> “Autologous platelet rich plasma has been used for a variety of indications for more than 30 years with effective results, and plays an important role in soft tissue healing.”

USING OBJECTIVE CRITERIA TO EVALUATE COSMETIC EFFECTS OF PLATELET RICH PLASMA

Gilbert Amgar, Christian Bonnet, Alain Butnaru and **Fabienne Herault-Bardin** discuss the results of a study which aimed to evaluate the efficacy of platelet rich plasma as a facial skin treatment

ABSTRACT
Autologous platelet rich plasma (PRP) plays an important role in soft tissue healing. The purpose of this study was to evaluate the efficacy of this facial skin treatment through objective parameters. Biometric parameters, including anisotropy, transepidermal water loss (TEWL), micro-relief, and hydration were used to determine the relative success of the treatment. The study, which included 37 female patients, demonstrated an average of 24% improvement in the anisotropy coefficient after the third week. The TEWL and micro-relief parameters also improved significantly ($P < 0.05$ in all parameters tested). In addition to these results, this study suggests that certain measured values may be used to predict the success of the aesthetic use of PRP.

HISTORICALLY, AUTOLOGOUS platelet rich plasma (PRP) has been used for a variety of indications for more than 30 years, with effective results. The first publication on this subject in 1975[1], deals with the use of platelets as physiological adhesives in corneal surgery. Since then, the literature has widely reported their functions and effects.

A search in PubMed using the terms 'platelet rich plasma' or 'PRP gel' identifies more than 300 references. A search on Google results in over 300 000 links. The popularity of the platelet concentrate gel was further developed by Marks et al[2] after demonstrating improved osseous regeneration in dental surgery using PRP. Cutaneous repair was the domain which followed this interest, along with the evaluation of the platelet concentrate gel to heal the ulcers in diabetic patients[3]. A series of later publications focused on the vascular phenomena of cellular migration, collagen synthesis, and structural improvement. A number of publications have identified the range of platelet factors and their functions during the repair phenomena. A 1996 study demonstrated its impact on healing large-scale burns[4], and another important publication specified healing mechanisms when applied to wounds[5]. This allowed confirmation of the role of platelet factors and fibroblasts in these phenomena, as well as that of metalloprotease, fibrin, and fibronectin. Subsequently, cosmetic surgery applications have been developed using PRP as a 'biological adhesive'[6]. Additional uses for the PRP gel have been identified in orthopaedics[7], cardiology[8], and rheumatology[9,10].

The use of PRP in cosmetic surgery dates back to 2005. The main indications include cutaneous regeneration, dark circles around the eyes[11], and hair growth. In the case of cutaneous revitalisation within cosmetology, only the subjective aspect has been traditionally evaluated based on the patient or physician's subjective level of satisfaction. However, objective, scientifically measurable evaluations have been rare. One such case meta-analysis was published in 2007 by Borzini and Mazucco[12].

Objectives of the study

This study suggests a way to use biometric parameters to mitigate the lack of objective, scientifically measurable means of assessing the results from the use of PRP injections, and to maximise its use by providing a means to qualify the most viable candidates prior to treatment.

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KEYWORDS
platelet rich plasma, anisotropy, revitalisation, biometrics

Figure 1 MyCells tubes

Materials and methods

MyCells Kits

Blood withdrawal, preparation of platelet concentrate and its subsequent injection were performed using the MyCells Autologous Platelet preparation Kit. The proprietary tubes, PPT-I and PPT-II (*Figure 1*), as well the necessary syringes and needles, are also included in the kit. The MyCells Kits were supplied by Kaylight Corporation, who designed them specifically to create a high yield and viable PRP.

IntuiSkin Professional WorkStation

The measurement of skin parameters was made using an integrated work station supplied by IntuiSkin (*Figure 2*). The validity of this equipment for the evaluation of biometric measurements has already been established[13, 14]. The WorkStation includes a video camera with 50x lens, USB sensors that are fully temperature and humidity compensated, and a Windows XP Pro-based portable computer. The system is capable of making simultaneous measurements of hydration, transepidermal water loss (TEWL), and surface temperature.

Objective measurements

The temperature of the stratum corneum is recorded along with a measurement of the ambient temperature. Both are expressed in degrees Celsius.

