Protocol

Title: Effect of autologous platelet-rich plasma on photoaged skin: A prospective randomized controlled trial (Phase B). MA081110

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LIST OF ABBREVIATIONS

PRP- platelet-rich plasma
PPP- platelet-poor plasma
UV- ultraviolet
FDA- Food and drug administration

STUDY TITLE: Effect of autologous platelet-rich plasma on photoaged skin: A prospective randomized controlled trial (Phase B).

1. STUDY OBJECTIVES

The primary objective of this study is to evaluate the effect of autologous platelet-rich plasma in the treatment of photoaging**.**

2. STUDY DESIGN

This is a prospective randomized controlled split face study that will evaluate the effect of autologous platelet-rich plasma on photoaged skin, specifically, fine lines, mottled pigmentation, tactile roughness, and sallowness. Subjects who meet the inclusion and exclusion criteria will be enrolled in this study. The subjects will be randomly assigned to receive one treatment session in which one side of the cheek will be treated with intradermal autologous platelet-rich plasma and the other side of will be treated with a normal saline intradermal injection as a control. Two blinded dermatologists will inspect the skin and provide a photoaging score. Standard digital and UVA photographs to evaluate skin surface morphology and photoaging will be taken. Evaluation and photos will take place during the treatment visit (before treatment), at the 2 week visit, and 3 and 6 months after treatment. The subjects will perform a blind self-assessment at the 2 week visit, and 3 and 6 month after treatment. Adverse events will be recorded. A telephone call at 12 months will also assess adverse events.

3. BACKGROUND AND RATIONALE

Platelets are anucleated cells that are derived from megakaryocytes in the bone marrow. The normal concentration of platelets in blood is approximately 140,000 to 400,000/mm^3. Inside platelets, there are three types of platelet secretory granules: α-granules which are most abundant, dense granules, and lysosomes.[1] Numerous proteins that strongly influence wound healing are contained within the α-granules of platelets, including platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, interleukin (IL)-1, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF) and insulin-like growth factor (IGF).[1-3]

Platelets are the first cells to appear after injury when damaged blood vessel walls expose the underlying collagen and extracellular matrix. Platelets are activated at the site of blood vessel injury by locally generated thrombin. During activation, the α-granules within platelets fuse with the platelet plasma membrane and release some of their protein contents to their surroundings, a process called degranulation. Secretory proteins are transformed to a bioactive state and secreted. Active proteins then bind to the transmembrane receptors of target cells e.g. mesenchymal stem cells, osteoblast, fibroblasts, endothelial cells, and epidermal cells.[3]

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma.[4] An 8-fold increase in platelet concentration was found in the platelet-rich plasma compared with that of whole blood.[5-6] It is not only a concentration of platelets, but also a concentration of at least 7 fundamental protein growth factors proven to be actively

secreted by platelets. These growth factors include 3 isomers of platelet-derived growth factor (PDGFαα, PDGFββ, and PDGFαβ), 2 of the numerous transforming growth factors-β (TGFβ1 and TGFβ2), vascular endothelial growth factor, and epithelial growth factor.[5-7] However, growth factor concentrations vary from patient to patient. The cytokines play important roles in cell proliferation, chemotaxis, cell differentiation, and angiogenesis. In addition, the platelets in PRP are delivered to the site of a clot, which contains several cell adhesion molecules including fibronectin, fibrin, and vitronectin. These cell adhesion molecules aid in cell migration by acting as scaffolding upon which cells adhere and begin the wound healing process. There is no standard recommendation for the concentration of platelets in platelet-rich plasma over baseline. Marx[4] suggested that a PRP platelet count of 1 million/µL in 6-mL of plasma is therapeutic PRP. Some investigators have suggested that platelet-rich plasma should achieve a 3 to 5-fold increase in platelet concentration over baseline. Weibrich et al.[8]suggest that different individuals may require different platelet concentration ratios to achieve a comparable biological effect.

