60%) were classified under group 3. None of the patients fell under Glogau groups 1 and 4 (Table IB).

None of the included patients had other illnesses or were taking medications during this study. None of the patients had undergone any surgical procedures on the face prior to this study. No patient developed a positive reaction to the intradermal test of the drug.

**Clinical efficacy**

A. Patient assessments

After the third week post-treatment, 23% of patients reported a slight improvement in fine lines and 77% reported a moderate improvement of the same parameter. For radiance and glow of skin 30% of patients reported slight improvement and 70% reported moderate improvement. Tightness of the skin was assessed to have moderately improved in 43% of patients and slight improvement was seen in 57% of patients.

At the end of the study ($6^{th}$ week), all patients reported either moderate or marked improvement of fine lines. Twenty percent of the patients gave an excellent rating for improvement, while 80% of the patients gave a moderate rating for the same parameter.

For radiance and glow of skin, 16.67% of the subjects gave a rating of marked improvement, and 83.33% of patients gave a rating of moderate improvement.

Improvement scores of week 3 and week 6 were compared. Further improvement in fine lines, radiance and tightness was reported by 37%, 47% and 60% of the patients, respectively. The mean improvement scores during

week 3 and week 6 were significantly different for line lines, radiance and tightness (p=0.0114, 0.001 and 0.0002, respectively) (Table 2-C)

**B. Physician assessments**

The subjective assessment of physicians likewise, had a universal moderate improvement for fine lines, radiance and glow and tightness of skin at the end of the study.

These valuations were again higher than during the 3rd week of the study (Table 3-C). When improvement scores of week 3 and week 6 were compared, investigators noted further improvement in fine lines, radiance and tightness in 33.3%, 40% and 97% of patients, respectively. The increase in mean scores from week 3 to week 6 were significant for all parameters (p=0.0054, 0.0024 and <0.0001, respectively) and most noticeable for skin tightness (Table 3C)

**C. Histopathologic Changes**

Evaluation in the increase of collagen was done by comparing hematoxyllin and eosin stained slides taken before and after completion of the study. A manual count of fibroblasts before and after injection of the test drug was done to quantify the increase in collagen density. A comparison of these slides revealed a slight improvement (10-30% increase) in collagen in 46.67% of patients and a moderate improvement (31-70% increase) in 40% of patients, while 13.33% of patients showed no change in collagen from baseline.

A similar procedure was employed in the assessment of elastic fibers. Elastic tissue stain was used to evaluate the increase in dermal elastic fibers at the end of the study. For this parameter, 26.66% of patients had slight increase (10-30% increase) in elastic fibers while 73.33% revealed no change in the number of elastic fibers compared to baseline.

Table 1A. Sociodemographic characteristics (n=30)

| **AGE** | **No.** | **%** |
|---|---|---|
| 35-45 | 4 | 13.3 |
| 46-55 | 18 | 60 |
| 56-65 | 8 | 26.7 |
| **GENDER** | | |
| MALE | 2 | 6.7 |
| FEMALE | 28 | 93.3 |

Table 1B. Clinical characteristics (N=30)

| | | |
|---|---|---|
| **GLOGAU CLASSIFICATION** | | |
| GROUP 1 | 0 | 0.0 |
| GROUP 2 | 12 | 40.0 |
| GROUP 3 | 18 | 60.0 |
| GROUP 4 | 0 | 0.0 |
| **WRINKLE SEVERITY** | | |
| MILD WRINKLING | 9 | 30 |
| EARLY WRINKLING (PARALLEL SMILE LINES) | 9 | 30 |
| WRINKLING PRESENT AT REST | 12 | 40 |
| **TEXTURE** | | |
| SLIGHTLY UNEVEN | 5 | 26.7 |
| MODERATELY UNEVEN | 14 | 46.7 |
| MARKEDLY UNEVEN | 11 | 36.7 |
| **FIRMNESS** | | |
| MILDLY SAGGING SKIN | 7 | 23.3 |
| MODERATE SAGGING | 13 | 43.3 |
| SEVERE SAGGING | 10 | 33.3 |

TABLE 2-A. No. and % of PDSC-PRP-treated patients with improvement at week 3 (Patient-assessed)

| PARAMETER | SLIGHT IMPROVEMENT (N=30) | | MODERATE IMPROVEMENT (N=30) | | MARKED IMPROVEMENT (N=30) | |
|---|---|---|---|---|---|---|
| | NO | % | No. | % | No. | % |
| FINE LINES | 7 | 23.33 | 23 | 76.67 | 0 | 0 |
| RADIANCE AND GLOW | 9 | 30 | 21 | 70 | 0 | 0 |
| TIGHTNESS | 17 | 56.67 | 13 | 43 | 0 | 0 |

TABLE 2-B. No. and % of PDSC-PRP-treated patients with improvement at week 6 (Patient-assessed)

| PARAMETER | SLIGHT IMPROVEMENT (N=30) | | MODERATE IMPROVEMENT (N=30) | | MARKED IMPROVEMENT (N=30) | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| FINE LINES | 0 | 0 | 24 | 80 | 6 | 20 |
| RADIANCE AND GLOW | 0 | 0 | 25 | 83.3 | 5 | 16.7 |
| TIGHTNESS | 0 | 0 | 17 | 56.7 | 13 | 43 |