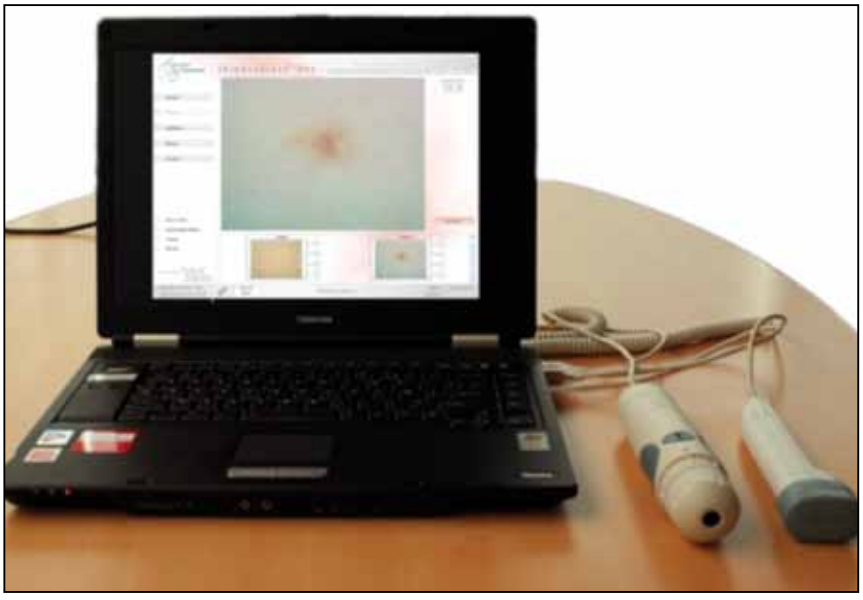
Skin hydration was made after compensating for the ambient humidity level. The hydration was expressed by a standard index. The results are expressed as a percentage and are categorised as:

- Over 50% is considered normal for the skin
- From 30–50% the skin is classified as tending towards dry or 'medium'
- Under 30% the skin is considered dry.

Following marked swelling of the skin, a number of micro-lines on the surface of the skin can be observed. The projection and distribution of these micro-lines two-dimensionally in all directions (360°) is indicative of young skin. A child will have lines oriented homogeneously in all directions, which is reflective of good skin elasticity. The older the skin, the more these lines will change orientation and become parallel. Anisotropy measures the distribution of micro-lines in 360°, and is expressed as a percentage. If the index reading is low, the micro-lines are at a 360° orientation (young skin), but if high, the micro-lines tend to be parallel (elderly skin). A decrease in this index is indicative of the degree to which a given treatment has had an anti-ageing effect. It is undoubtedly the most significant parameter when evaluating the anti-ageing effect of a beauty product or a morpho-aesthetic therapy.

TEWL is a non-invasive measurement of the amount of epidermal water lost or evaporated. It is expressed in grams per hour per square metre of cutaneous surface. The range of the measurement is 0–100 gram/m²/hr. TEWL reflects the integrity of the stratum corneum, which is the barrier the body has in preventing water loss. The measurement method uses two solid-state micro-sensors placed at two different positions. The difference between the vapour concentrations at these two points allows a 'gradient' to be calculated. Values generally range from 10 to 20 and are considered most accurate when the indicator is below 10. Higher readings may indicate that the ability of the skin to provide a barrier to water loss has been compromised.

Figure 2 The IntuiSkin WorkStation



Pigmentation is measured using the probe and expressed in levels of red, green and blue (RGB) luminescent intensity. These readings provide reference points that enable the system to be consistently calibrated from one use to the next.

Cutaneous micro-relief (*Figure 3*) measurements performed using the micro-relief module are based on definitions of metrology. The readings are expressed in gray levels (GL). This method provides the data required to transform a 2D picture of height and depth to a 3D image. The GL changes from one image to the next to represent the dynamic of biometric properties of the skin. The range of measurement is 0–255. *Figure 3* refers to the following parameters:

- Average distance from tuberose 'Ra' (GL)
- Maximal depression depth 'Rv' (GL)
- Depth of peaks 'Rp' (GL)
- Total height 'Rt' (GL)
- Density of peaks 'Pc' (mm-1)
- Average space between irregularities 'SM' (µm).

The 2D parameters are expressed in mm or µm. The calculated surfaces are expressed in mm². In addition to information related to anisotropy, pigmentation, luminance, and other variables of tuberosity, both skin photography and a 3D reconstruction of skin's surface are provided.