Preparation of PRP begins by addition of citrate to whole blood to bind the ionized calcium and inhibit the clotting cascade. Citrate phosphate dextrose (CPD) or acid citrate dextrose (ACD) is generally used as an anticoagulant. Blood is collected with anticoagulant and is immediately processed by 2 centrifugation steps. The first step (hard spin) separates the blood into three layers: red blood cells (specific gravity1.09) at the bottom, platelet-poor plasma (specific gravity 1.06) as the supernatant, and a buffy coat layer (specific gravity 1.06) consisting of platelets and white blood cells in between. The second (soft) spin is an attempt to discard both the RBC layer and the platelet-poor plasma (PPP) to collect only the buffy coat layer, which settle sat the bottom because of its high specific gravity.[9] (Fig.1) Finally, to activate platelets in order to release growth factors from platelet α-granules, calcium chloride and thrombin will be added to the platelet concentrate. Thrombin directly activates platelets and calcium ions replenish the calcium that was bound to the acid citrate dextrose anticoagulant.[3] (Fig.2) The PRP must then be clotted to allow for delivery to the desired site. Platelets begin actively secreting these proteins about10 minutes after clotting, with more than 95% of the pre-synthesized growth factors secreted within 1 hour. Platelets continue to synthesize and secrete additional growth factor for the remaining 7 days of their life span.[4]

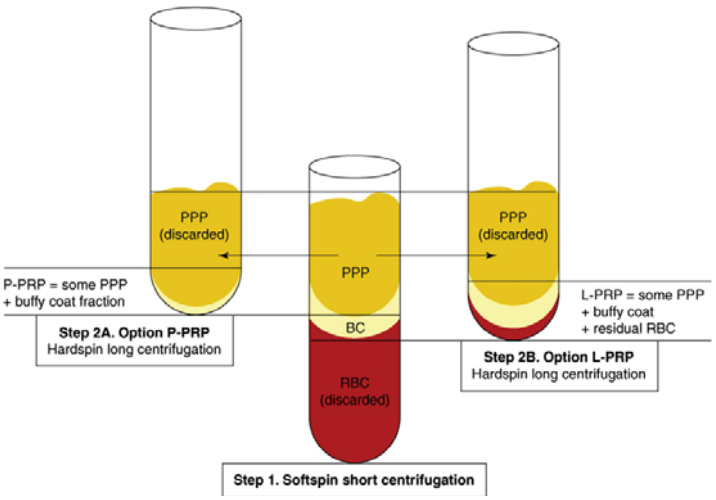


Fig . 1 Classical manual platelet-rich plasma protocol using a two-step centrifugation procedure.[10]

