Preliminary Report

Introducing Platelet-Rich Stroma: Platelet-Rich Plasma (PRP) and Stromal Vascular Fraction (SVF) Combined for the Treatment of Androgenetic Alopecia

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Abstract

Background: Androgenetic alopecia (AGA) is characterized by miniaturization of the hair follicles gradually causing conversion of terminal hairs into vellus hairs, leading to progressive reduction of the density of hair on the scalp. Approved therapeutic options are limited and show side effects.

Objectives: To evaluate injections of stromal vascular fraction (SVF), which is rich in adipose-derived stromal cells (ASCs) in combination with platelet-rich plasma (PRP) in the upper scalp as a new autologous treatment option for AGA.

Methods: Ten male patients (age range, 25-72 years), suffering from AGA at stage II to III according to the Norwood-Hamilton scale, have been treated with a single injection of autologous PRS (ACP^{SVF}: combination of PRP and SVF) in the upper scalp. Preinjection and 6 and 12 weeks postinjection changes in hair density were assessed using ultra high-resolution photography (Fotofinder).

Results: Hair density was significantly increased after 6 weeks and 12 weeks postinjection ($P = 0.013$ and $P < 0.001$). In hair-to-hair matching analyses, new hair grew from active follicles. Furhtermore nonfunctioning hair follicles filled with hyperkeartotic plugs, up to today assumed incapable of forming new hair, proved to grow new hair. No side effects were noted after treatment.

Conclusions: A single treatment of platelet-rich stroma injected in the scalp of patients with AGA significantly increased hair density within 6 to 12 weeks. Further research is required to determine the optimal treatment regimen. Preferred options to our opinion include the repetition of PRS or additional treatments with PRP.

Level of Evidence: 4

Editorial Decision date: January 25, 2018.

Androgenetic alopecia (AGA) is a genetically determined and androgen influenced progressive condition, which is characterized by progressive hair loss of the scalp. AGA develops in a typical way, affecting the temples, vertex scalp, and mid-frontal scalp.[1] The prevalence of AGA varies by age, genetics, and race.[2-4] AGA is reported to be more common in Caucasian men whereas 30% of men are affected by the age of 30 years and up to 50% by the age of 50 years.[5]

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The pathogenesis of AGA is based on miniaturization of the hair follicle and alterations in the hair cycle. This process is known to cause gradual conversion of terminal hairs into vellus hairs.[6] Simultaneously, the telogenic and anagenic stages shorten, resulting in a progressive reduction of thickness, density, and total numbers of both of these hair types.[7]

Currently, minoxidil and oral finasteride are the only two therapeutic drugs approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of AGA. Treatment with finasteride, a 5α-reductase inhibitor, or minoxidil, of which its action is not yet fully understood, must be taken lifelong and daily, as its interruption is followed by gradual return of hair loss. Other currently available nonsurgical treatments have limited effectiveness, making AGA a remaining unsolved problem.[8,9]

Platelet-rich plasma (PRP) seems a new promising strategy for the treatment of AGA.[10-14] PRP can be derived from whole centrifuged autologous blood easily and presents a higher concentration of platelets than unprocessed blood plasma.[11,12] Activation of platelets releases a myriad of growth factors (GF), including platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and interleukin (IL)-1. These GFs, in turn, activate processes including angiogenesis and differentiation of cells in the direct microenvironement. In recent studies it is hypothesized that growth factors may act on the dermal papilla (DP) and stem cells in the bulge area of the follicles, stimulating the development of the follicular unit and promoting neovascularization.[12-14] Taking these facts together, we assume that, on a cellular level, AGA is a highly similar condition to tissue damage. Repair processes, which are activated upon tissue damage, are positively influenced by growth factors which in turn stimulate proccesses including chemotaxis and subsequent "homing" of cells needed for repair. We theorized that this process could be amelioreted by also adding the desired cellular component for optimal wound healing and regeneration: the regenerative cells, localised in their niche within the autologous stromal vascular fraction (SVF). Formerly referred to as adipose-derived stem cells, we now like to address this cellular component of the repair process as adipose-derived stromal cells (ASCs).[15] These ASCs have been shown to stimulating follicle regrowth and modulation of the hair cycle also, possibly by their own production and secretion of GFs.[15-17]

Impressive results of this combination of PRP plus SVF (referred to as platelet-rich stroma [PRS]) have been observed frequently in the practice of the corresponding author over the last 2 years, varying from unprecedented regneration of scars in the skin[18] to unprecedented reduction of pain from osteoartrosis in the knee,[19] hip, and wrist (papers in progress).

Since we have developed and published an inexpensive and quick method to obtain SVF from autologous adipose tissue by mechanical fractionation,[18] this treatment modality is tested for multiple soft tissue lesions including damaged facial skin. AGA being a skin related, parallel developing, nearby condition was perceived as a perfect new target. In these case reports we share the findings of ten patients with AGA treated consecutively with a single injection of autologous PRP in combination with SVF from adipose tissue turning this procedure in an easy and quick treatment in an office-based setting.

METHODS

Patient Consent

The study was conducted between January and December 2016. Informed consent and specific approval for this treatment was obtained from all patients preceding the procedure, respecting the Declaration of Helskini.

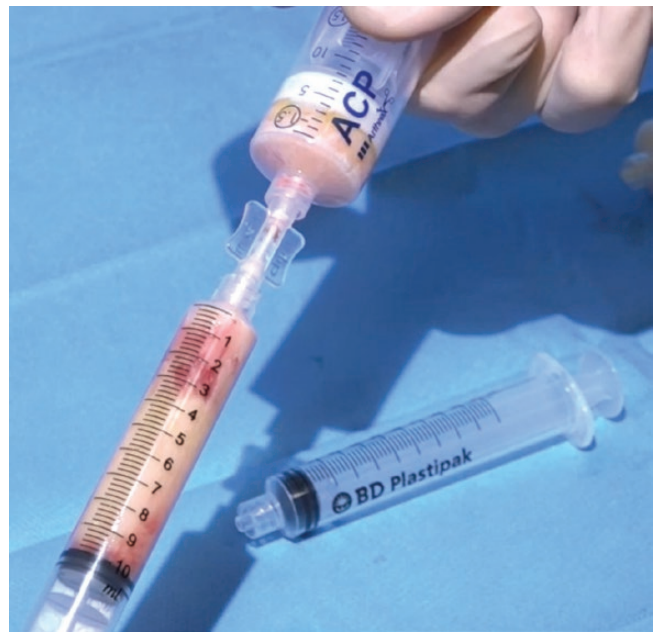
Inclusion and Exclusion Criteria

The inclusion criteria for this study were as follows: male patients, no females, with alopecia androgenica from stage IIa to stage VII according to the Norwood-Hamilton classification and a maximum of 1 hour travelling time to the clinic. Exlcusion criteria were patients with platelets disorders, thrombo-cytopenia, cancer, sepsis, antiaggregating therapy, as well as smokers. Furthermore, we excluded patients that have been treated for male pattern hair loss in the previous 12 months or used a hormone replacement therapy previously.