Table 2-C Mean improvement scores of fine lines, radiance, and tightness on week 3 and week 6 (Patient assessed)

| | **FINE LINES** | | | **RADIANCE AND GLOW** | | | **TIGHTNESS** | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Mean Diff | | | Mean Diff | | | Mean Diff |
| | 3 weeks | 6weeks | | 3weeks | 6weeks | | 3weeks | 6 weeks | |
| Mean | 1.9 | 2.2 | 0.3 | 1.7 | 2.1 | 0.4 | 1.5 | 2.3 | 0.8 |
| SD | 0.43 | 0.41 | | 0.47 | 0.35 | | 0.51 | 0.47 | |

Table 3-A. Mean improvement scores of fine facial wrinkles, radiance and skin laxity at 3 weeks post treatment (Investigator-assessed)

| PARAMETER | SLIGHT IMPROVEMENT (N=30) | | MODERATE IMPROVEMENT (N=30) | | MARKED IMPROVEMENT (N=30) | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| FINE LINES | 21 | 70 | 9 | 30 | 0 | 0 |
| RADIANCE AND GLOW | 19 | 63.3 | 11 | 36.7 | 0 | 0 |
| TIGHTNESS | 24 | 80 | 6 | 20 | 0 | 0 |

Table 3-B. No. and % of PDSC-PRP-treated patients with improvement on fine facial wrinkles, radiance, and skin laxity 6 weeks post-treatment (Investigator assessed)

| PARAMETER | SLIGHT IMPROVEMENT (N=30) | | MODERATE IMPROVEMENT (N=30) | | MARKED IMPROVEMENT (N=30) | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| FINE LINES | 0 | 0 | 30 | 100 | 0 | 0 |
| RADIANCE AND GLOW | 0 | 0 | 30 | 100 | 0 | 0 |
| TIGHTNESS | 0 | 0 | 30 | 100 | 0 | 0 |

Table 3-C. Mean improvement scores at week 3 and week 6 (Investigator assessed)

| | **FINE LINES** | | | **RADIANCE** | | | **TIGHTNESS** | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3weeks | 6weeks | Mean Diff | 3 weeks | 6 weeks | Mean Diff | 3 weeks | 6weeks | Mean Diff |
| Mean | 1.7 | 2 | 0.3 | 1.6 | 2 | 0.4 | 1 | 2 | 1 |
| SD | 0.47 | 0 | | 0.49 | 0 | | 0.19 | 0 | |

Table 4. No. and % patients with adverse events reported immediately after treatment

| PARAMETER | MILD (n=30) | % | MODERATE (n=30) | % | SEVERE (n=30) | % |
|---|---|---|---|---|---|---|
| PRURITUS | 18 | 60 | 4 | 13.33 | 0 | 0 |
| ERYTHEMA | 0 | 0 | 0 | 0 | 0 | 0 |
| DISCOMFORT | 0 | 0 | 0 | 0 | 0 | 0 |
| PAIN | 14 | 46.66 | 5 | 16.66 | 0 | 0 |

*Mild = VAS of 1-3, Moderate = VAS of 4-7, Severe = VAS of 8-10

TABLE 5. Histologic changes in the dermis comparing baseline to week 6 post-treatment

| PARAMETER | NO INCREASE (n=30) | % | SLIGHT INCREASE (n=30) | % | MODERATE INCREASE (n=30) | % | GREAT INCREASE (n=30) | % |
|---|---|---|---|---|---|---|---|---|
| COLLAGEN | 7 | 23.33 | 16 | 53.33 | 7 | 26.92 | 0 | 0 |
| ELASTIN | 18 | 60 | 12 | 40 | 0 | 0 | 0 | 0 |

*No improvement = 0-9% increase, Slight improvement = 10-30% increase
Moderate improvement = 31-70% increase, Great improvement = 71-100% increase

TABLE 6. Effects of PDSC-PRP treatment on complete blood count, fasting blood sugar, blood urea nitrogen, creatinine, cholesterol, uric acid, serum alkaline phosphatase and serum alkaline aminotransferase, comparing baseline to week 6 post treatment

| PARAMETER | Patients with elevation (n) | Patients with elevation (%) | Range of increase (units) | Range of increase (%) |
|---|---|---|---|---|
| HEMOGLOBIN | 0 | 0 | 0 | 0 |
| HEMATOCRIT | 0 | 0 | 0 | 0 |
| RBC | 0 | 0 | 0 | 0 |
| WBC | 0 | 0 | 0 | 0 |
| FBS | 0 | 0 | 0 | 0 |
| BUN | 0 | 0 | 0 | 0 |
| CREATININE | 0 | 0 | 0 | 0 |
| CHOLESTEROL | 0 | 0 | 0 | 0 |
| URIC ACID | 0 | 0 | 0 | 0 |
| SGPT | 2 | 6.67 | 8.5-43.66 | 21.25% to 109.15% |
| SGOT | 2 | 33.33 | 4.74-12.59 | 11.85 to 31.48% |