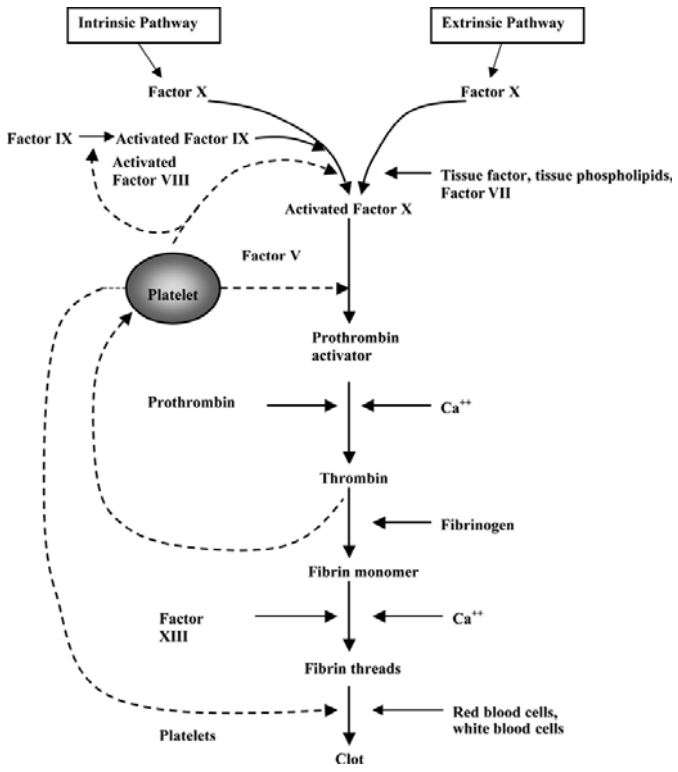


Fig.2 Diagram of platelet clot formation

The use of PRP was proposed to provide a microenvironment for the orchestration of the sequential process of hard and soft tissue regeneration involving migration, proliferation and differentiation of mesenchymal cells. In recent years, the application of platelet-rich plasma has been widely extended in diverse medical and surgical procedures, especially in the fields of wound healing, orthopedic surgery,[11] periodontic surgery,[12] maxillofacial surgery,[13-14] plastic surgery,[15-17] thoracic surgery,[18-19] and ophthalmology.[20-21] In a meta-analysis of oral and maxillofacial surgery which analyzed four studies with 153 patients suffering from chronic periodontitis, a significant improvement in depth reduction was seen in the group treated with PRP. These results suggest that patients with severe chronic periodontitis could benefit from PRP.[22-24] A systematic review found that treatment of skin ulcers with PRP increased the percentage of total recovery but the results were not statistically significant.[24] Powell and colleagues[25] used a split-face study design where each side of face received either experimental PRP or control. This was a randomized controlled trial using autologous PRP in patients undergoing face-lifts. The patients that received PRP after face-lift surgery showed decreased ecchymosis and edema compared to the control group, although results were not statistically significant. Recently, Redaelli and colleagues[26] published a case series of 23 consecutively treated patients with PRP injection into the face and neck. The results were evaluated by photos taken from a dermoscope connected to a digital camera. The average improvement was 'moderate to good' and every patient was satisfied with the overall quality of the treatment. In addition, patients observed that acne scars in the treated areas also improved.

Skin photoaging refers to the effects of long-term ultraviolet exposure and sun damage superimposed on intrinsically aged skin.[27] The clinical signs of photoaging are fine lines, dyspigmentation, skin laxity, yellow hues, wrinkles, and telangiectasias. Histologically, photodamage is manifested as the disorganization of collagen fibrils and the accumulation of

abnormal, amorphous, elastin-containing material representing solar elastosis. Although the predominant component of elastosis is fragmented, thickened elastic fibers, immunochemistry studies have demonstrated the presence of abnormal, fragmented collagen fibers as well.[28-29] In addition, collagen production is reduced and collagen degradation is increased in photoaged skin. Activator protein-1 (AP-1) and transforming growth factor (TGF-β) are involved in this UV-mediated down-regulation of collagen synthesis. There is a decreased expression of TGF-β, an important promoter of collagen synthesis, and its receptor throughout the epidermis and dermis after UV irradiation.[27] UV light also causes damage to collagen and other skin structures through the formation of free radicals. Similar to wound healing, this cellular damage from free radicals is repaired, but in photodamaged skin, the damage from free radicals occurs over time and the injury is ongoing due to continued exposure to UV light. Photodamaged skin may thus be thought of as a chronic wound. A pilot study evaluated topical application of a combination of growth factors and cytokines and the effect on photoaged skin. 11 out of 14 patients showed clinical improvement in at least one facial area and a significant change in objective measurements by optical profilometry indicating a decrease in the depth and number of fine lines. In addition, new collagen formation was observed in biopsy specimens.[30] Repair of photodamaged skin involves the same mechanisms as wound repair, making the enhancement of this process by topical growth factors seems logical.

PRP can be prepared either by a blood bank or by point-of-care table top devices. Compared to table top devices, the preparation of PRP by blood banks through discontinuous plasmapheresis methods might be limited because of higher production costs, multiple transfers, required larger volumes of blood, and the delayed availability of PRP. With table top devices, a similar protocol of a hard and soft spin are followed. Standard cell separators and salvage devices can be used to produce platelet-rich plasma. These devices operate on a unit of blood and typically use a continuous-flow centrifuge bowl or continuous-flow disk separation technology and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations 2-4 times greater than baseline.