Liposuction, Harvesting, and Preparation of Adipose Tissue

After local infiltration with 500 mL of saline with 30 mL of lidocaïne 2% plus epinephrine 1:200.000 plus 3 mL of bicarbonate (6.8%), 30 mL of lipoaspirate from the lower abdomen was harvested into two Arthrex ACP double syringes using the disposable Arthrex ACA kit.The Arthrex ACP double syringes filled with 15 mL of decanted lipoaspirate each, were centrifuged at 2500 rpm with a swing out rotor centrifuge (Hettich Rotofix 32, benchtop, swing out rotor, Kirchlengern, Germany) for 4 minutes at room temperature. Subsequently, 20 mL of condensed lipoaspirate was obtained and transferred into two 10 mL luer-lock syringes using a 3-way cock. Fractionation was performed by swooshing the condensed lipoaspirate 40 times forward and back over the 3-hole reusable fractionator (3x 1.4mm

Figure 1. Platelet-rich stroma (ACP^{SVF}) was produced by combining platelet rich plasma (PRP, syringe on the left) with stromal vascular fraction (SVF, syringe on the right side). The stromal vascular fraction (ACA^{SVF}) was prepared using the Arthrex ACA kit and for the platelet-rich plasma (ACP) the ACP double syringe (Arthrex, Munich, Germany) was utilized.



Video 1. Watch now at https://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjy029

hole, luer-to-luer transfer, Tulip). A second round of centrifugation using the same parameters yielded 4 fractions: disrupted adipocytes turned into oil (85-vol%); 1 mL of tissue-SVF (10-vol%) and a liquid fraction of the infiltration fluid with a small pellet (5-vol%, Figure 1 and Video 1).

Blood Withdrawal and Preparation of PRP

Simultaneously, 15 mL of whole blood was drawn from the patient using the Arthrex ACP double syringe and processed according to the manufacturer's instructions in the same centrifuge. No citrate was added to the blood sample as the prepared PRP was injected within 10 minutes after preparation. A total of 5 mL of ACP (PRP) and 1 mL of ACA^{SVF} (SVF) was combined into 1 syringe, gently emulsified and turned into 6 mL of ACP^{SVF} (Figure 1 and Video 1). Both these quantities are the standardized volumes obtained from standaridised procedures previously described, allowing easy reproduction.[18] After local anesthetics (lidocaine 1%) were used to place a sensory block around the treatment area, ACP^{SVF} was injected using a 1 mL syringe and a 20 gauge needle, equally distributing it at the level of the hair follicles intradermaly in the designated area (Figure 2) in small droplets of 0.01 mL at 0.4 cm apart over an average surface of approximately 100 cm^2.

Treatment and Evaluation of AGA

All 10 patients in this consecutive series received the same treatment. The area treated was the central and anterior part of the scalp. The occipital region remained untreated and served as a control (Figures 1 and 2). Hair status was assessed preinjection and at 6 and 12 weeks after injection, using Fotofinder epiluminescense microscopy in combination with their Trichoscan digital image analysis (Fotofinder, Bad Birnbach, Germany). Three spots on the scalp were photographed with high magnification each time in each patient for trichogram analysis (at 20× magnification, 3 days after shaving back hair to 1 mm length). Two spots were located in the treated area on the right and left temporal margin, one spot was located occipitally in the nontreated area.

This allowed us to assess changes and distribution in hair density, hair diameter, and growth speed. Fotofinder recently further improved computer analysis of trichoscale data allowing for exact follicle-to-follicle matching. This provides new insights in changes occurring at single follicular level. Now, differentiation can be made between the origin of regrowing terminal or vellus hairs from either active follicular units or previously inactive empty ones. From each patient, stromal vascular fraction viability was assessed on a separate sample of SVF obtained in parallel to the SVF sample that was injected. On SVF samples of patients 1 to 5, cell isolation and culture were performed.

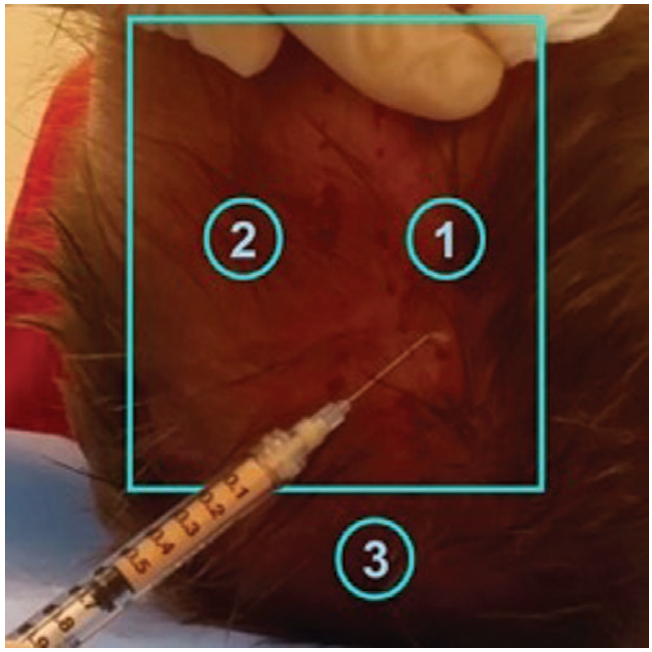


Figure 2. A 48-year-old man was treated with ACP^{SVF} by sharp needle injection (20 gauge green needle) at the level of the hair follicles in the predesigned area (blue rectangle: anterior two thirds of the affected area of the scalp). Measuring spots for Thrichoscopy were circles 1 and 2 (treated area) and circle 3 (nontreated area).

Processing, seeding, culturing, and testing of morphology of the samples was performed as described earlier.[18] Using a colony formation assay as described here also, colony area and intensity were analyzed using a plug in for imageJ (Guzman C. 24647355). Colony intensity takes both the area covered and the colony intensity into account. Images were taken using TissueFAXS microscope.

