Growth factor concentrations in platelet rich plasma for androgenetic alopecia: an intra-subject, randomized, blinded, placebo controlled, pilot study

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Abstract

**Background:** Platelet rich plasma (PRP), processed from autologous peripheral blood, is used to treat androgenetic alopecia (AGA). **Objective:** To determine the efficacy of PRP for hair growth promotion in AGA patients in a randomized, blinded, placebo controlled, pilot clinical trial (NCT02074943). **Methods:** The efficacy of an 8 week, 5 session, PRP treatment course was determined by measuring hair density and hair caliber changes in 10 AGA affected patients. For each PRP sample, the concentrations of selected growth factors were determined using a multiplex assay system. The clinical results were then correlated to the growth factor concentrations in PRP. **Results:** At 16 weeks, 8 weeks after the last PRP injection, treated areas exhibited increased mean hair density (+12.76%) over baseline compared to placebo (+0.99%). Mean hair caliber decreased in both treated and placebo regions (-16.22% and -19.46% respectively). Serial analysis of PRP significant variability in concentrations between patients. Overall, there was a positive correlation between GDNF concentration and hair density (p=0.004). Trends, though not statistically significant, were also observed for FGF2 and VEGF. **Limitations:** Small sample size and lack of comparative cohorts receiving protocol variations limit confidence in the study data. **Conclusions:** This small pilot clinical trial suggests PRP treatment may be beneficial for AGA. However, the variable hair growth responses between patients indicate there is a significant opportunity to improve PRP therapy protocols for hair growth promotion. The variability in growth factor concentration in PRP suggests standardization of growth factors post-processing might improve hair growth responses.

**Key words**

platelet rich plasma, androgenetic alopecia, growth factors, Glial cell line-derived neurotrophic factor
Introduction

Androgenetic alopecia (AGA) is the most common cause of hair loss in both men and women.\(^1\) Treatment with autologous platelet rich plasma (PRP) is an innovative therapy that has gained considerable attention in diverse fields of medicine such as orthopedic interventions\(^2\) and oral surgery.\(^3\) In dermatology, PRP has been used to treat chronic diabetic wounds, scarring, and for cosmetic skin augmentation and rejuvenation, where repeated application shows some success.\(^4\)-6 More recently, several clinical reports have proposed the use of PRP in the treatment of hair loss.\(^7\)-11

Platelets are non-nucleated blood cells which provide hemostasis primarily by means of clot formation. Typically, PRP treatment involves a patient's own autologous plasma that has been mechanically centrifuged to increase the concentration of platelets 3-8 times as compared to whole blood.\(^12\) This high concentration of platelets is capable of delivering more platelet associated growth factors to a localized area at supra-physiological levels with the intent of promoting accelerated tissue growth and regeneration.

PRP contains many growth factors, and other components,\(^13\) with the potential to have a significant effect on hair growth. Growth factors present in typical PRP preparations,\(^14\),\(^15\) such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and several fibroblast growth factors (FGFs), should be beneficial for hair growth.\(^16\) However, PRP also contains factors such as transforming growth factor beta (TGFβ, TGF2),\(^17\) that have been shown to inhibit hair growth.\(^18\) The characterization of PRP suggests any efficacy is likely based on the net effect of positive and negative modulators of hair growth promotion.

PRP can be further processed to produce “activated” PRP. Activation generally involves encouraging the release of growth factors from platelets into the plasma component prior to injection and can be achieved using a variety of methods.\(^19\) Activated PRP has been showed to promote, faster telogen-to-anagen transition and increase the proliferation of dermal papilla cells, potentially via fibroblast growth factor 7 (FGF7) signaling.\(^20\)-22 Activated PRP has also been
shown to have a positive effect on hair follicle formation in skin reconstitution assays. Notably, a recent study examining hair growth in response to activated PRP, with analysis of PDGF, EGF, and VEGF, was unable to identify a correlation to growth factor concentration.