**DISCUSSION**

Aging is a universal occurrence. This process affects all cells of the human body in several different ways. In the skin, specifically, a number of age-related changes may become perceptible thru time. Among these changes, those observed in the epidermis include a flattened dermal epidermal junction combined with loss of regularity of the dermal papillary architecture which corresponds to the gross skin changes of wrinkles. In addition to this, epidermal turnover rate decreases by 30-50% between the third and eighth decades of life, resulting in a prolongation of the rate of replacement of the stratum corneum . Other features of epidermal aging include variable thickness of the epidermis and dermis, variable cell size and shape, occasional nuclear atypia, fewer melanocytes and fewer Langerhan's cells. (1) In the dermis, age-related changes include loss of dermal thickness, fewer fibroblasts, fewer mast cells and blood vessels, shortened capillary loops and abnormal nerve endings. (1)

Many studies have been done to document the role of collagen, elastin and the dermal ground substance in aging skin. These studies show that biochemical changes in these structures during fetal and early postnatal development are far greater that those described with advancing age. It has also been found that collagen content per unit area of skin surface decreases approximately 1 percent per year throughout adult life. Histopathologically, the remaining collagen fibers in the elderly population appear disorganized, more compact and granular. (1)

On the other hand, elastic fibers decrease in number and diameter, and by old age, they often appear fragmented with small cysts and lacunae in the dermal-epidermal junction. It has been proposed that enzymatic degradation of elastin may be a mechanism for normal dermal aging. These microscopic processes result in progressive loss of elastic recovery and marked prolongation time required for excised skin to return to its original thickness after compression. (3) As the patient ages, UV damage to the elastic fibers becomes chronic, and the inherent snap back quality of the skin becomes impaired, hence the appearance of wrinkles.

Aging skin may also appear rough and uneven, which can be explained histologically by an increased compaction of the stratum corneum, increased thickness of granular cell layer, reduced epidermal thickness and reduced epidermal mucin content. Aging produces a profound loss of subcutaneous fat in the perioral area, the temporal fossae, the premolar areas, china and forehead. The older face has a flattened quality to the cheekbones, a sunken appearance of the lips, a bulging of the inferior fat pads of the eye, and in general, a loss of fullness and roundness of youth. (3)

All organs of the body including the skin undergo degenerative changes as we age. Cells in the epidermis and dermis including collagen and elastic fibers, undergo continuous wear and tear by bio-oxidative processes and environmental factors. Collagen and elastic fibers are important components in the dermal matrix. They give the fuller look and suppleness of the skin. At a younger age the synthesis of collagen and elastic fibers predominates, whereas after about age of 40, their degradation pick up speed. Therefore, steps to boost their synthesis and reduce degradation may well be the answer to a youthful skin.

PDSC-PRP, an autologous fractionated plasma extract delivered by mesotherapy was used in this study . PRP has been shown to enhance production of collagen synthesis but not that of elastin... It contains a variety of growth factors, (epidermal and fibroblast growth factors, etc.), that are able to stimulate a variety of dermal cells to induce a wound healing or cosmetic effect…..

Our results indicated that subjective assessments, both from the pateints' and the investigators' observations have shown moderate to marked improvements in skin tightening, radiance, and decrease of fine wrinkles. Objectively,

histologic measurements of fibroblasts/collagen were slight to moderately increased while elastic fibers were slightly increased, after a period of only 6 weeks. It is possible that a longer period of observation may allow more regeneration of these structures to have a more visible change. Our photographs did not show well appreciable differences due to some technical problems.

Although there were patients whose serum alanine phospatase and aminotransferase were elevated, no clinical sign of the disease was observed. They were aysmptomatic during treatment and upon completion of the study, none of the said patients required systemic treatment for the elevations in liver enzymes. Further investigation conducted two weeks after the study showed these responses to be transient. No other adverese reactions were noted except for local pain which was transient.

**CONCLUSIONS**

1. Administration of PDSC-PRP moderately improves perceptible signs of skin aging (fine lines, radiance and glow, tightness)
2. Slight to moderate increase of dermal collagen was observed in 80% of patients after 6 weeks of treatment with PDSC-PRP.
3. Slight increase in dermal elastin was noted in 40% of patients after 6 weeks of treatment with PDSC-PRP
4. No severe adverse reactions are associated with administration of PDSC-PRP
5. Local reactions experienced are well tolerated and did not result in discontinuation of or withdrawal from treatment
6. Administration of PDSC-PRP may result in mild transient elevations of SGOT.

**RECOMMENDATIONS**

The investigators recommend the following for future study:

1. Repeat biopsy 6 weeks after completion of study (D84) to allow more time for collagen and elastic fibers regeneration to take place
2. Randomized controlled trial (placebo vs treatment)
3. Upgrade technology of photograph taking