Immunohistochemistry

For patients 6 to 10, the SVF samples were formalin fixed and embedded in paraffin only and no cell isolation and culturing were performed. Quality control of the obtained samples can be confirmed to be equally effective with this method rather than with the more expensive and elaborative classical technique of cell culturing.[20]

To confirm the FAT-procedure was adequately performed, quality of the processed SVF samples (slices of 4 microns) was performed by assessing presence of remaining adipocytes (Perilipin A staining, 1:200, ab3526, Abcam, Cambridge, UK), as well as smooth muscle cells (alpha-smooth muscle actin,(α-SMA) staining, 1:200, ab7817, Abcam, Cambridge, UK) and endothelial cells (von Willebrand Factor, vWF, 1:200, A0082, Dako, Glostrup, Denmark). Prior to this, antigen retrieval was performed by overnight buffering with 0.1 M Tris/HCL (pH 9.0). For α-Smooth Muscle Actin (α-SMA) and Perilipin A, von Willebrand Factor (vWF) staining was preincubated with 10 mM Tris/1 mM EDTA buffer (pH 9.0). Secondary antibodies used were, respectively; a) rabbit anti-mouse and consecutively swine anti-rabbit peroxidase conjugated antibodies (P0260 and P0217, 1:100, Dako, Glostrup, Denmark) for α-SMA; b) swine anti-rabbit peroxidase conjugated antibody (P0217, 1:100, Dako, Glostrup, Denmark) for vWF; and c) goat anti-rabbit peroxidase conjugated antibody (P0448, 1:100, Dako, Glostrup, Denmark) for perilipin. More technical details have been described previously.[18]

Statistical Analysis

Results from Trichogram analysis were tested for significance comparing all postinjection data per (un)-treated spot to the same preinjection spots using a paired *t* test (*P*-value of 0.05). As the treated area held two separate spots for measuring, also the combined average value of these 2 spots was tested for significance using a paired *t* test (*P*-value of 0.05).

Descriptive statistics were used to evaluate cell numbers, α-SMA, and vWF expression, and colony area and intensity. Data were expressed as mean ± standard deviation (SD). The *t* tests were performed using Graphpad Prism, version 5.01 (Graph Pad Software Inc., Los Angeles, CA).

RESULTS

Between January and December 2016, 10 male patients with an average age of 45.2 ± 14.5 years (range, 25-72 years) were included in this study. All patients sufferd from AGA, which was evaluated according to the Hamilton-Norwood scale. None of them had ever used hormone replacement therapy, suffered from any hormonal or hematological disease, diabetes, cancer or hypertension, or ever smoked. All patients were treated with PRP in combination with SVF from adipose tissue in the same clinic by the senior author himself. None of the patients were lost to follow up and no complications or adverse events could be observed. Patient satisfaction was not assessed in this case series. However, future intended larger studies will include also patient reported outcome measures (PROMs).

Hair Density

Hair density (numbers of hairs/cm^2) was assessed by Fotofinder analysis according to the scheme in Table 1 (see also Figure 3). Results are summarized in Table 2. The nontreated occipital areas did not show any overall significant changes in hair density (or any of the other parameters measured with trichogram analysis) throughout the entire period of 3 months follow up.

Table 1. Scheme for Assessment of Hair Density With Fotofinder Analysis Preinjection and at 6 and 12 Weeks Postinjection

Date towards day of surgery = day 0	–3 days	Day 0	6 weeks – 3 days	6 weeks	12 weeks – 3 days	12 weeks
Photo number	FF-3	FF0	FF6-3	FF6	FF12-3	FF12
Trichogram	3	3	3	3	3	3
Trichoscopy	9	9	—	9	—	9
Total photgraphs	12	12	3	12	3	12

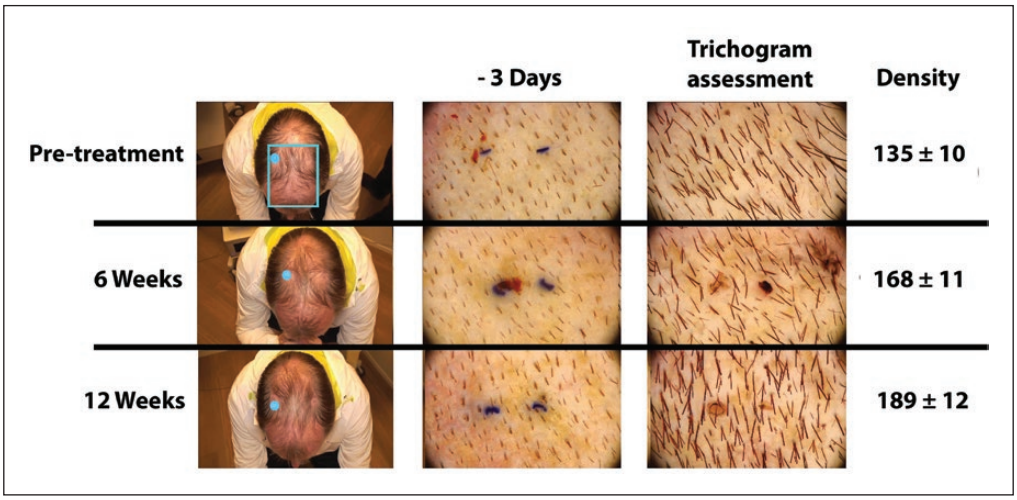


Figure 3. Photographs used for trichogram analysis of a representative 48-year-old man (the same patient from Figure 2) of AGA treated with platelet-rich stroma (ACP^{SVF}). The treated area is marked as a blue square. Within this area, one right and one left sided spot were used for analysis. The occipital region outside this square was nontreated; in the midline in this area a third spot was used for analysis. Three days prior to Fotofinder Trichogram analysis, the hair was shaved down to 1 mm around the marked area (right temporal treated spot). Three days later at a magnitude of 7 times, on the same spot a second photo is taken and computer analysis is used to make a trichogram analysis, measuring density.

A significant increase in density was observed at 6 weeks after injection on the treated right temporal side ($P < 0.03$; n = 7, Figure 4). Density was significantly increased on both the treated right as well as the left side at 12 weeks after injection ($P < 0.02$ and $P < 0.01$ respectively, n = 10, Figures 4 and 5).

Pooling the average number of hairs of the right and left treated sides (hence, one average value is obtained for analysis per patient), the increase in density was significant at 6 weeks postinjection ($P = 0.013$), with an increase of significance at 12 weeks postinjection ($P < 0.001$, Figure 6). This average hair density improved with a mean of 30.7 hairs per cm^2 (range, 5-59 hairs per cm^2) in the target area compared to baseline, while hair density did not change significantly in the occipital regions (Figure 7).

Follicle-to-Follicle Matching Analysis

In Figures 8 and 9, exact follicle-to-follicle matching of two different skin samples is demonstrated, comparing preinjection (left) with 12 weeks postinjection (right). Hair loss (postinjection) is depicted as red colored hairs preinjection (220, 127, 132). New hairs postinjection are depicted in green. The different origins of the new hair become visible with this technique and 455 is a new terminal hair regrowing from a previously inactive, clearly visible empty follicle. When such empty follicles are filled with a hyperkeratotic plug, they are referred to as yellow dots, having previously always been considered a folliculair opening lacking any hair shaft but filled with sebum or keratotic material.[20]

Regrowth from such an assumed nonvital follicle has never been reported before, to our knowledge. Numbers 391 and 392 are new vellous hairs growing from previously invisible (or new?) isolated follicles. Numbers 388, 389, 390, and 395 are new terminal hairs regrowing within existing follicular units.