While early journal publications on PRP largely focused on case series reports, some comparative control studies have been conducted in humans, albeit using a variety of treatment regimens. Arguably, the most relevant trial design for local scalp injections of PRP is an intra-control, defined area, quantitative analysis study. For example, an intra-control half head treatment study on AGA patients using PRP and placebo showed a statistically significant improvement in the number of anagen hairs in the target area and in total hair density post-treatment. Pilot studies have also evaluated the efficacy of PRP for treating alopecia areata and were found to improve hair growth. Recent reviews of PRP for the treatment of hair loss conclude that, though clearly still in a nascent stage, the use of PRP for alopecia is promising and the side effects are relatively limited.

With the current study, we aimed to study the effects of intradermal PRP injections on androgenetic alopecia (AGA), and quantify select growth factors in PRP. We used an intra-control, defined area, quantitative analysis, placebo controlled design in a pilot study to determine the effects of PRP on hair growth in AGA subjects. We hypothesized that concentrations of specific growth factors present in PRP may correlate to the degree of hair growth observed in patients.

Materials and Methods

Subjects

This study was approved by the Institutional Review Board of the University of British Colombia (H13-03126) and was listed on the clinicaltrials.gov website (NCT02074943). All subjects provided informed, written consent. Inclusion and exclusion criteria were as described
In a pilot cohort treated with non-activated PRP, a total of 10 subjects (9 female, 1 male) were enrolled; age ranged from 20-55 years (average 35.5 years). All subjects were clinically diagnosed with AGA by an experienced dermatologist and hair specialist (Dr. J. Shapiro) (supplementary table SII).

**Hair growth assessment**

Clinical evaluation, photographs and hair measurements were taken before the PRP treatment regime started (visit 1; baseline) during the treatment course (visit 4; immediately prior to the third injection of PRP; 4 weeks after the start of PRP treatments) and after (visit 7; 8 weeks after the final PRP treatment session) completion of the PRP regime (Table I). The assessments included photography of the region injected using a dermatoscope (Folliscope®, LeadM, Seoul, South Korea), hair density counting, and hair caliber measurement using the associated software.

**PRP preparation**

PRP was prepared using a modified, non-proprietary protocol based on previous publications. Briefly, 20 mL of venous blood was centrifuged at 300g at 18°C for 5 minutes; the upper fraction (PRP1) was separated and transferred into a sterile tube without disturbing the buffy coat, the PRP1 volume was then quantified (typically 6-9.5 ml). Subsequently, PRP1 was centrifuged at 700g at 18°C for 17 minutes, the upper part, designated as PPP (platelet poor plasma) was transferred into a sterile tube. The platelet pellet obtained from centrifugation of PRP1 was resuspended in 1200 μl PPP to produce PRP2. 250 μl of PRP2 was saved for growth factor detection and another 10 μl of PRP2 was used for the quantification of platelet concentration in a subset of samples. The PRP was not separately activated prior to injection.

**PRP injection**

After processing, the PRP was injected into one side of the fronto-temporal scalp in an area of approximately 3 cm² with a volume of approximately 0.1 mL/cm². An equivalent volume of normal saline was injected in the contra-lateral frontal region of the scalp. Physicians marked the
scalps before injection, the center of the 3 cm² region of the injections was 12cm distance from lateral angle of the eye, and 9cm distance from top of the ear. The intervals of repeat injections was 2 weeks, with 5 injections total (Table I), based on a previously published protocol. 35

Determinant of growth factor concentration

The samples of PRP were processed to evaluate concentrations of: Fibroblast growth factor beta (FGFβ; FGF2), Epidermal growth factor (EGF), Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Glial cell line-derived neurotrophic factor (GDNF), and Platelet-derived growth factor BB (PDGF-BB) by Luminex® xMAP technology (FlowMetrix; Luminex, Austin, TX) using manufacturer’s protocols.

Statistical analysis

Paired t test and Pearson correlation analysis were employed by using a combination of SPSS statistics 24 (IBM Corp, Armonk, NY) and Excel (Micosoft Corp, Redmond, WA). A p-value of less than 0.05 was considered statistically significant.