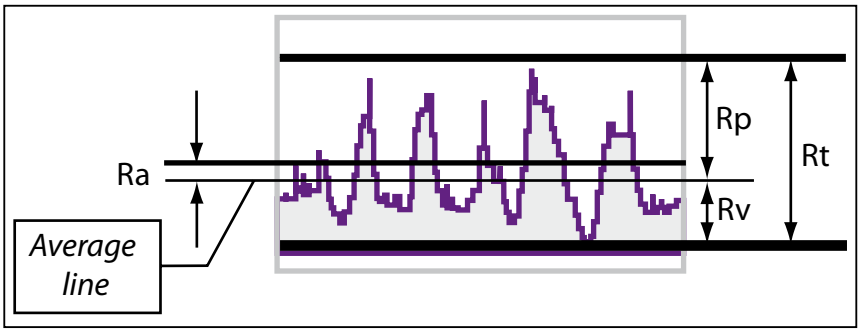
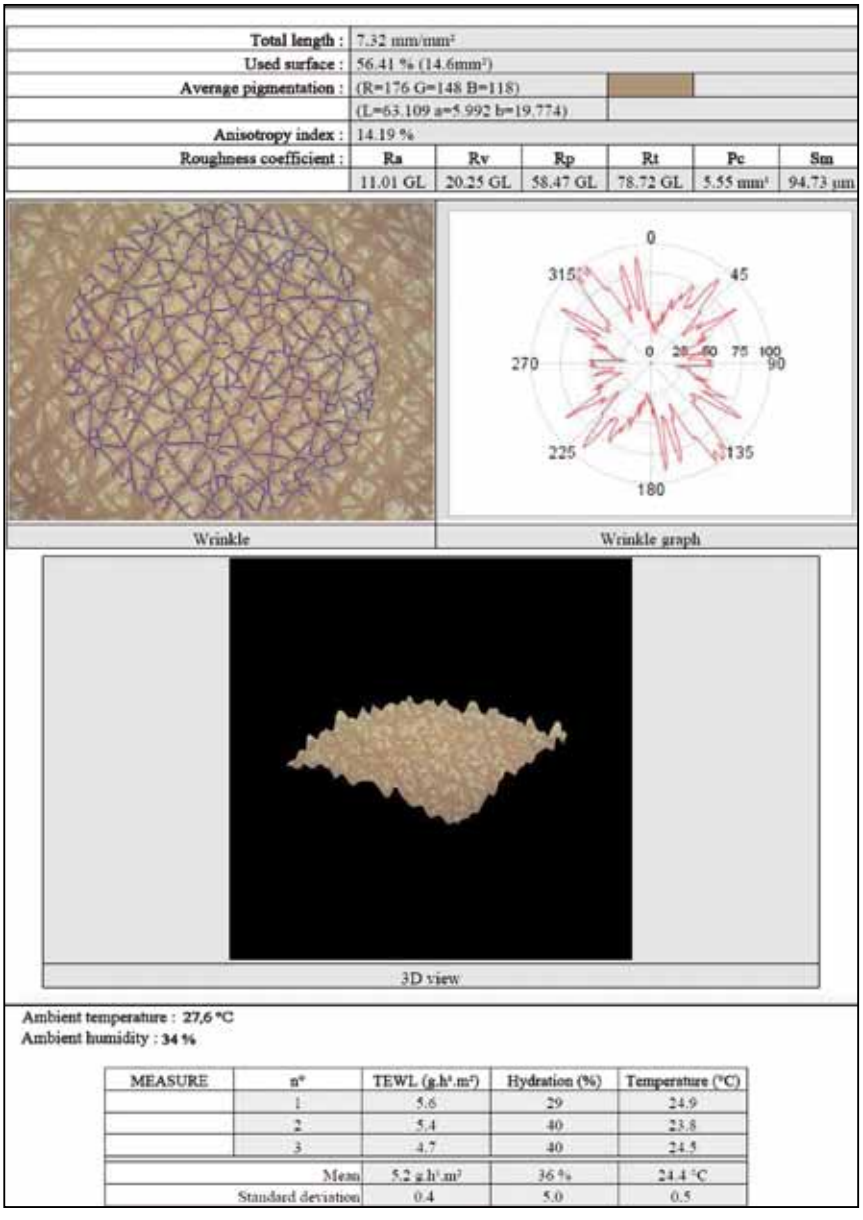


Figure 3 Micro-relief values

Figure 4 Information provided by the IntuiSkin WorkStation



Previous studies

The current study is a confirmation of preliminary, unpublished work carried out by Amgar and Bersand[15] in 2009. It included 18 volunteer participants (six men and 12 women) aged between 30 and 75 years. This preliminary study demonstrated that the best results are obtained in the age group of 40–60 years, with a more effective response observed in women. For subjects younger than 40 years and older than 60 years, little or no improvement was observed, with an occasional occurrence of deterioration. The conclusions from the preliminary study were used to assist in the formulation of the inclusion/exclusion criteria for the present study.

Inclusion criteria were as follows:

- Female
- Aged 40–60 years
- Class 2 or 3 on the Glogau scale of photoageing (moderate or advanced wrinkles)
- No previous PRP treatments
- Signed informed consent.

Exclusion criteria were:

- History of cancer
- Use of anticoagulants or blood products
- Active facial skin infection
- Haematological diseases
- Pregnancy.