SVF Viabilty

Enzymatic isolation and cell culturing of the ACA^{SVF} resulted in a mean cell count of $1.275 \times 10^6 \pm 1.1 \times 10^6$ per 1 mL

Table 2. Hair Density Data, Measured With Fotofinder. Analysis of 10 Separate Consecutive Cases Treated with ACP^{SVF} for AGA

Case	Occipital side, density in n/cm² (SD)			Right side, density in n/cm² (SD)			Left side, density in n/cm² (SD)		
	Preinjection	6 weeks	12 weeks	Preinjection	6 weeks	12 weeks	Preinjection	6 weeks	12 weeks
1	157 (11)	177 (12)	185 (12)	135 (10)	168 (11)	189 (12)	129 (10)	178 (12)	181 (12)
2	129 (10)	208 (13)	211 (13)	127 (10)	202 (13)	186 (12)	117 (10)	153 (11)	169 (12)
3	132 (10)	137 (10)	130 (10)	148 (11)	158 (11)	168 (11)	150 (11)	182 (12)	162 (11)
4	177 (12)	—	202 (13)	208 (13)	—	225 (13)	187 (12)	—	226 (13)
5	144 (11)	179 (12)	128 (10)	130 (10)	169 (12)	189 (12)	159 (11)	129 (10)	140 (10)
6	274 (15)	—	336 (16)	224 (13)	—	278 (15)	251 (14)	—	275 (15)
7	144 (11)	123 (10)	112 (9)	86 (8)	113 (9)	116 (10)	114 (9)	128 (10)	118 (10)
8	129 (10)	145 (11)	111 (9)	234 (14)	246 (14)	214 (13)	174 (12)	222 (13)	222 (13)
9	273 (15)	—	309 (16)	333 (16)	—	302 (15)	274 (15)	—	315 (16)
10	291 (15)	260 (14)	341 (16)	352 (17)	339 (16)	439 (19)	317 (16)	335 (16)	348 (17)

In each case two spots were analyzed within the treated area on the right and left temporal side respectively (Figure 1). On the occipital side a nontreated spot was included for assessing hair density. Standard deviation (SD) is placed in between brackets for each value in numbers per cm².

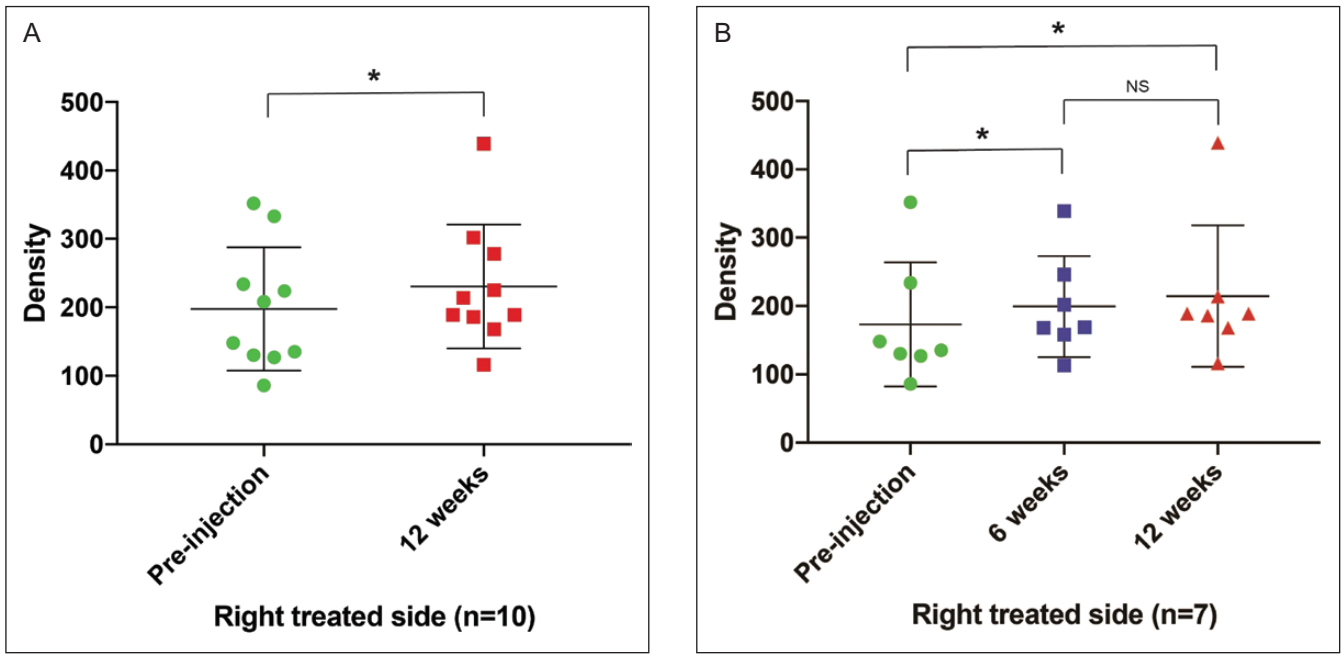


Figure 4. Hair density before and after the treatment of the right side of the scalp with ACP^{SVF}. (A) Whole study group (n = 10). (B) Patients that were analyzed 6 weeks and 12 weeks after the ACP^{SVF} treatment (n = 7). * $P < 0.05$. NS, not significant.

(range, 0.53×10^6 - 3.15×10^6) (Table 3, n = 5, samples #1-5). Assessment of colony forming units allowed for the confirmation that the ACA^{SVF} injected held viable ASCs able to grow and differentiate as described in the original FAT paper (Table 3, n = 4, samples 1-5, except for 4).[17] This was most likely due to a technical problem as increase in hair density after injection of ACA^{SVF} occurred in this patient also and was significant (Table 3, case 4, 14% increase in number of hairs at 12 weeks after injection). No clear relation could be observed between the number of colony forming units (CFUs) and the increase in density in this case series.