The majority of clinical trials reported encouraging outcomes of autologous platelet-rich plasma, but to date there is no randomized controlled clinical trial which can provide evidence of PRP in dermatology. This study will determine whether the intradermal injection of autologous platelet-rich plasma results in the reversal of facial photodamage.

4. STUDY POPULATION

4.1 Inclusion criteria

1. Subjects of either sex (Male or Female), 18-70 years old.
2. Subjects are in good health.
3. Bilateral cheek wrinkles “in motion” with severities of ≥ type II of Glogau photoaging classification (APPENDIX I).
4. Subjects who are willing and have the ability to understand and provide informed consent for participation in the study and are able to communicate with the investigator.
5. Subject requests cosmetic improvement of facial wrinkles.

4.2 Exclusion criteria

1. Pregnant or lactating.
2. Subjects who are unable to understand the protocol or to give informed consent.

3. Subjects who have a history of blood or platelet disorders e.g. anemia, thrombocytopenia, coagulopathy, hypofibrinogenemia, or are on anticoagulant or anti-platelet therapy.
4. Subjects who have had topical or oral tretinoin, chemical peeling, botulinum toxin injection or laser and light treatment for facial rhytides or rejuvenation within past 6 months or planning to undergo treatments as described in the next 3 months.
5. Facial surgery in the lower 2/3 of the face or semi-permanent dermal fillers within 1 year prior to study enrollment
6. Subjects who have history of recurrent facial or labial herpes simplex infection.
7. Subjects who have active skin disease or skin infection in treatment area.
8. Subjects who are allergic to lidocaine or prilocaine.
9. Subjects who have a history of hypertrophic scars and keloids.
10. Subjects who have any requirement for the use of local or systemic steroids or immunosuppressive agents.
11. Subject notes that he/she is HIV positive
12. Subjects with history of skin cancer or actinic keratosis
13. Uncooperative patients or patients with neurological disorders who are incapable of following directions or who are predictably unwilling to return for follow-up examinations.
14. Skin conditions that interfere with wrinkle assessment/treatment (excessive dermatochalasis, inability to lessen the wrinkles by physically spreading the area apart).
15. Excessive exposure to the sun, such as jobs requiring constant outdoor exposure.
16. Known genetic disorders affecting fibroblasts or collagen, such as achondroplasia, osteogenesis imperfecta, etc.

4.3 Criteria for terminating study participation

- Subject wishes to stop being in the study
- Subject no longer qualifies based on inclusion/exclusion criteria
- Adverse events occur that, in the opinion of the investigator, put the subject at increased risk and it is not in the best interest of the subject to continue the study

4.4 Recruiting/Screening

- The investigator will select subjects presenting to the Department of Dermatology Clinic at Northwestern University who meet the inclusion criteria. Those expressing an interest in the study will be contacted for a verbal explanation of the protocol by an investigator.
- Written announcements for participation in the study will also be posted on the internet and around the Northwestern University Feinberg School of Medicine. Volunteer participants will come for a screening visit. If they meet inclusion/exclusion criteria, the participant will be consented.
- Consent for participation will be obtained in writing by signature on a consent form by the study staff. Before consent, the consent will be read by the subject and the subject will be encouraged to ask any questions for clarification. A copy of the subject's signed consent form will be retained in the study file.

5. STUDY PROCEDURES

5.1 Screening visit

- Subjects who meet the inclusion/exclusion criteria will be enrolled and consented.
- The subject's demographic data, medical history, and current usage of any topical drug or cream on the face will be recorded**.**
- Appropriate laboratory testing will be performed (CBC, PT/PTT/INR, liver function, creatinine). All blood will be taken to the Northwestern Memorial Hospital Laboratories on the 18th floor of the Galter Pavilion or the 2nd floor of Arkes Pavilion for processing and storage.