Schedule

The protocol included three separate visits.

Visit 0 (V0) on Day 0 (D0)

- Administrative data collection (e.g. medical history, family history)

- Subjective skin evaluation
- Biometric measurements
- First PRP treatment.

Visit 1 (V1) between Day 21 (D21) and Day 28 (D28)

- Subjective skin evaluation
- Biometric measurements
- Discussion of possible side-effects
- Second PRP treatment.

Visit 2 (V2) between Day 90 (D90) and day 120 (D120)

- Subjective skin evaluation
- Biometric measurements
- Report of side-effects
- End of the study.

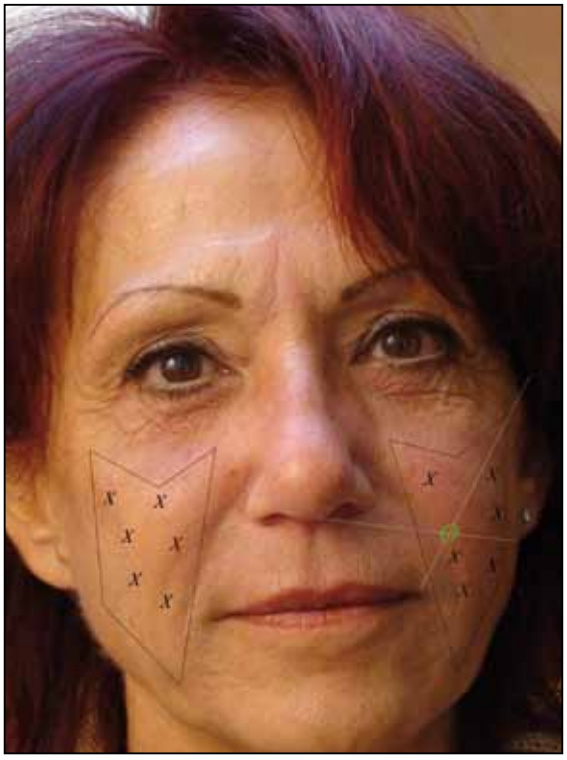
Treatment

The treatment protocol consisted of injecting 4cc of PRP. The injections were made to the lower part of the face (*Figure 5*), and to designated intra-dermal points (black crosses in *Figure 5*). The injections were intended to cover the measurement zones.

The preparation of the PRP was made in accordance with the operating instructions of the kit manufacturer. The blood was collected and spun in a centrifuge at 1200g (4000 RPMs) for 7 minutes. After discarding the platelet-poor plasma phase, the buffy layer was resuspended in approximately 4mL of plasma fluid. Following resuspension, the filter sleeve was used to assist in the transfer into of the PRP into a syringe fitted with a with 30G needle. No calcium chloride was added to the PRP prior to reinjection.

All measurements were made with the following conditions:

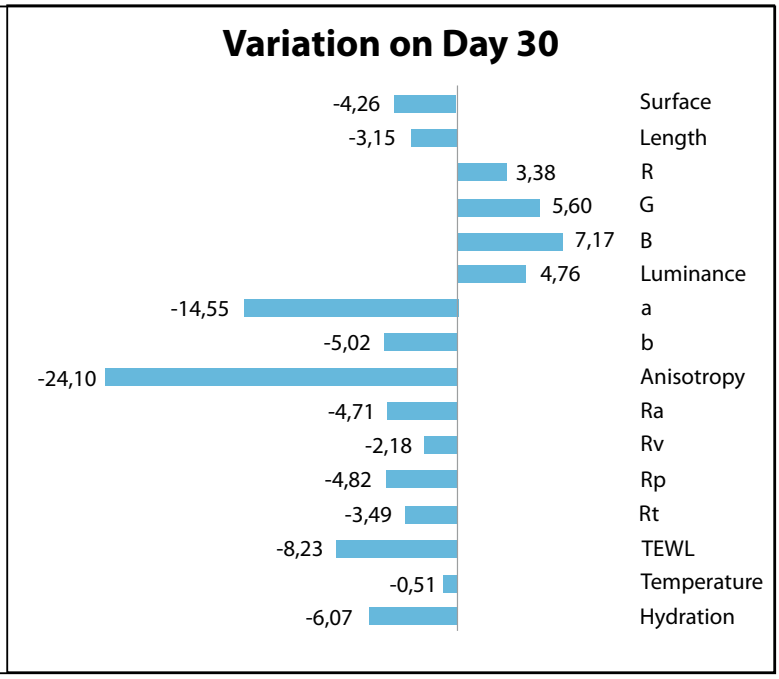
Figure 5 Zones of injection and measurement



- No make-up in the measurement area
- Any make-up removal was performed at least 1 hour prior to measurement
- No cosmetic or beauty products should be applied within the 2 hours prior to measurement
- The zone of the measurement was defined by the intersection of a line from the ear to the labial fold and another line from the bottom of the ear to the nostril (green circle in *Figure 5*).