In patients 6 to 10 the SVF samples were formalin fixed and embedded in paraffin. Presence of vessel rich extra cellualr matrix was confirmed by presence of smooth

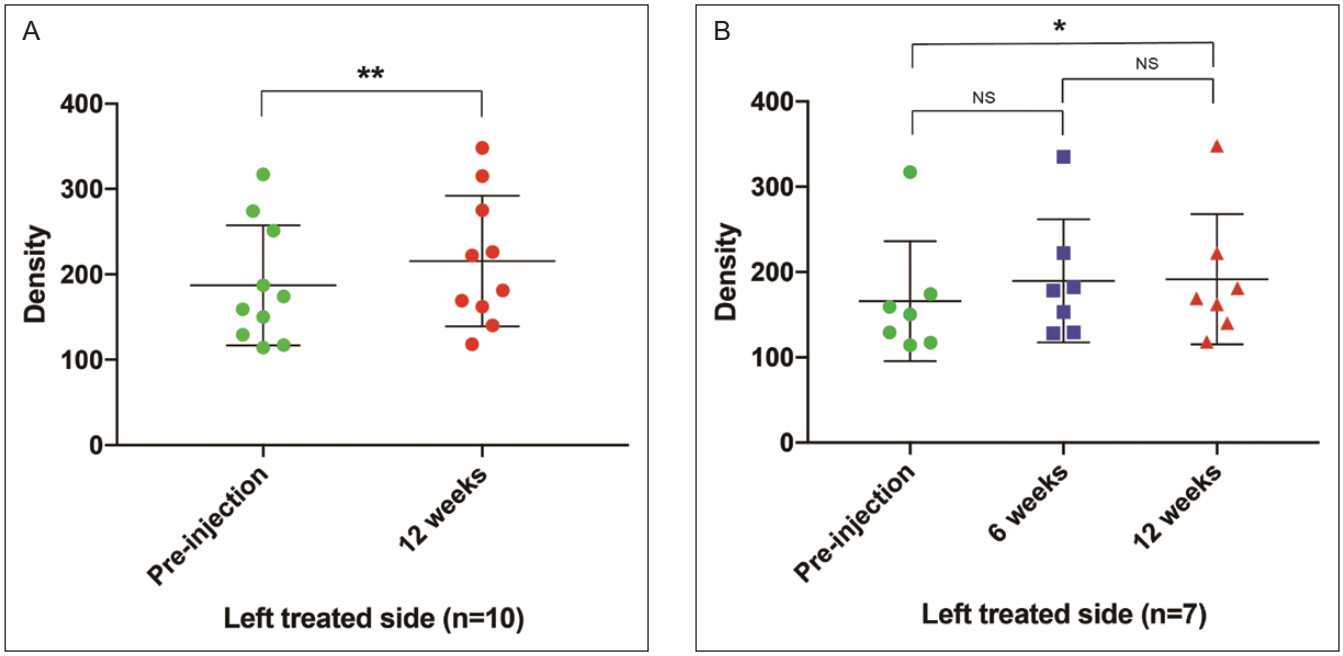


Figure 5. Hair density before and after the treatment of the left side of the scalp with ACP^{SVF}. (A) Whole study group (n = 10). (B) Patients that were analyzed 6 weeks and 12 weeks after the ACP^{SVF} treatment (n = 7). * $P < 0.05$, ** $P < 0.01$. NS, not significant.

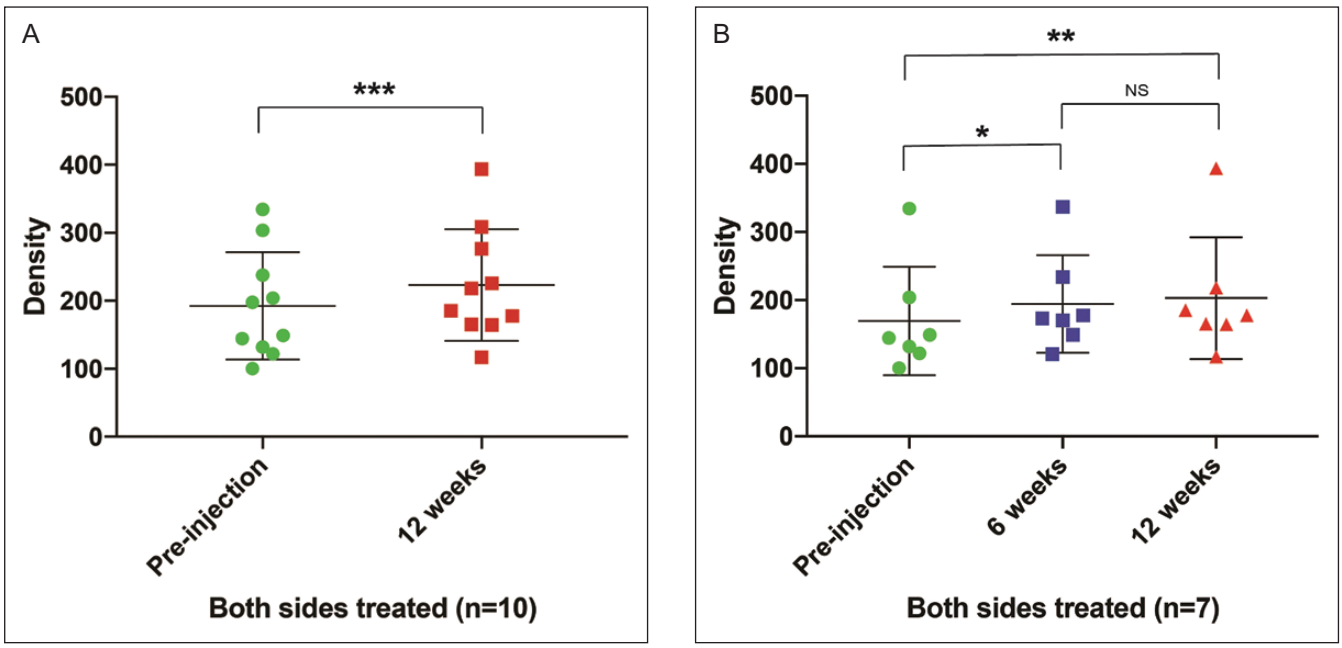


Figure 6. Hair density before and after the treatment of the left and the right side of the scalp with ACP^{SVF}. (A) Whole study group (n = 10). (B) Patients that were analyzed 6 weeks and 12 weeks after the ACP^{SVF} treatment (n = 7). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS, not significant.

muscle cells (Alpha-Smooth Muscle Actin staining) and endothelial cells (von Willebrand Factor, Figure 10). The sample with the lowest number of vessel related intensity (Table 3, 9) also had the lowest contribution to increase in hair density (2%). Presence of some remaining adipocytes was confirmed by Perilipin A staining, numbers were not different from study results published earlier (Table 3, n = 4, samples 6-10, Figure 10).