5.2 Treatment Visit

- Laboratory results from screening visit will be reviewed. If all labs are within normal limits, the subject will proceed to the treatment phase. If not, the subject will be withdrawn from the study.
- Subjects will be permitted to apply their usual skin care products and will be requested not alter their usual routine skin care during the study period.
- Subjects will be instructed to avoid the use of any therapeutic agents for photoaging or rejuvenation of the skin during the study period.
- Baseline digital and UV-A photographs (*en face* and 45° oblique) will be taken. Two dermatologists will inspect these photos in order to assign a photoaging score.
- Subjects will receive sunscreen to apply to the whole face during the study period.
- Adverse events will be recorded.

Treatment randomization

Before the study begins, a random number table will be generated for each study participant according to subject number. The number will indicate whether treatment or control will be taken on the right cheek. "0" represents intradermal injection with sterile normal saline whereas "1" represents intradermal injection with autologous platelet-rich plasma.

Standard digital photographing will be performed in the same location for standardized *en face* and 45° oblique views of both sides of face at each visit. The subjects will be photographed 10 minutes after the face is washed. Photos will be taken with a standard clinic camera using ambient lighting for one set of photos per subject and then ultraviolet A lighting for a second set of photos per subject at all visits that indicate photos will be taken

- A topical anesthetic (EMLA, a 5% emulsion preparation, containing 2.5% each of lidocaine and prilocaine, AstraZeneca, Wilmington, DE, USA) will be applied to both cheeks approximately one hour before the treatment begins.
- Each subject will receive an intradermal injection of platelet-rich plasma on one cheek and an intradermal injection of sterile normal saline on the other cheek.

A **Procedure sterile pack** is intended for use with the SmartPReP Platelet Concentrate System.

- All of the following processes will be performed strictly following aseptic techniques.
- Subject's blood will be drawn just prior to start the procedure.

SmartPReP Procedure Pack includes:

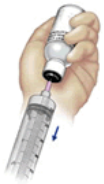
- 19-gauge butterfly catheter
- Acid citrate dextrose-A anticoagulant
- A 20 ml syringe for the blood draw
- 2 10 ml syringes: one for the platelet-poor plasma draw which will be discarded, one for the platelet-rich plasma
- Process disposable (PD)
- Sterile plastic cup

Fig3: SmartPReP Procedure Pack

Preparation of the venipuncture site

A suitably large peripheral vein free of lesions will be selected, typically the antecubital vein.
1. Blood will be drawn by using a 19-gauge butterfly access catheter to avoid trauma to platelets.
2. Tourniquet will be applied and a venipuncture site will be identified.
3. The area at least 4 cm in all direction from the intended site of venipuncture will be scrubbed with an aqueous solution of iodophor compound for a minimum of 30 seconds.
4. The area will be covered with dry, sterile gauze and this area will not be touched again until venipuncture.

Process Steps for platelet-rich plasma



1. Draw 3 ml of acid citrate dextrose A (ACD-A) as an anticoagulant in a 20 ml syringe.

2. Transfer 1 ml ACD-A from the 20 ml syringe into the Plasma Chamber of the Process Disposable (PD)

3. Draw venous blood using acid a citrate dextrose-A (ACD-A) syringe to the ¾ oz mark (approximately 22 ml).

4. Transfer total syringe volume into the blood chamber of the PD (**red port**).

5. Load the SmartPReP® with the PD, close the lid, and press the green start button. To prevent system imbalance, the correct balance weight will be used.

The first spin (hard spin) will separate red blood cells from the plasma that contains platelets, white blood cells, and clotting factors. The second spin (soft spin) will finely separate the platelet concentrate (PRP) from the platelet poor plasma (PPP). The dual spin will be in one time in the separation system. Remove PD(s) when cycle is complete.

6. After processing, use the syringe with spacer and withdraw platelet-poor plasma (PPP) from **white port** until air bubbles are present. Discard the syringe of PPP.