Figure 6 4-week results. R, G, B and Luminance, a, b are calorimetric parameters; Rt, Rp, Rv, Ra are micro-relief parameters

PRP	V0	V1-V0	Var 1
Surface	39.21	-1.67	-4.26
Length	6.51	-0.21	-3.15
R	175.16	5.92	3.38
G	147.30	8.24	5.6
B	123.22	8.84	7.17
Luminance	62.94	2.99	4.76
a	6.83	-0.99	-14.55
b	16.66	-0.84	-5.02
Anistropy	28.96	-6.98	-24.1
Ra	15.28	-0.72	-4.71
Rv	40.15	-0.88	-2.18
Rp	79.18	-3.82	-4.82
Rt	119.27	-4.17	-3.49
TEWL	14.09	-1.16	-8.23
Temperature	28.03	-0.14	-0.51
Hydration	57.3	-3.48	-6.07



No modifications to normal beauty care routines were allowed between visits. Patients were not permitted to undergo any resurfacing applications (peeling, filling, laser, LED, RF frequency) during the study.

Review of side-effects

The questionnaire on side-effects checks for the following:

- Pain
- Bruises
- Redness
- Oedema
- Pruritis
- Granuloma
- Pigmentation
- Unexpected side-effects.

Statistical analysis

Quantitative variables were evaluated by calculating the mean and standard deviation. The comparison of the pre- and post-treatment mean was made using the Student t-test for paired data. The correlation was evaluated via linear regression, while the significance between these tests, was determined when $P<0.05$ (VisualStat software).

Results and discussion

Visit 1

Thirty-seven female subjects were enrolled in the study, and were treated with PRP. As a control group, two additional patients underwent full evaluation and received injections with saline rather than PRP. The values of parameters at V1 between Day 21 (D21) and Day 28 (D28) were reported and were compared to the initial values (V0). They are expressed as a percentage variation (*Figure 6*). Two parameters demonstrated meaningful improvement—anisotropy and TEWL. The anisotropy significantly improved, showing a decrease of -6.98 points, so -24.1%.

Historically, this is a reliable value that relates to an observable anti-ageing effect. Subsequent improvement of the indicator was observed after a few months of treatment. This data was also observed over a short period (3 weeks) and is associated with an improvement in the micro-relief parameters with RT of -3.49% and length -3.15%.

In parallel, the TEWL indicator showed significant improvement with a variance of -8.23%. This value correlates with the repair effect of PRP treatments. Specifically, the TEWL is interpreted as a capacity of the skin to retain its water levels.

The calorimetric parameters demonstrated only minor improvement in luminance (up to +4.76%). Other variations were minor and within the scale values set at 255 (RGBab). No change was observed in skin temperature.

The final evaluation point, hydration, showed reduced levels at -6.07%. However, this dehydration proved transient and disappeared over the 3-month period.