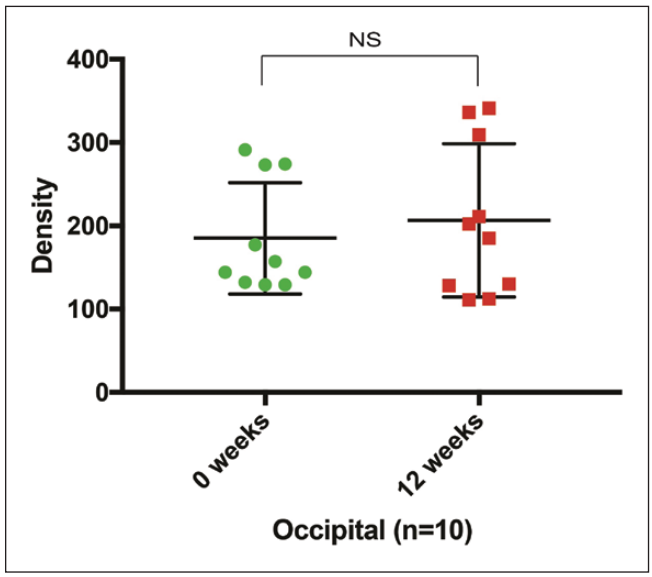


Figure 7. Hair density of the non-treated occipital region during the 12 weeks period of analysis (n = 10). NS, not significant.

DISCUSSION

This report describes the potential effect of the combination of platelet-rich plasma and adipose-derived stromal vascular fraction (ACA^{SVF}) on androgenetic alopecia (AGA). Ten consecutive cases of AGA were treated with the combination of these two biological components of wound healing, the cellular and intercellular one respectively. This combination is named platelet-rich stroma (PRS) as a generic term. In this case series ACP^{SVF} was used, specifying the fabrication process of making PRS. Previously, we have demonstrated that mechnical fractionation of adipose tissue can deliver nonmanipulated SVF within 45 minutes of operating time.[18] This SVF holds a 7 to 8 times higher number of vital stromal cells after selective crushing of adipocytes and removing them by centrifugation (fractionation of adipose tissue).[18] The combination of PRP and SVF is a potent repair agent with promising results in ageing skin, damaged tissue from irradiation, and numerous other indications.[18,19] Only recently, this combination was used to improve healing of posttraumatic lower extremity ulcers,[21] degenerative disc disease,[20] and by our group to treat osteoarthrosis of the knee,[19] hip, and CarpoMetaCarpal-joint in the wrist (unpublished data). Although AGA is often described as a condition resulting from different factors, we believe that AGA is a process involving tissue damage of hair follicles as well as the surrounding tissue.

In this case series, the potential effect of a single dose of ACP^{SVF} on AGA was tested, showing a clear significant increase of density of hair growth within 6 weeks after injection. Within 12 weeks the significance increased to $P < 0.0001$. In a follicle-to-follicle hair matching anaylsis (Fotofinder) it was demonstrated that density increased by new terminal hairs growing from within excisting follicular units. But also new terminal hair was regrowing from previously inactive empty follicles that were still clearly visible. New vellous hairs were also growing from previously invisible (or new?) isolated follicles. To our knowledge this is an unprecedented finding. Previous studies have evaluated the effect of PRP alone on AGA and have suggested that repeated injections of PRP are required to induce hair growth.[22,23] Our results with a single injection of ACP^{SVF} are comparable to the before mentioned outcomes, indicating that the combination of PRP with SVF is much more effective than PRP alone. A treatment of AGA with ACP^{SVF} plus 3 additional ACP injections with ACP might be even more powerful.

Colony forming unit (CFU) assessment confirmed the ACA^{SVF} that was injected contained viable adipose-derived stromal cells able to grow and differentiate as described in the original paper.[17] One sample showed no cell counts, most likely due to a technical problem, as increase in hair density after injection of PRS also occurred in this patient and was significant (Table 3, case 4, 14% increase in number of hairs at 12 weeks after injection). No clear relation could be observed between the number of CFUs and the increase in hair density in this study, suggesting in all cases the number of regenerative cells needed to induce a change was above threshold.

The finding that hair densitity could also increase on some of the nontreated spots (though not significant at 6 weeks (n = 7, $P > 0.05$), nor at 12 weeks postinjection, n = 10, $P > 0.05$) was somewhat surprising (Table 2). This result might suggest that ASCs migrated close to the injection site migration, enabling hair growth. Alternatively, ASCs might be capable of migration by making use of the local circulation.

To better understand our reason for preferring PRS to PRP or SVF alone we summarize some of their individual properties first. Second, we postulate that PRS to us seems the ideal way to treat soft tissue damage in general. Considering AGA a process with damaged hair follicles, PRS could be considered for treating early hair loss without the need for long-term oral medication with all of the potential side effects or procedures leaving scars. ACP^{SVF} proved to be an ideal way of making PRS ensuring a swift procedure, necessating one type of centrifugator only for the making of PRP as well as SVF in a 100% closed system, using disposables exclusively.

PRP has been already used treating AGA and different mechanisms for how PRP stimulates hair growth have been postulated.[24-29] PRP induces proliferation and survival of the dermal papilla (DP) cells, known to nourish the hair follicle by phosphorylation of extracellular signal-regulated kinases (ERK).[24,25] PRP also activates several antiapoptotic regulators, such as the Akt signaling and Bcl2-protein, leading to prolonged cell survival

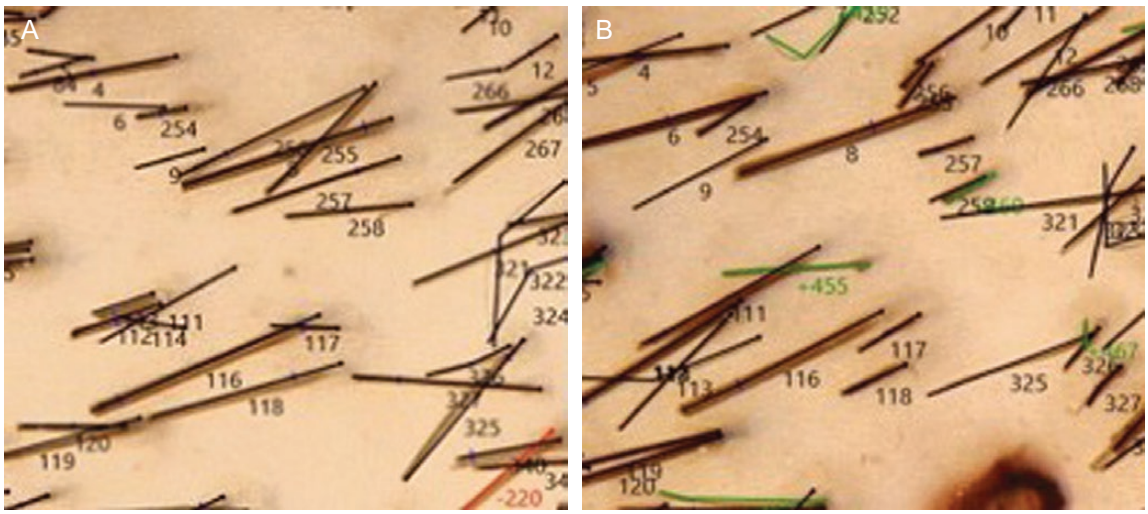


Figure 8. Shows the exact follicle-to-follicle match of a sample of skin of a 49-year-old man, comparing preinjection (A) with 12 weeks postinjection (B) per area. Hair missing postinjection is colored red preinjection (220). New hairs postinjection are colored green. Different origins for new hair become visible with this technique. Number 455 is a new terminal hair regrowing from a previously inactive, but clearly visible, empty follicle.