**APPENDICES: RAW DATA**

A. PATIENT'S SUBJECTIVE ASSESSMENT FOR FINE LINES

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 2 | 2 | Moderate |
| 2 | 1 | 2 | Moderate |
| 3 | 1 | 2 | Moderate |
| 4 | 2 | 2 | Moderate |
| 5 | 2 | 2 | Moderate |
| 6 | 2 | 2 | Moderate |
| 7 | 2 | 2 | Moderate |
| 8 | 2 | 2 | Moderate |
| 9 | 2 | 2 | Moderate |
| 10 | 2 | 3 | Marked |
| 11 | 2 | 3 | Marked |
| 12 | 2 | 3 | Marked |
| 13 | 2 | 3 | Marked |
| 14 | 2 | 3 | Marked |
| 15 | 2 | 3 | Marked |
| 16 | 2 | 2 | Moderate |
| 17 | 1 | 2 | Moderate |
| 18 | 1 | 2 | Moderate |
| 19 | 1 | 2 | Moderate |
| 20 | 2 | 2 | Moderate |
| 21 | 2 | 2 | Moderate |
| 22 | 2 | 2 | Moderate |
| 23 | 2 | 2 | Moderate |
| 24 | 2 | 2 | Moderate |
| 25 | 1 | 2 | Moderate |
| 26 | 2 | 2 | Moderate |
| 27 | 1 | 2 | Moderate |
| 28 | 2 | 2 | Moderate |
| 29 | 2 | 2 | Moderate |
| 30 | 2 | 2 | Moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvement

B. PATIENT'S SUBJECTIVE ASSESSMENT FOR RADIANCE AND GLOW

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 2 | 3 | Marked |
| 2 | 2 | 3 | Marked |
| 3 | 2 | 3 | Marked |
| 4 | 2 | 2 | Moderate |
| 5 | 2 | 2 | Moderate |
| 6 | 2 | 2 | Moderate |
| 7 | 2 | 2 | Moderate |
| 8 | 2 | 2 | Moderate |
| 9 | 2 | 2 | Moderate |
| 10 | 2 | 3 | Marked |
| 11 | 2 | 2 | Moderate |
| 12 | 2 | 3 | Marked |
| 13 | 1 | 2 | Moderate |
| 14 | 1 | 2 | Moderate |
| 15 | 2 | 2 | Moderate |
| 16 | 2 | 2 | Moderate |
| 17 | 2 | 2 | Moderate |
| 18 | 2 | 2 | Moderate |
| 19 | 2 | 2 | Moderate |
| 20 | 1 | 2 | Moderate |
| 21 | 1 | 2 | Moderate |
| 22 | 2 | 2 | Moderate |
| 23 | 2 | 2 | Moderate |
| 24 | 2 | 2 | Moderate |
| 25 | 1 | 2 | Moderate |
| 26 | 1 | 2 | Moderate |
| 27 | 2 | 2 | Moderate |
| 28 | 1 | 2 | Moderate |
| 29 | 1 | 2 | Moderate |
| 30 | 1 | 2 | Moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvement

C. PATIENT'S SUBJECTIVE ASSESSMENT FOR TIGHTNESS

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 1 | 2 | moderate |
| 2 | 2 | 3 | Marked |
| 3 | 2 | 3 | Marked |
| 4 | 2 | 3 | Marked |
| 5 | 2 | 3 | Marked |
| 6 | 2 | 3 | Marked |
| 7 | 2 | 3 | Marked |
| 8 | 1 | 3 | Marked |
| 9 | 1 | 3 | Marked |
| 10 | 1 | 3 | Marked |
| 11 | 1 | 3 | Marked |
| 12 | 1 | 3 | Marked |
| 13 | 1 | 3 | Marked |
| 14 | 1 | 3 | Marked |
| 15 | 1 | 2 | moderate |
| 16 | 1 | 2 | moderate |
| 17 | 1 | 2 | moderate |
| 18 | 1 | 2 | moderate |
| 19 | 1 | 2 | moderate |
| 20 | 2 | 2 | moderate |
| 21 | 2 | 2 | moderate |
| 22 | 2 | 2 | moderate |
| 23 | 1 | 2 | moderate |
| 24 | 2 | 2 | moderate |
| 25 | 1 | 2 | moderate |
| 26 | 2 | 2 | moderate |
| 27 | 1 | 2 | moderate |
| 28 | 1 | 2 | moderate |
| 29 | 2 | 2 | moderate |
| 30 | 2 | 2 | moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvement

D. PHYSICIAN'S SUBJECTIVE ASSESSEMENT FOR FINE LINES

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 1 | 2 | moderate |
| 2 | 1 | 2 | moderate |
| 3 | 1 | 2 | moderate |
| 4 | 2 | 2 | moderate |
| 5 | 2 | 2 | moderate |
| 6 | 2 | 2 | moderate |
| 7 | 2 | 2 | moderate |
| 8 | 1 | 2 | moderate |
| 9 | 2 | 2 | moderate |
| 10 | 2 | 2 | moderate |
| 11 | 2 | 2 | moderate |
| 12 | 1 | 2 | moderate |
| 13 | 1 | 2 | moderate |
| 14 | 2 | 2 | moderate |
| 15 | 2 | 2 | moderate |
| 16 | 1 | 2 | moderate |
| 17 | 1 | 2 | moderate |
| 18 | 1 | 2 | moderate |
| 19 | 1 | 2 | moderate |
| 20 | 1 | 2 | moderate |
| 21 | 1 | 2 | moderate |
| 22 | 1 | 2 | moderate |
| 23 | 1 | 2 | moderate |
| 24 | 1 | 2 | moderate |
| 25 | 1 | 2 | moderate |
| 26 | 1 | 2 | moderate |
| 27 | 1 | 2 | moderate |
| 28 | 1 | 2 | moderate |
| 29 | 1 | 2 | moderate |
| 30 | 1 | 2 | moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvement

E. PHYSICIAN'S ASSESSMENT FOR RADIANCE AND GLOW

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 1 | 2 | moderate |
| 2 | 2 | 2 | moderate |
| 3 | 2 | 2 | moderate |
| 4 | 2 | 2 | moderate |
| 5 | 1 | 2 | moderate |
| 6 | 2 | 2 | moderate |
| 7 | 2 | 2 | moderate |
| 8 | 1 | 2 | moderate |
| 9 | 2 | 2 | moderate |
| 10 | 2 | 2 | moderate |
| 11 | 2 | 2 | moderate |
| 12 | 2 | 2 | moderate |
| 13 | 1 | 2 | moderate |
| 14 | 1 | 2 | moderate |
| 15 | 1 | 2 | moderate |
| 16 | 1 | 2 | moderate |
| 17 | 1 | 2 | moderate |
| 18 | 1 | 2 | moderate |
| 19 | 2 | 2 | moderate |
| 20 | 1 | 2 | moderate |
| 21 | 1 | 2 | moderate |
| 22 | 1 | 2 | moderate |
| 23 | 1 | 2 | moderate |
| 24 | 1 | 2 | Moderate |
| 25 | 1 | 2 | Moderate |
| 26 | 1 | 2 | Moderate |
| 27 | 2 | 2 | Moderate |
| 28 | 1 | 2 | Moderate |
| 29 | 1 | 2 | Moderate |
| 30 | 1 | 2 | Moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvemet

F. PHYSICAN'S ASSESSMENT FOR TIGHTNESS

| PATIENT | DAY 21 | DAY 42 | CHANGE |
|---|---|---|---|
| 1 | 1 | 2 | moderate |
| 2 | 2 | 2 | moderate |
| 3 | 2 | 2 | moderate |
| 4 | 1 | 2 | moderate |
| 5 | 1 | 2 | moderate |
| 6 | 1 | 2 | moderate |
| 7 | 1 | 2 | moderate |
| 8 | 1 | 2 | moderate |
| 9 | 2 | 2 | moderate |
| 10 | 1 | 2 | moderate |
| 11 | 1 | 2 | moderate |
| 12 | 1 | 2 | moderate |
| 13 | 1 | 2 | moderate |
| 14 | 1 | 2 | moderate |
| 15 | 1 | 2 | moderate |
| 16 | 1 | 2 | moderate |
| 17 | 1 | 2 | moderate |
| 18 | 1 | 2 | moderate |
| 19 | 2 | 2 | moderate |
| 20 | 1 | 2 | moderate |
| 21 | 1 | 2 | moderate |
| 22 | 1 | 2 | moderate |
| 23 | 1 | 2 | moderate |
| 24 | 1 | 2 | moderate |
| 25 | 1 | 2 | moderate |
| 26 | 1 | 2 | moderate |
| 27 | 2 | 2 | moderate |
| 28 | 2 | 2 | moderate |
| 29 | 1 | 2 | moderate |
| 30 | 1 | 2 | moderate |

LEGEND

1 = very slight improvement

2 = moderate improvement

3 = marked improvement

G. ADVERSE DRUG REACTIONS (PRURITUS)

| PATIENT | DAY 0 | DAY 21 | DAY 42 |
|---|---|---|---|
| 1 | Mild | N | N |
| 2 | Mild | N | N |
| 3 | Mild | N | N |
| 4 | Mild | N | N |
| 5 | N | N | N |
| 6 | N | N | N |
| 7 | moderate | N | N |
| 8 | moderate | N | N |
| 9 | N | N | N |
| 10 | N | N | N |
| 11 | Mild | N | N |
| 12 | N | N | N |
| 13 | moderate | N | N |
| 14 | Mild | N | N |
| 15 | moderate | N | N |
| 16 | N | N | N |
| 17 | N | N | N |
| 18 | Mild | N | N |
| 19 | Mild | N | N |
| 20 | N | N | N |
| 21 | Mild | N | N |
| 22 | Mild | N | N |
| 23 | Mild | N | N |
| 24 | Mild | N | N |
| 25 | Mild | N | N |
| 26 | mild | N | N |
| 27 | mild | N | N |
| 28 | mild | N | N |
| 29 | mild | N | N |
| 30 | mild | N | N |

LEGEND

N = none

Mild = VAS of 1-3

Moderate = VAS of 4-7

Severe = VAS of 8-10

## H. ADVERSE DRUG REACTIONS (ERYTHEMA)

| PATIENT | DAY 0 | DAY 21 | DAY 42 |
|---|---|---|---|
| 1 | N | N | N |
| 2 | N | N | N |
| 3 | N | N | N |
| 4 | N | N | N |
| 5 | N | N | N |
| 6 | N | N | N |
| 7 | N | N | N |
| 8 | N | N | N |
| 9 | N | N | N |
| 10 | N | N | N |
| 11 | N | N | N |
| 12 | N | N | N |
| 13 | N | N | N |
| 14 | N | N | N |
| 15 | N | N | N |
| 16 | N | N | N |
| 17 | N | N | N |
| 18 | N | N | N |
| 19 | N | N | N |
| 20 | N | N | N |
| 21 | N | N | N |
| 22 | N | N | N |
| 23 | N | N | N |
| 24 | N | N | N |
| 25 | N | N | N |
| 26 | N | N | N |
| 27 | N | N | N |
| 28 | N | N | N |
| 29 | N | N | N |
| 30 | | | |