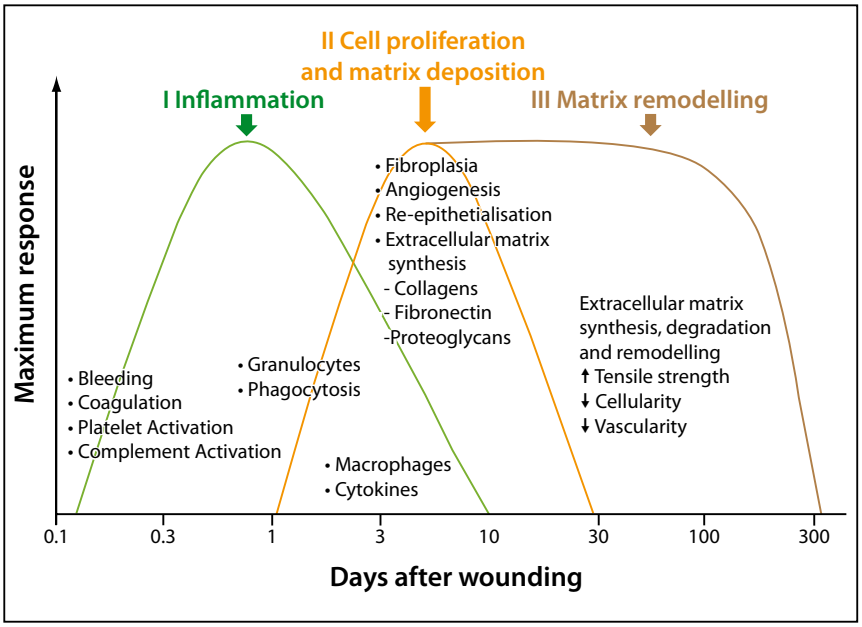


Figure 7 Phases of the wound healing process

Table 1 Results from the control group

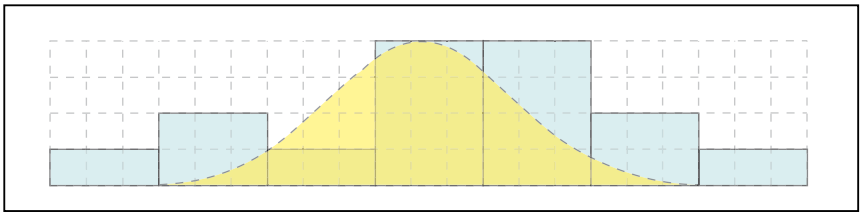
	Length	Surf	Lum	Anis	Rt	TEWL	Temp	Hydr.
Control (V1-V0)	0.45	-2	0.1	0.57	-3.1	1.1	-0.3	-4
Study (V1-V0)	-0.2	-1.7	3	-6.98	-4.2	-1.2	-0.1	-3.5

In referring to the classic model which describes a number of phases of the wound healing process (*Figure 7*), it is clear that the measurements taken within 3 weeks of treatment indicate the inflammatory phase. It may be that improvement of dermal hydration, perhaps by osmotic effect, results in a minor loss of hydration of the stratum corneum.

As a control, two individuals underwent the same treatment regimen, but with saline rather than PRP. These individuals experienced the same injection treatments. Therefore, the results of their treatment served as an objective test with regard to the effect of PRP compared with skin injected with plasma or other non-platelet containing solutions. The control group correlated well with effects attributable to inflammation and did not vary significantly from the experimental group, with the exception of decreased hydration.

Based on the initial results, an in-depth cross-analysis of results was conducted.

Figure 8 Age distribution ($P<0.05$)



Descriptive statistics for the samples

T-test for significance of group size

Age repartition shows a symmetric distribution with an average of 49.8 (*Figure 8*); while anisotropy repartition shows a symmetric distribution with an average of 28.9 (*Figure* 9). This demonstrates that the size of the groups chosen is statistically acceptable and is representative of the general female population within the chosen age group.

Predictive values for the success of the treatment

The average anisotropy rate in the study group was -24.1%. In order to find predictive values for the success of this treatment, the study population was divided into two groups. The first division was based on age, and the second on initial anisotropy.

Age-based group

The study population was divided into two separate groups by age: 40–50 years, and 50–60 years. The anisotropy levels of these two age groups remained at an average level. Thus, it can be concluded that the selection criterion of the 40–60-year age group can not serve as a predictive value, and that these two age groups constitute a homogeneous class (except for hydration).

Anisotropy-based group

The study population was segregated according to the initial anisotropy rate: below and above 30% anisotropy.

Here, the data demonstrated its most significant effective value. Specifically, patients whose initial anisotropy levels are above 30% (or in other terms, to those who would most benefit from the treatment), showed an excellent rate of improvement (-12 points versus -2.61 points), so a 33% variation *versus* 11%. Therefore, the parameter is validated as a predictor of the treatment efficacy.

Hydration-based group

In order to further investigate predictive values for the success of the treatment, a study group based on the initial hydration rate (less than 50%, between 50% and 70%, and above 70% initial hydration) was investigated.

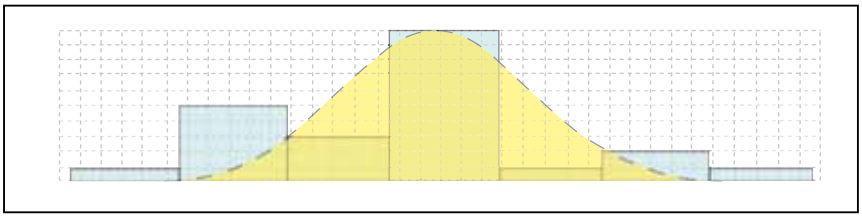


Figure 9 Anistropy distribution (*P*<0.05)

Table 2 Results from the age-based group

Age	Length	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydr.	nbr
40–50	-0.1	-0.9	1.56	-7.1	-3.5	-0.7	0.01	-0.3	21
50–60	-0.4	-2.8	5.09	-6.81	-5.2	-1.8	-0.4	-8.2	16
Average	-0.2	-1.7	2.99	-6.98	-4.2	-1.16	-0.1	-3.5	