Results

Platelet enrichment

The protocol described provided an average 6.96 fold enrichment in PRP platelet density. Mean platelet counts varied between patients and within the same patient at each PRP processing and injection point (Supplemental fig 1). Consequently, the achieved rate of platelet enrichment was also variable. Linear regression analysis indicated the starting concentration of platelets in the collected peripheral blood correlated to the end platelet fold change enrichment in the processed PRP (p=0.017, r=0.53).
Hair growth changes in response to PRP treatment

We examined the hair density and hair diameter of patients at baseline, prior to the first injection of PRP, after 4 weeks (immediately prior to the 3rd PRP treatment session), and 8 weeks after the final PRP treatment. For the 10 subjects, mean baseline hair density counts were similar (treatment area 114.7; placebo area 114.2). After 4 weeks, an apparent increase in hair density in both treatment and placebo areas was observed (134.4 versus 136 respectively). At the final visit, 8 weeks after the final PRP injection, a difference was observed with the treatment site having a higher hair density (129.3) as compared to the placebo site (115.3). The treatment and placebo site hair densities were higher than observed at baseline (increased by 12.7% and 0.96% respectively) however, the difference was not statistically significant (p=0.126). The same hair fibers counted for hair density were also measured to determine hair diameter. Somewhat unexpectedly, hair caliber in both the treated and placebo areas exhibited an average decrease over time from baseline to the final measurement at visit 7; a decrease of -16.22% and -19.46% respectively (p=0.067). Global photographs did not suggest any significant overall improvement within the area of injection by subjective observation (Fig 1).

Variation in growth factor concentration in PRP

The relative concentration of each respective growth factor in PRP was quite varied. PDGF-BB was the highest concentration of growth factor injected, with patients receiving an average 7437 pg/ml. The lowest growth factor concentration of those tested was for VEGF (mean 125 pg/ml) (Fig 2). Within each subject, the growth factor concentrations in PRP produced at the 5 treatment time points remained relatively consistent (Supplemental fig 2). However, comparing the PRP obtained between different subjects revealed there were considerable variations in the total quantity of specific growth factors injected per subject over the course of the study (Supplemental fig 3). For example, the plasma of subject 4 consistently yielded concentrations of greater than 1000 pg/ml of FGF2 at every PRP collection, processing and injection session (mean, 1545 pg/ml). In contrast, the plasma of subject 8 consistently yielded concentrations of less than 50 pg/ml of FGF2 at every PRP collection, processing and injection session (mean, 38
pg/ml). Consequently, the nature of PRP injection therapy is likely unique to each individual patient.

**Change in hair growth parameters in relation to growth factor concentration**

In comparing hair growth parameters to growth factor concentration, multiple linear regression analysis did not identify a significant correlation between patient hair density or caliber changes and combined growth factor concentrations. However, correlation of hair density increase against FGF2 concentration alone approached significance (p=0.087, r=0.60). Correlation of hair density increase against GDNF concentration alone was significant (p= 0.004, r=0.85). For VEGF, linear regression analysis of all subjects completing the study suggested no correlation (p=0.833, r=0.08). However, removal of one outlier subject, exhibiting a 5 fold greater VEGF concentration as compared to other patients, enabled significant correlation (p=0.030, r=0.756) (Supplemental fig. 4). For correlation to hair caliber, only VEGF concentration approached significance (p=0.071, r=0.63) (supplemental fig. 5).

**Discussion**

**Safety and efficacy**

In this pilot study, we observed repeated, autologous PRP application to be a generally safe treatment protocol; no significant side effects were observed. Some subjects did report mild pain and a temporary stinging sensation in the scalp region of PRP injection, which disappeared within a few hours after the treatment application. Statistically significant clinical hair growth changes were not achieved. This was likely due to the small cohort of 10 subjects in this pilot study, the wide variation in hair growth responses observed between subjects, and the short 2 month duration of the PRP treatment application protocol. Consequently, the increase in hair count density did not translate into clear macroscopic visual improvements. However, the data suggest a trend for an increased hair density in response to injection of non-activated PRP which
is consistent with previous studies.\textsuperscript{25} Our data also suggest that activation of platelets with exogenous compounds may not be needed for treating hair loss. This could be relevant in some jurisdictions, such as the United Kingdom for example, where the addition of compounds to activate PRP prior to injection is a regulatory grey area.

\textit{Low platelets}

Unsurprisingly, low platelets in a subject’s whole blood was highly correlated to a lower degree of platelet enrichment in the processed PRP. This could partly account for such large variations in the end concentration of growth factors in PRP for different subjects. There has been at least one attempt to use homologous PRP in place of autologous PRP for treating hair loss.\textsuperscript{36} Such an approach may overcome low platelet count issues in some patients, and would also provide an on-demand treatment without the need for in-clinic PRP processing, though the safety and jurisdiction regulatory status of such an approach is open to question.