7. Using a new syringe without the spacer, resuspend the platelet-rich plasma with remaining PPP.

8. The final 3 ml of autologous platelet-rich plasma will be immediately injected to the subjects within next 7 minutes.

Injection techniques and wound care

- Injections will be performed intradermally (approximately at the level of the mid-dermis) using a serial puncture technique with a 25-gauge needle. Injections should be approximately 1 cm apart.
- Aliquots of approximately 0.02 ml per puncture will be placed on the designated cheek from zygomatic area to the mandibular area and from the nasolabial folds to the preauricular areas.
- The investigator will prepare 3 ml of sterile normal saline and inject the contralateral cheek in the same manner.
- The subject's eyes will be covered during the procedure in order that they remain blinded to the procedure so that they may do a blinded self-assessment at a later visit.
- Immediately after treatment, the skin will have minimal bleeding and serum secretion that will be controlled by gentle pressure with gauze for five minutes. The skin will then be soaked with saline swabs for 15 minutes.
- For wound care, Aquaphor healing ointment (Eucerin®, Beiersdorf company Germany) will be recommended for continual application for 7 days post-treatment.

5.3 Follow up visits

- **2 week follow up visit**
  - Digital and UV-A photographs (en face and 45° oblique) will be taken. Two dermatologists will inspect these photos in order to assign a photoaging score.
  - Subjects will complete a self-assessment questionnaire.
  - Adverse events will be recorded.

- **3 month follow up visit**
  - Digital and UV-A photographs (*en face* and 45° oblique) will be taken.Two dermatologists will inspect these photos in order to assign a photoaging score.
  - Subject will complete a self-assessment questionnaire.
  - Adverse events will be recorded.

- **6 month follow up visit**
  - Digital and UV-A photographs (*en face* and 45° oblique) will be taken. Two dermatologists will inspect these photos in order to assign a photoaging score.
  - Subject will complete a self-assessment questionnaire.
  - Subject will complete an overall subject satisfaction questionnaire.
  - In the event of an uneven response, subject will be offered an optional standard of care treatment chemical peel (standard of care treatment) to treat the unevenness of the skin.
  - Adverse events will be recorded.

- **12 month telephone follow up**
  - Adverse events will be recorded.

6. DATA COLLECTION AND REPORTING

6.1 Primary Outcome Measures

1. Photoaging[31] scores will be recorded for each cheek by two blinded dermatologists at the treatment visit (before treatment)and during the 2 week, 3 month, and 6 month post-treatment visits. Individual scores for each variable (fine lines, mottled pigmentation, roughness, and sallowness) will be recorded by the blinded dermatologists.

6.2 Secondary Outcome Measures

1. A subject self-assessment of each cheek will be performed at the 2 week, 3 month and 6 month follow up visits.
2. A subject overall satisfaction questionnaire will be performed at the 6 month follow up visit.
3. Adverse events will be recorded.

7. STUDY EQUIPMENT

7.1 **Harvest PRP Separation System** is a table-top centrifuge previously approved by the FDA. It is a table-top, closed process system, with a self-decanting, swinging bucket centrifuge and processing disposable designed to allow rapid automatic separation of plasma and platelets. It is used for the safe and rapid preparation of autologous platelet-rich plasma from a small sample of blood at the patient's point of care.

Fig.3 Harvest PRP Separation System

8. DATA DISCLOSURE AND SUBJECT CONFIDENTIALITY

Subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than the principal investigator and the co-investigators is prohibited. All reports and communications relating to subjects in this study will identify each subject only by their initials and study identification number. Medical information resulting from a subject's participation in this study may be given to the subject's personal physician or to the appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection upon request by the Food and Drug Administration, other government regulatory agency auditors, and the Northwestern University Institutional Review Board (IRB). The information developed in this clinical study will be used to reassess the treatments for photoaging of the skin.

9. EFFICACY ASSESSMENT

Efficacy Assessment will be done based on the primary and secondary outcome measures mentioned above.