Table 3 Results from the anisotrophy-based group

Init.Aniso.	Length	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydr.	nbr
Aniso<30	-0.5	-3.3	2.59	-2.61	-6.7	-1.3	-0.6	-1.6	22
Aniso>30	0.1	0	3.43	-12	-1.5	-1	0.28	-5.5	15
Average	-0.2	-1.7	3	-6.98	-4.2	-1.2	-0.1	-3.5	

Table 4 Results from the hydration-based group

Initial hydration (%)	Length	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydra	nbr
<50	-0,6	-3,9	2,49	-6,58	-1,2	0,22	0,12	4,88	12
Hydr=50–70	0,07	0,19	2,56	-9,17	-4	-1	-0,7	-3,3	17
Hydr>70	-0,2	-2	4,45	-3,61	-8,5	-3,3	0,46	-15	9
Average	-0,2	-1,7	3	-6,98	-4,2	-1,2	-0,1	-3,5	

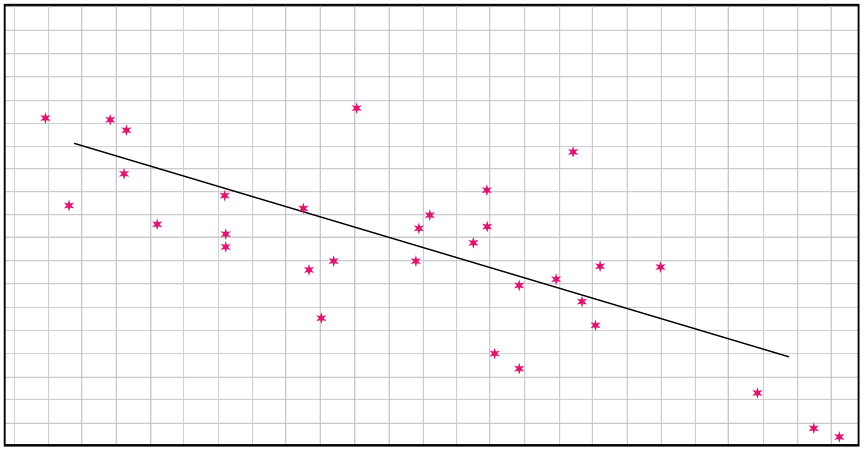


Figure 10 Linear regression for anistropy at 3 weeks. Aniso v1-v0 = 12.1903 - 0.6618 * Aniso V0

For patients with poorly hydrated skin (<50%), the treatment was found to improve the hydration values by 4.8 points. Conversely, in patients with naturally well-hydrated skin (>70%), the treatment resulted in significant dehydration of -15 points.

In conclusion, 3 weeks after treatment evidenced a good anti-ageing effect (anisotropy of -24.1%). This progressed into an excellent effect (-33%) provided the treatment group was restricted to women who would benefit the most (anisotropy >30%). Similarly, in women with initial poorly hydrated skin (<50%), in addition to the anisotropy effect, an impressive hydration effect can be seen. In women with well-hydrated skin, some dehydration is observed. PRP repairs more effectively provided there is something to repair.

A paired test found the V1-V0 variation of anisotropy significant (*P*<0.05) and variation of hydration insignificant (*P*=0.2).

Linear regression use indicates the relationship between the anisotropy at 3 weeks and the initial anisotropy show significant correlation (*Figure 10*).

PRP	V0	V2-V0	Var 2
Surface	39.21	-0.46	-1.17
Length	6.51	-0.20	-3.13
R	175.16	10.04	5.73
G	147.30	10.26	6.97
B	123.22	7.41	6.01
Luminance	62.94	3.77	5.99
a	6.83	-0.60	-8.76
b	16.66	1.29	-7.72
Anistropy	28.96	-4.90	-16.9
Ra	15.28	0.54	3.52
Rv	40.15	2.67	6.65
Rp	79.18	-5.51	-6.96
Rt	119.27	0.93	0.78
TEWL	14.09	-0.53	-3.79
Temperature	28.03	-0.16	-0.57
Hydration	57.3	0.89	1.55