\textit{Changes in hair density and caliber}

We observed an increase in hair density in the PRP treated area by the end of the study. The apparent increase in hair density at 4 weeks in placebo injected skin may simply be a consequence of measurement error. However, the regions for injection were carefully demarcated by the same individual using measurements from the lateral angle of the eye and from top of the ear. It is also possible that the observed increase is specific and a response to injury from the repeated injection process. Skin injury is a known stimulator of hair growth; the phenomena has been exploited with the development of micro-needling techniques to promote hair growth for example.\textsuperscript{37,38} While a significant visible improvement in hair growth was not achieved, the trend for increased hair count density in the PRP treated areas suggests that our 8 week treatment protocol duration was likely too short. Treatment with PRP over a longer period of time might allow further improvement to hair density.
In contrast to other studies evaluating clinical responses to PRP treatment, we observed a reduction in mean hair caliber over time. This apparent reduction in hair diameter might be due to the underlying AGA effects and ongoing miniaturization. It is also possible that the increase in hair fiber density in the treatment area comprised fibers derived from intermediate hair follicles, leading to a net reduction in average diameter. Of note, the reduction in caliber was relatively less in the treated area versus the placebo area. Most other studies on PRP as a hair growth promoting treatment evaluate hair caliber over a much longer time period, well beyond 8 weeks. It is possible that further improvements in hair diameter would be seen with treatment over a longer time period beyond.

**Growth factor concentrations and variation**

Significant variations in growth factor concentration between different subjects were observed for all of the factors tested at all time points. However, within the same subject there was a relative consistency in the respective concentrations of growth factors over the 5 treatment sessions. Consequently, although the PRP produced from each subject could be variable in terms of growth factor concentration, mean values for all subjects over time demonstrated relatively more consistent results. As blood processing and treatments were performed in an overlapping sequence, with 5 blood samples processed from each subject at different time points, and PRP processing was conducted by the same individuals with the same equipment each time, the variation in observed concentrations is less likely to be due to variability in equipment or materials,\textsuperscript{25} processing error, or progressive skills/experience development in processing. We suggest the variations in growth factor concentration are more likely to reflect real differences in PRP quality between patients. Consequently, it is conceivable that the net stimulatory effects on hair growth could be equally variable between patients. An equal volume of PRP produced from different patients is not necessarily comparable in terms of growth factor concentration, and therefore efficacy, in terms of hair growth promotion ability.

When comparing hair growth responses to growth factor concentrations, the degree of increase in hair density positively correlated to GDNF concentration in PRP (p= 0.004, r=0.85). The direct
effect of GDNF on hair growth has not been investigated in detail. However, GDNF and its receptors are expressed in hair follicle cells and tissues, while studies suggest it promotes hair follicle cell proliferation and retards hair follicle catagen regression. In addition, there was a trend, though not statistically significant, suggesting a potential positive correlation between hair density and FGF2 concentration. FGF2 is known to have a significant positive effect on hair follicle growth.

Hair density may also improve in response to VEGF. VEGF has been extensively investigated in relation to hair growth and cycling regulation and has been revealed as a potent hair growth promoting growth factor, likely due to its ability to stimulate angiogenesis. To identify a statistically significant relationship to VEGF, an outlier was subsequently removed from the analysis. This subject presented with a mean VEGF concentration that was 5 fold greater than the mean observed for the other 9 subjects. Notably, the same subject did not exhibit any hair growth response in the PRP treatment region. Cytokines and growth factors as treatments can have negative side effects and it is possible that high concentration VEGF toxicity can occur. Potentially, PRP with very high concentrations of certain growth factors may be detrimental to hair growth. If so, then there is likely an optimal window of growth factor concentration in PRP that enables maximal hair growth while avoiding detrimental effects. As such, activation of PRP may not be appropriate; at least not without subsequent titration of the activated PRP to an optimal concentration of growth factors.