LEGEND

N = none

Mild = VAS of 1-3

Moderate = VAS of 4-7

Severe = VAS of 8-10

## I. ADVERSE DRUG REACTION (DISCOMFORT)

| PATIENT | DAY 0 | DAY 21 | DAY42 |
|---|---|---|---|
| 1 | N | N | N |
| 2 | N | N | N |
| 3 | N | N | N |
| 4 | N | N | N |
| 5 | N | N | N |
| 6 | N | N | N |
| 7 | N | N | N |
| 8 | N | N | N |
| 9 | N | N | N |
| 10 | N | N | N |
| 11 | N | N | N |
| 12 | N | N | N |
| 13 | N | N | N |
| 14 | N | N | N |
| 15 | N | N | N |
| 16 | N | N | N |
| 17 | N | N | N |
| 18 | N | N | N |
| 19 | N | N | N |
| 20 | N | N | N |
| 21 | N | N | N |
| 22 | N | N | N |
| 23 | N | N | N |
| 24 | N | N | N |
| 25 | N | N | N |
| 26 | N | N | N |
| 27 | N | N | N |
| 28 | N | N | N |
| 29 | N | N | N |
| 30 | | | |

LEGEND

N = none

Mild = VAS of 1-3

Moderate = VAS of 4-7

Severe = VAS of 8-10

## J. ADVERSE DRUG REACTION (PAIN AT INJECTION SITE)

| PATIENT | DAY 0 | DAY 21 | DAY 42 |
|---|---|---|---|
| 1 | moderate | N | N |
| 2 | N | N | N |
| 3 | mild | N | N |
| 4 | moderate | N | N |
| 5 | N | N | N |
| 6 | moderate | N | N |
| 7 | moderate | N | N |
| 8 | moderate | N | N |
| 9 | mild | N | N |
| 10 | N | N | N |
| 11 | mild | N | N |
| 12 | mild | N | N |
| 13 | mild | N | N |
| 14 | N | N | N |
| 15 | mild | N | N |
| 16 | N | N | N |
| 17 | N | N | N |
| 18 | N | N | N |
| 19 | mild | N | N |
| 20 | mild | N | N |
| 21 | N | N | N |
| 22 | N | N | N |
| 23 | mild | N | N |
| 24 | mild | N | N |
| 25 | N | N | N |
| 26 | moderate | N | N |
| 27 | mild | N | N |
| 28 | mild | N | N |
| 29 | mild | N | N |
| 30 | N | N | N |

LEGEND

N = none

Mild = VAS of 1-3

Moderate = VAS of 4-7

Severe = VAS of 8-10

## K. OBJECTIVE ASSESSMENT OF COLLAGEN

| H&E | Fibroblasts Day 0 | Fibroblasts Day 42 | % Increase in collagen |
|---|---|---|---|
| | | 60-70 | 1 (20%) |
| 1 | 50-60 | | |
| | | 30-40 | 0 |
| 2 | 30-40 | | |
| | | 35-45 | 1 (15%) |
| 3 | 30-40 | | |
| | | 50-55 | 1 (25%) |
| 4 | 40-50 | | |
| | | 40-50 | 2 (33%) |
| 5 | 30-40 | | |
| | | 30-40 | 1 (30%) |
| 6 | 25-35 | | |
| | | 40-50 | 0 |
| 7 | 50-60 | | |
| | | 40-50 | 2 (33%) |
| 8 | 30-40 | | |
| | | 50-60 | 2 (67%) |
| 9 | 30-40 | | |
| | | 25-35 | 1 (20%) |
| 10 | 20-30 | | |
| | | 40-50 | 2 (33%) |
| 11 | 30-40 | | |
| | | 35-45 | 1 (15%) |
| 12 | 30-40 | | |
| | | 40-50 | 2 (50%) |
| 13 | 20-30 | | |
| | | 35-45 | 2 (40%) |
| 14 | 25-35 | | |
| | | 35-45 | 1 (15%) |
| 15 | 30-40 | | |
| | | 15-25 | 1 (25%) |
| 16 | 10-20cells | | |
| | | 30-40 | 0 |
| 17 | 30-40 | | |
| | | 30-40 | 2 (60%) |
| 18 | 15-25 | | |
| | | 20-30 | 0 |
| 19 | 20-30 | | |
| | | 35-45 | 1 (17%) |
| 20 | 30-40 | | |
| | | 25-35 | 1 (25%) |
| 21 | 20-30 | | |
| | | 15-20 | 0 |
| 22 | 15-20 | | |
| | | 25-35 | 1 (25%) |
| 23 | 20-25 | | |
| | | 35-45 | 1 (17%) |
| 24 | 30-40 | | |
| | | 25-35 | 1 (25%) |
| 25 | 20-25 | | |
| | | 30-35 | 0 |
| 26 | 30-35 | | |
| | | 30-35 | 1 (20%) |
| 27 | 25-30 | | |
| | | 20-30 | 1(20%) |
| 28 | 20-25 | | |
| | | 20-25 | 0 |
| 29 | 20-25 | | |
| | | 25-35 | 1 (25%) |
| 30 | 20-30 | | |