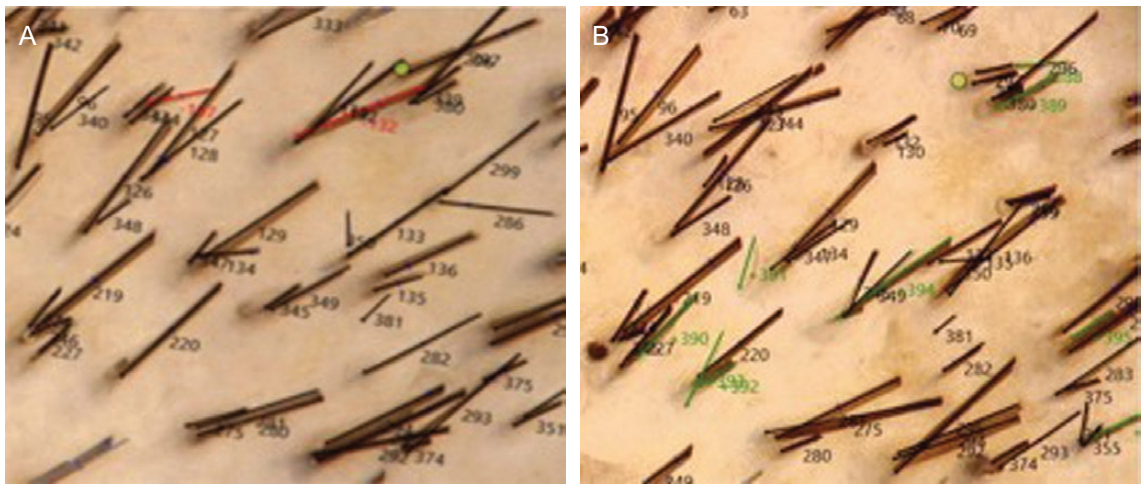


Figure 9. Shows the exact follicle-to-follicle match of a sample of skin of a 43-year-old man, comparing preinjection (A) with 12 weeks postinjection (B) per area. Hairs missing postinjection are colored red preinjection (127, 132). New hairs postinjection are colored green. Different origins for new hair become visible with this technique. Numbers 391 and 392 are new vellous hairs growing from previously invisible (or new?) isolated follicles. 388, 389, 390, and 395 are new terminal hairs regrowing within existing follicular units. After quality control, hair 394 seemed to be a nondetected vellus hair.

contributing to hair growth.[24,26,27] PRP increases β-catenin activity and FGF-7 expression in the DP cells.[26,27] β-catenin is mainly expressed in the outer root sheath at the bulge region, where the stem cell nich of human hair follicle tissue is located.[26] Upregulated β-catenin activity seems to induce the differentiation of stem cells into hair follicle cells. Expression of FGF-7 in DP cells prolongs the anagen phase of the hair growth cycle.[27]

The relevance of adding cellular therpeutical components from adipose tissue to hair follicles has been suggested before. Festa et al reported that adipose lineage cells, including mature adipocyte and preadipocytes, have been defined as skin niche cells that regulate hair follicle stem cell activity.[28] They also report that the number of adipocyte precursor cells changes with the hair cycle, the cell number peaks in the skin during follicular stem cell activation (anagen) and decreases during the catagen phase. The differentiation as well as production and secretion of growth factors that activate neighboring cells are also mentioned as relevant functions of ASCs. These growth factors include vascular endothelial growth factor (VEGF), transfroming growth factor (TGF-β), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), placental growth factor (PlGF), and basic fibroblast growth factor (bFGF).

Table 3. Enzymatic Isolation and Cell Culturing of the ACA^{SVF}

Case	Average of both sides treated										
	Nucleated cell count/ml	CFU-intensity (100 cells seaded)	CFU-intensity (1000 cells seaded)	CFU area covered (100 cells seaded)	CFU area covered (1000 cells seaded)	Vessel related intensity #	Adipocytes (n/mm²)###	Vessel related intensity ##	Pre-injection (n/mm²)###	6 weeks density n/cm²	12 weeks density n/cm²
1	$3.15*10^6$	0.39	0.51	0.79	1.13	—	—	—	132	173	185
2	$0.875*10^6$	0.75	0.98	1.78	2.21	—	—	—	122	177.5	177.5
3	$0.7*10^6$	0.70	1.84	1.83	4.12	—	—	—	149	170	165
4	$0.125*10^6$	TE	TE	TE	TE	—	—	—	197.5	—	225.5
5	$0.525*10^6$	0.22	0.51	0.44	1.81	—	—	—	144.5	149	164.5
6	—	—	—	—	—	1.35	0.77	2.47	237.5	—	276.5
7	—	—	—	—	—	TE	TE	TE	100	120.5	117
8	—	—	—	—	—	3.77	0.33	6.11	204	234	218
9	—	—	—	—	—	0.10	0.18	9.26	303.5	—	308.5
10	—	—	—	—	—	2.36	0.45	2.24	334.5	337	393.5
						#vonWille-brand	##alpha-SMA	###perilip-pine			

Alpha-SMA, alpha-smooth muscle actin; CFU, colony forming unit; TE, Technical Error.

The expression of these growth factors allows ASCs to have an angiogenic capacity and the ability to induce tissue neovascularization, which show that ASCs may contribute to a microenvironment with an abundant blood supply for hair cells to regenerate hair follicles (or for regeneration of any other damaged tissue for that matter). ASCs are also immunomodulatory and/or immunosuppressive via direct cell-to-cell interaction or secreted cytokines such as prostaglandin E2 (PGE2), leukemia inhibitory factor (LIF), and kynurenine.[29]

Won et al also described comparable findings after the treatment of DP cells with adipose tissue-derived stem cells (ASCs).[16] Their group demonstrates that ASCs and its medium enable an increase in proliferation of DP cells and activation of the anagen phase in hair cycles. In the bulge area, primitive stem cells of ectodermal origin are found, giving rise to epidermal cells and sebaceous glands. In the matrix, germinative cells of mesenchymal origin are found at the dermal papilla. Interactions between these two cell types as well as with binding growth factors (PDGF, TGF-β, and VEGF) activate the proliferative phase of the hair, giving rise to the future follicular unit.[14] Khatu et al and Gkini et al report that using solely PRP might also be a treatment option for androgenetic alopecia AGA.[13,14]

However, we believe that the sole addition of growth factors to any damaged tissue will only lead to the desired result when a vital cellular component for regeneration component (eg, vital hair follicle cells or injected cells from the SVF) is still present. Taking everything into consideration, we believe that the hallmarks of tissue damage are also present in AGA. Addressing the combination of both cellular as well as intercellular aspects of wound repair as an alternative treatment of AGA seems to deserve further attention.