**Limitations**

There are several limitations specific to the current study, and to the use of PRP for treating hair loss in general. The pilot study presented here is significantly limited by the small number of subjects treated, and the relatively short treatment course. PRP treatment protocols for alopecia are many and varied, largely designed based on trial and error by individual dermatologists within their own respective clinics. The frequency of PRP procedures and the duration of the overall treatment protocol likely have a significant effect on hair growth response. The concentration of PRP/platelets, whether to activate PRP or not, the nature of activation, the
density, volume, and depth of PRP injections, are all variables that could have a significant impact on PRP efficacy in treating alopecia. The subject too is a possible source of significant variability. Pattern, extent, and duration of the hair loss, age, sex, and genetic background of the individual could all play a role in defining the degree of response to PRP. Never-the-less, the data from this small pilot study does suggest it may be possible to determine the most significant growth factors, and their optimal concentrations for efficacy, with larger trials involving comparative cohorts. Attempts to objectively investigate the effects of PRP, with quantification of hair growth and comparison to defined PRP characteristics, should be a priority.32,51,52

Conclusions

This small randomized, placebo-controlled pilot clinical trial suggests a potential beneficial effect for PRP treatment of AGA. The variable hair growth responses, indicate there is a significant opportunity to improve PRP therapy protocols for hair growth promotion. The variability in growth factor concentration in PRP suggest standardization of growth factor concentration post processing could aid control of hair growth responses. A larger cohort study may help with development of new and improved PRP protocols for AGA.

Acknowledgements

This study was supported in part by unrestricted funds from Replicel Life Sciences Inc. (Canada).
References


Figures

**Figure 1. Hair density in the region of PRP application.** Hair density prior to first PRP injection (non-activated PRP; cohort 1) for a subject with Ludwig I (A). Hair density in the same subject at 16 weeks, 8 weeks after the final injection of PRP (B).

**Figure 2. Variation in growth factor concentration in PRP.** Measurement of growth factor concentration in PRP identified PDGF-BB as the most prevalent, with VEGF having the lowest mean concentration. Bars indicate standard deviation.
Tables

Table I. Clinical trial timeline

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Disclosures

Dr Siah, Conflicts of interest: none.

Dr Guo, Conflicts of interest: none.

Dr Chu, Conflicts of interest: none.

Dr Santos, Conflicts of interest: none.

Dr Nakamura, was an employee of Replicel Life Sciences Inc. at the time the research study was conducted.

Dr Shapiro has received shares in Replicel Life Sciences Inc as a company co-founder. He has received payment for his participation in advisory boards for Replicel Life Sciences Inc. and Regen Lab SA.

Dr McElwee has received contract research support for his role as an investigator from Replicel Life Sciences Inc. He has received shares in Replicel Life Sciences Inc as a company co-founder and for consultation and participation in advisory boards. He has received payment for his role as his role as Chief Scientific Officer of Replicel Life Sciences Inc.

Author contribution statement:

TWS, TC, LS, and JS designed the clinical study protocol; HG, HN, GL, and KJM designed the research protocol; TWS, TC, LS, and JS performed clinical assessments, data collection, blood collection and clinical treatment procedures; HG, HN, and GL performed the laboratory experiments; HG, LS and KJM analysed the data; HG and KJM wrote the manuscript. All authors have read and accepted the final manuscript.
Corresponding author
Kevin J. McElwee. Centre for Skin Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK.

IRB approval status
Reviewed and approved by the Institutional Review Board for the University of British Columbia, Vancouver, Canada (H13-03126). Clinical trials.gov database identifier: NCT02391935

Abbreviations
AGA – androgenetic alopecia; EGF, Epidermal growth factor, FGF2, Fibroblast growth factor 2/beta; GDNF, Glial cell line-derived neurotrophic factor; HGF, Hepatocyte growth factor; PDGF-BB, Platelet-derived growth factor BB; VEGF, Vascular endothelial growth factor.
Growth factor concentrations in platelet rich plasma for androgenetic alopecia: an intra-subject, randomized, blinded, placebo controlled, pilot study


Supplemental file

Supplemental Figures

Supplemental figure 1. Mean platelet enrichment in 5 individual subjects with PRP processing

Mean platelet counts for whole blood and in PRP post processing are shown for 5 individual subjects. Bars indicate standard deviation.
Supplemental figure 2. Mean PRP growth factor concentrations for all subjects at each treatment visit session