LEGEND

0 = no increase

1 = slight increase

2 = moderate increase

3 = marked increase

## L. OBJECTIVE ASSESSMENT FOR CHANGE IN ELASTIC FIBER DENSITY

| PHYSICIAN'S OBJECTIVE ASSESSMENT (ELASTIC) | %CHANGE |
|---|---|
| 0 | |
| 1 | 0 |
| 2 | 0 (5%) |
| 3 | 0 |
| 4 | 1 (10%) |
| 5 | 1 (10%) |
| 6 | 0 |
| 7 | 0 (5%) |
| 8 | 0 |
| 9 | 1 (10%) |
| 10 | 0 |
| 11 | 1 (10%) |
| 12 | 0 |
| 13 | 0 |
| 14 | 1 (10%) |
| 15 | 1 (10%) |
| 16 | 1 (20%) |
| 17 | 1 (10%) |
| 18 | 1 (20%) |
| 19 | 1 (10%) |
| 20 | 1 (20%) |
| 21 | 0 (5%) |
| 22 | 0 |
| 23 | 0 |
| 24 | 0 (5%) |
| 25 | 0 |
| 26 | 0 |
| 27 | 0 (5%) |
| 28 | 1 (10%) |
| 29 | 0 |
| 30 | |

**FIGURE 1**

**PATIENT CONSENT FORM FOR "The Efficacy and safety of an autologous fractionated plasma extract (PDSC-PRP) on skin rejuvenation: An open-label clinical trial"**

I, _____, of legal age, frequently residing at _____, agree to participate in the research project titled "**PDSC-PRP, an autologous fractionated plasma extract delivered by mesogun: An Open Label Study on its Efficacy and Safety against Skin Aging"** being conducted by Rhett Bosnich MBBS, Ashley Granot MBBS, Vasilis Paspaliaris, MD PhD and George Kolios MD PhD, at the Newin Institue and Ashley Centres (Me Clinics). I have been informed that the purpose of the study is to determine the efficacy and safety of PDSC-PRP for skin aging.

I understand that if I agree to participate in this study, I will be asked to do the following:

1. Have my picture taken during the first visit, on follow-up and upon completion of the study
2. Have a 3mm skin punch biopsy taken from the inferior portion of the mandible during the first visit and upon completion of the study
3. Undergo an intradermal skin test prior to receiving the treatment
4. Have 100cc of blood taken by venipuncture.
5. Undergo injection of PDSC-PRP via mesotherapy on my face
6. Use the only the cleanser and sunscreen provided by the investigators for the duration of the study
7. Agree to publication of my pictures and biopsy results, and
8. Follow-up every two weeks for one month, which is the entire duration of this study.

I am aware that my participation is voluntary and may be withdrawn at any time without penalty or prejudice, and that if I have any additional questions concerning this study, I may contact Dr Rhett Bosnich at Newin Institue. I understand that if I wish further information regarding my rights as a research subject, I may contact the above-mentioned numbers.

I understand that the intended benefits of this study include decreasing fine lines and wrinkles on the areas treated, with a more radiant and brighter glow of my skin, giving me a more youthful look.

I have been informed that potential risks and/or discomforts I could experience during this study including an allergic reaction to the medication, if I have hypersensitivity to any known component. I understand that all information gathered during this experiment will be kept confidential by the investigators. My name or any contact information will not be disclosed to anyone not involved in this study. However, I also understand that data gathered in this study, including my picture or part of my picture and skin punch biopsy may be published.

I understand that my participation is fully voluntary and that I may withdraw from this study on my own free will.

I understand that my consent to participate in this project does not constitute a waiver of any legal rights or redress I might have as a result of my participation, and I acknowledge that I have received a copy of this consent form.

_____

Signature of Subject/Date

**FIGURE 3: SITES TREATED WITH PDSC-PRP VIA MESOTHERAPY**

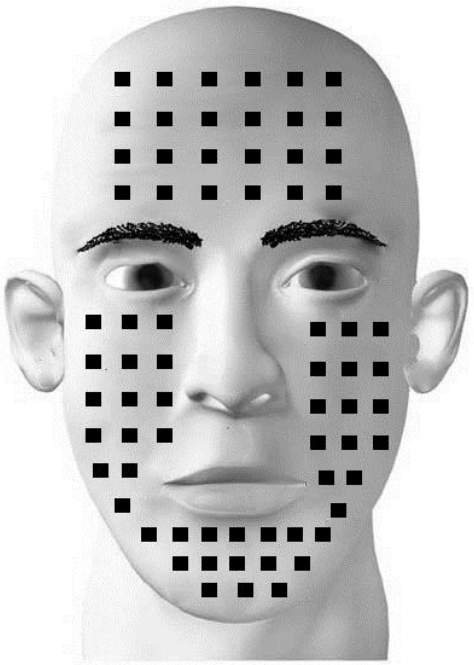

**FIGURE 4-A**

**PATIENT'S SUBJECTIVE ASSESSMENT SCALE**

Please rate the parameters according to the scale below.
0 = no improvement
1 = slight improvement
2 = moderate improvement
3= great improvement

| Parameter | Day 0 | Day 14 | Day 30 | Comments |
|---|---|---|---|---|
| Fine lines | | | | |
| Radiance and Glow of skin | | | | |
| Tightness or fullness of skin | | | | |