10. SAFETY ASSESSMENT

Because of its autologous origin, PRP does not hold any risk of immunological reaction or transmissible diseases. Risks from needle injection include transient pain, bleeding, bruising, and hyperpigmentation. Bleeding is usually easily stopped with compression. Subjects will be given instructions for proper wound care and phone numbers for answering any questions. Treatment for adverse events will be per standard of care from dermatologists in the Department of Dermatology or at the subject's physician of choice. The subject will be financially responsible for these treatments.

11. STATISTICAL CONSIDERATIONS

Data will be analyzed under the supervision of Mary Kwasny, ScD, in the Department of Preventive Medicine. With a sample of 30 patients, we are limited to non-parametric testing, however, as the patients undergo both the PRP and Saline, we should have adequate power to detect any clinically relevant differences.

11.1 Primary Outcome

For the primary endpoint of difference between PRP and Saline in change at 2 weeks for any of the 4 photoaging subscales, a sample of 30 individuals will provide 80% power to detect an effect size of 0.85 at type I error rate of 1.2% (adjusted for multiple comparisons) using Wilcoxon Sign Rank tests. The same effect size would be detected in comparing change from 2 weeks to 3 months to 6 months to determine if any change is sustained.

11.2 Secondary Outcomes

For differences between self-assessment, or satisfaction, a sample of 30 patients will provide 80% power to detect effect sizes as small as 0.68 using Wilcoxon Sign Rank tests at a type I error rate of 5%. Similar power/effect sizes will be detectable in determining if the change in either of these measures is sustainable at 3 and 6 months.

Fisher's exact test will be used to determine if the rate of any adverse events (if reported) differs between PRP and Saline. Assuming independence, if the rate of an adverse event is 3% (1/30) in the control group, we have 80% power to detect rates in the PRP group of 30% (9/30). Regardless, any adverse events will be reported.

12. STUDY SITES

Northwestern University Dermatology Clinic
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13. ETHICAL CONSIDERATIONS

13.1 Human Subjects Protection

A periodic review must be submitted to the IRB at least once per year. The IRB must be notified of completion of the study. After study completion or termination, a final report must be provided to the IRB to close the study. The investigator must maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted. Adverse events that are reported to the FDA as IDE Safety Reports must be submitted promptly to the IRB per IRB guidelines.

At least once per year, the IRB must review and give written approval in order to continue the study. This trial will be conducted in accordance with Good Clinical Practices and the Declaration of Helsinki.

13.2 Consent Form

Prior to study entry, a written informed consent must be obtained from the subject. A copy of the subject's signed consent form must be retained in the study file.

13.3 Protocol Amendments

All changes must be submitted to the IRB. Protocol modifications that impact subject safety or the validity of the study must be approved by the IRB before initiation.

13.4 Retention of Records

Food and Drug Administration and Good Clinical Practice guidelines require that an Investigator retain subject identification codes, subject files, and source data for the maximum period of time permitted by the hospital, institution, or private practice, but not less than 15 years after the completion or discontinuation of the trial.

13.5 Use of Information and Publication

The Principal Investigator or sub-investigators may publish the results of this study in conjunction with appropriate scientific and medical personnel.

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APPENDIX I Glogau photoaging classification

Title: Effect of autologous platelet-rich plasma on photoaged skin: A prospective randomized controlled trial.

Type I no wrinkles

Early photoaging
Mild pigmentary changes
No keratoses
Minimal wrinkles
Patient age: Twenties or thirties
Minimal or no makeup

Type II wrinkles in motion

Early to moderate photoaging
Early senile lenfigines visible
Keratoses palpable but not visible
Parallel smile lines beginning to appear
Patient age: late thirties or forties
Usually wears some foundation

Type III wrinkles at rest

Advanced photoaging
Obvious dyschromia, telanglectasia
Visible keratoses
Wrinkles even when not moving
Patient age: fifties or older
Always wears heavy foundation

Type IV "only wrinkles"

Severe photoaging
Yellow-gray color of skin
Prior skin malignancies
Wrinkled throughout, no normal skin
Patient age: sixties or seventies decade