Mean growth factor concentrations are shown for all subjects as calculated for each treatment session; treatment 1 (visit 2), treatment 2 (visit 3), treatment 3 (visit 4), treatment 4 (visit 5), and treatment 5 (visit 6). Bars indicate standard deviation.
Supplemental figure 3. Mean growth factor concentration per subject

Mean FGFb concentration in PRP

Mean EGF concentration in PRP

Mean HGF concentration in PRP

Mean VEGF concentration in PRP

Mean GDNF concentration in PRP

Mean PDGF-BB concentration in PRP

Mean growth factor concentrations are shown for each subject as calculated for all 5 treatment sessions. Bars indicate standard deviation.
Supplemental figure 4. PRP growth factor concentration correlation to hair density

Mean FGF2 concentration correlation to hair density change

\[ y = 0.1104x - 22.167 \]
\[ R^2 = 0.3616 \]

Mean EGF concentration correlation to hair density change

\[ y = 0.03x - 12.414 \]
\[ R^2 = 0.0447 \]

Mean HGF concentration correlation to hair density change

\[ y = 0.0272x - 20.821 \]
\[ R^2 = 0.2536 \]
Mean VEGF concentration correlation to hair density change

\[ y = -0.0025x^2 + 1.2386x - 67.441 \]
\[ R^2 = 0.5022 \]

Mean VEGF concentration correlation to hair density change - with outlier removed

\[ y = 0.9121x - 60.312 \]
\[ R^2 = 0.5712 \]

Mean GDNF concentration correlation to hair density change

\[ y = 0.1291x - 12.341 \]
\[ R^2 = 0.7204 \]
Mean PDGF-BB concentration correlation to hair density change

\[ y = -0.0059x + 56.95 \]

\[ R^2 = 0.122 \]
Supplemental figure 5. PRP growth factor concentration correlation to hair caliber

Mean FGF2 concentration correlation to hair caliber change

\[ y = -4 \times 10^{-6}x - 0.007 \]
\[ R^2 = 0.0041 \]

Mean EGF concentration correlation to hair caliber change

\[ y = -2 \times 10^{-5}x + 0.0067 \]
\[ R^2 = 0.1181 \]

Mean HGF concentration correlation to hair caliber change

\[ y = -5 \times 10^{-6}x - 0.0019 \]
\[ R^2 = 0.0684 \]
Mean VEGF concentration correlation to hair caliber change

\[ y = 8 \times 10^{-5}x - 0.0178 \]

\[ R^2 = 0.3929 \]

Mean VEGF concentration correlation to hair caliber change - with outlier removed

\[ y = 2 \times 10^{-6}x - 0.0118 \]

\[ R^2 = 4 \times 10^{-5} \]

Mean GDNF concentration correlation to hair caliber change

\[ y = -7 \times 10^{-6}x - 0.0069 \]

\[ R^2 = 0.0178 \]
Mean PDGF-BB concentration correlation to hair caliber change

$y = -1E-06x + 0.0023$

$R^2 = 0.0524$
### Supplemental Table SI. Study inclusion and exclusion criteria

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<thead>
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<th>Inclusion criteria</th>
<th>Details</th>
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<tr>
<td>Males and females in good general health, age &lt;18 years old, &gt;70 years old</td>
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<tr>
<td>Patients with mild to moderate androgenetic alopecia (Ludwig alopecia score I and II; Hamilton-Norwood score 1 to 4).</td>
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<td>Not on other treatments for AGA for at least 12 weeks prior to the start of the study or during the study period</td>
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<td>Have platelet disorders, anemia and/or bleeding disorders</td>
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<td>Are known to be HIV or hepatitis B or C positive or otherwise immunocompromised</td>
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<td>Have active skin disease or skin infection in treatment area</td>
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<td>Patients with a propensity for keloid scarring</td>
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<td>Are on non-steroidal anti-inflammatory medications. Examples of these medications include ibuprofen, naproxen, indomethacin and aspirin. (Non-steroidal medications work by blocking the action of some of the growth factors present in PRP, thus may render the injection ineffective).</td>
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<td>Patients who are unable to understand the protocol or to give informed consent. Or are otherwise deemed unsuitable candidates</td>
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Supplemental Table SII. Study cohort demographic and clinical characteristics at baseline

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