**PATIENT'S ADVERSE DRUG REACTION FORM**

| Parameter | D0 | D14 | D30 | Comments |
|---|---|---|---|---|
| Itchiness | | | | |
| Redness | | | | |
| Discomfort | | | | |
| Pain at injection site | | | | |
| Others | | | | |

FIGURE 4-B

**PHYSICIAN'S SUBJECTIVE ASSESSMENT SCALE**

Please rate the parameters according to the scale below.
0 = no improvement
1 = slight improvement
2 = moderate improvement
3= marked improvement

| Parameter | Day 0 | Day 14 | Day 30 | Comments |
|---|---|---|---|---|
| Fine lines | | | | |
| Radiance and Glow of skin | | | | |
| Tightness or fullness of skin | | | | |

**PHYSICIAN'SADVERSE DRUG REACTION FORM**

| Parameter | D0 | D14 | D30 | Comments |
|---|---|---|---|---|
| Pruritus | | | | |
| Erythema | | | | |
| Discomfort | | | | |
| Pain at injection site | | | | |
| Others | | | | |

FIGURE 5

**PHYSICIAN'S SUBJECTIVE ASSESSMENT SCALE**

Please rate the parameters according to the scale below.
0 = no improvement
1 = slight improvement
2 = moderate improvement
3= marked improvement

| **Parameter** | **Photograph 1 (Day 0)** | **Photograph 2 (Day 14)** | **Photograph 3 (Day 30)** | **Comments** |
|---|---|---|---|---|
| Fine lines | | | | |
| Radiance and Glow of skin | | | | |
| Tightness or fullness of skin | | | | |

**GLOGAU'S CLASSIFICATION**

GROUP 1 Mild, usually age 28-35 years
- No keratoses
- Little wrinkling
- No scarring
- Little or no make-up

GROUP 2 Moderate, usually age 35-50 years
- Early actinic keratoses – slight yellow skin discoloration
- Early wrinkling – parallel smile lines
- Mild scarring
- Little make-up

GROUP 3 Advanced, usually age 50-65 years
- Actinic keratoses – obvious yellow skin discoloration
- Wrinkling – present at rest
- Moderate acne scarring
- Make-up always worn

GROUP 4 Severe, usually age 50-65 years
- Actinic keratoses and skin cancers have occurred
- Wrinkling – actinic, gravitational and dynamic
- Severe acne scarring
- Make-up is worn, does not cover but cakes on skin

FIGURE 6

**PHYSICIAN'S OBJECTIVE ASSESSMENT SCALE**

Please rate the parameters according to the scale below.
0 = no increase (0-9%)
1 = slight increase (10-30%)
2 = moderate increase (31-70%)
3= great increase (71-100%)

| **Parameter** | **Day 0** | **Day 30** | **Comments** |
|---|---|---|---|
| Collagen | | | |
| Elastin | | | |

FIGURE 7-A

**CASE RECORD FORM**

| Day | Date of Visit | Status | Procedure | Comments |
|---|---|---|---|---|
| | | | Signing of Consent forms<br><br>Distribution of Materials (cleanser, sunscreen and post procedure instructions)<br><br>Baseline blood chemistry determination | |
| | | | Intradermal skin test<br><br>Initial Photograph<br><br>Initial Punch biopsy<br><br>PDSC-PRP administration<br><br>Subjective assessment questionnaire (accomplished by physician)<br><br>Self-assessment questionnaire (accomplished by patient) | |
| | | | Follow-up Photograph<br><br>Subjective assessment questionnaire (accomplished by physician)<br><br>Self-assessment questionnaire (accomplished by patient) | |
| | | | Final Photograph<br><br>Final Punch biopsy<br><br>Final blood chemistry determination<br><br>Subjective assessment questionnaire (accomplished by physician)<br><br>Self-assessment questionnaire (accomplished by patient) | |

FIGURE 7-B

**PATIENT DIARY**

| **DAY** | **COMMENTS** |
|---|---|
| **1** | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |
| 11 | |
| 12 | |
| 13 | |
| 14 | |
| 15 | |
| 16 | |
| 17 | |
| 18 | |
| 19 | |
| 20 | |
| **21** | |

| DAY | COMMENTS |
|---|---|
| 22 | |
| 23 | |
| 24 | |
| 25 | |
| 26 | |
| 27 | |
| 28 | |
| 29 | |
| 30 | |
| 31 | |
| 32 | |
| 33 | |
| 34 | |
| 35 | |
| 36 | |
| 37 | |
| 38 | |
| 39 | |
| 40 | |
| 41 | |
| 42 | |

**References:**

1. Fitzpatrick's Dermatology in General Medicine: Sixth Edition. Freedberg et al.,McGraw-Hill, 2003.

2. Fisher GJ. "The Pathophysiology of Photoaging of the Skin." Cutis, 2005 Feb;75(2S):5-9.

3. Dermatology. Bolognia J, Jorizzo J, Rapini R., Mosby, 2003.

4. Reisman NR. "Ethics, legal issues and consent for fillers." Clin Plast Surg. 2006 Oct;33(4):505-10.