Limitations

We are aware that this study has limitations. First, this is a case series with a small patient number and without any control group. Larger randomized studies are required to compare the hair-modulatory effects of ACP^{SVF} to a placebo group and a group treated with PRP or SVF only. Furhtermore previous studies using PRP only suggested that repeated injections are required to achieve positive outcomes. The present study could not clarify if additional ACP injections would improve the results of ACP^{SVF} on AGA. A single ACP^{SVF} injection should be compared with a treatment with ACP^{SVF} plus an additional ACP(PRP)-booster injection (eg, three times in a three or four week interval), in order to further optimize the current treatment protocol.

CONCLUSIONS

In conclusion, we present platelet-rich stroma (the combination of PRP + SVF) as a new treatment option for early alopecia when repair of the soft tissue damage leading to this condition is more likely to occur than when the

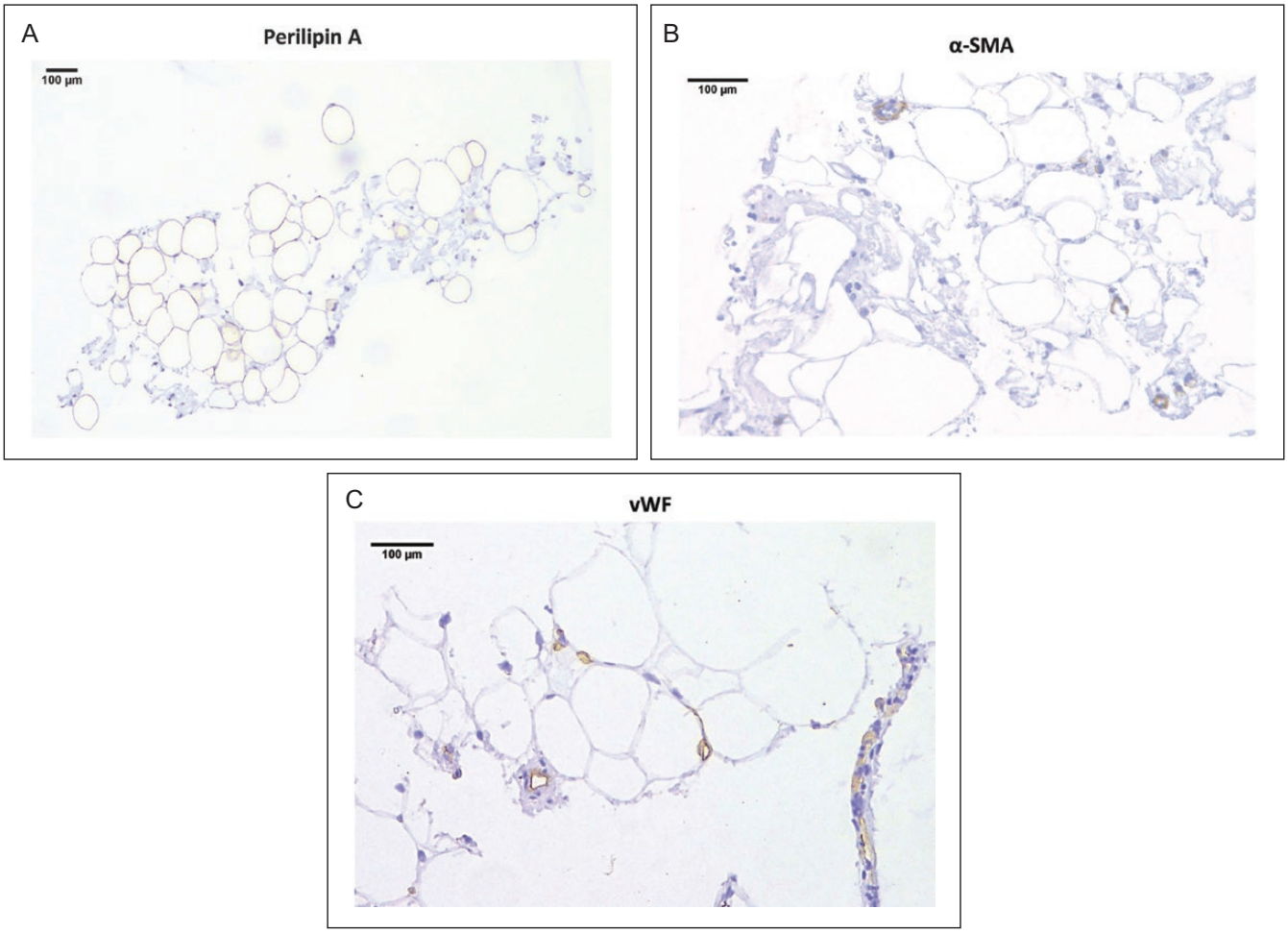


Figure 10. Exemplarily light microscope images of (A) Perillipin A, (B) α-SMA, and (C) vWF stained ACA^{SVF} samples. ACA^{SVF} samples of patients 6 to 10 were formalin fixed and embedded in paraffin. The presence of vessel rich extra cellular matrix was demonstrated by the staining of smooth muscle cells (α-SMA = alpha-smooth muscle actin) and endothelial cells (vWF = von Willebrand factor). Perilipine A staining confirmed the presence of remaining adipocytes.

damage is more advanced. Using the Arthrex ACP double syringe, respectively the Arthrex ACA kit to make ACP^{SVF} by combining ACP (PRP) and ACA^{SVF} (SVF), this procedure can be performed in an out-patient office under local anaesthetics and is a closed system, allowing treatment of an area of 100 cm^2 within 45 minutes. A single injection of ACP^{SVF} at the level of the hair follicles had a positive therapeutic effect on male AGA by increasing hair density significantly within 6 to 12 weeks without any side effect. Given that ACP^{SVF} is a nonhormonal treatment both males and females could benefit from such an early PRS treatment, not having to endure daily oral medication of hormones and its side effects, nor would they have to consider accepting scars from hair transplant surgery.

Supplementary Material

This article contains supplementary material located online at www.aestheticsurgeryjournal.com.

Disclosure

Dr Stevens is a senior consultant for Arthrex (Munich, Germany) and together they developed the ACP^{SVF} system, for which he has royalties and honorarium. Drs Donners and de Bruign declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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