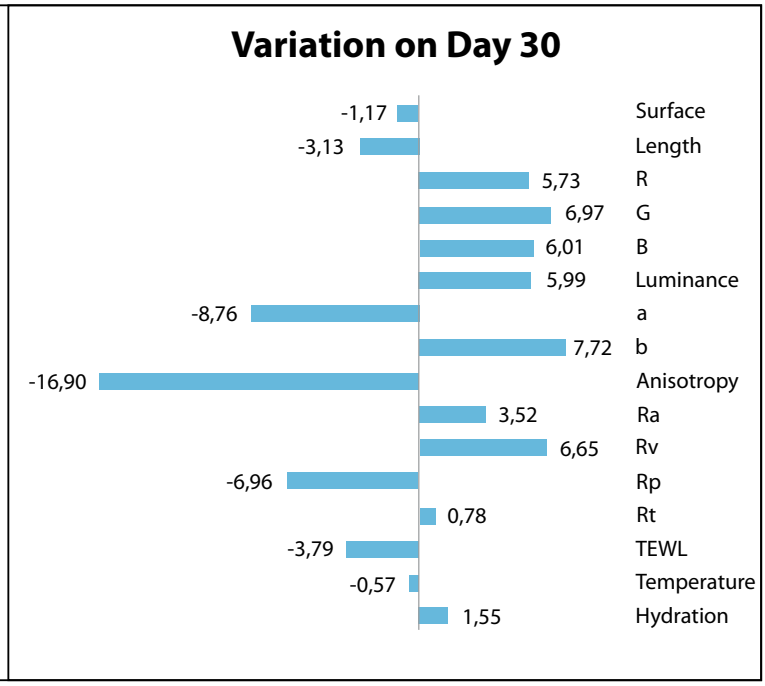


Figure 10 graphs the correlation between the initial anisotropy (ordinate) and the decrease in anisotropy along time (V1-V0), resulting from PRP treatment (abscissa). Clearly, the decrease in anisotropy correlates with the initial elevated anisotropy ($P<0.05$).

Visit 2

After three months (V2, between day 90 and day 120), the study participants had their final follow-up visit (*Figure 11*). The anisotropy remained improved and showed a decrease of -16.9%. On average, the skin lost the 30% of its initial gain [(24.1 – 16.9)/24.1]. If this decline were to progress in a continuous, linear manner, an estimate of the duration of effect of one PRP treatment session would be approximately 10 months. These figures correspond to the duration of the 'regeneration' within the healing phase of the referred model. The improvement of the cumulative length of lines remained stable at -3.13. The TEWL remains moderately improved with the -3.79% value. The luminance (L) demonstrated an improvement of +5.99%. The temperature was stable and unchanged. Hydration demonstrated a normal value. Therefore, it can be concluded that the initial dehydration was transient.

A t-test found the V2-V0 anisotropy test to be significant ($P<0.05$). In resuming the cross-analysis, the variations based on age remained insignificant, thereby confirming the homogeneity of the group of 40-60 years.

A cross-analysis based on the initial anisotropy revealed that if the treatment is applied to patients who would most benefit (anisotropy above 30%), the value still improves, reaching -14 points (-39.7% variation). Conversely, when applied to the study group with anisotropy under 30%, a minor decrease is seen.

A cross-analysis off the initial hydration status was also performed, and results similar to anisotropy were observed. Patients with poorly hydrated skin (<50%) achieved a hydration improvement of +9.24%. In contrast, subjects with naturally well-hydrated skin demonstrated a significant dehydration of -14%.

Figure 11 3-month results. R, G, B and Luminance, a, b are calorimetric parameters; Rt, Rp, Rv, Ra are micro-relief parameters

A statistical analysis of these results does not show significance; therefore, conclusions with regard to the nature of these hydration effects cannot be made ($P=0.7$).

It is important to note, however, that the side-effects reported in the study were bruises of less than 1 cm^2 (two cases), and some post-treatment red patches which

Table 5 Variation based on age

Age	Length	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydr.	Nbr
40-50	0.0	0.0	4.41	-5.6	-1.9	-1.7	-0.2	0.06	20
50-60	-0.9	-2.5	1.54	-2.5	11	3.71	-0.1	3.77	7
Average	-0.2	-0.5	3.77	-4.9	0.93	-0.5	-0.2	0.89	

Table 6 Variation based on initial anisotropy

Init.Aniso.	Length	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydr.	Nbr
Aniso<30	-0.4	-2.4	4.21	3.72	-0.6	-1	-0.3	0.39	14
Aniso>30	0.01	1.55	3.3	-14.17	2.56	-0.1	-0	1.42	13
Average	-0.2	-0.5	3.77	-4.9	0.93	-0.5	-0.2	0.89	

Table 7 Variation based on initial hydration

Init. Hydrat.	Lentgh	Surf	Lum	Aniso	Rt	TEWL	Temp	Hydr.	Nbr
Hyd <50	-0.1	-1.1	4.5	-5.2	-0.2	0.63	-0	9.24	9
Hyd 50-70	-0.5	-1.1	1.13	-4.8	5.59	1.53	-0.6	3.51	11
Hyd >70	0.1	1.21	6.98	-4.6	-4.9	-5.3	0.34	-14	7
Average	-0.2	-0.5	3.77	-4.9	0.93	-0.5	-0.2	0.89	

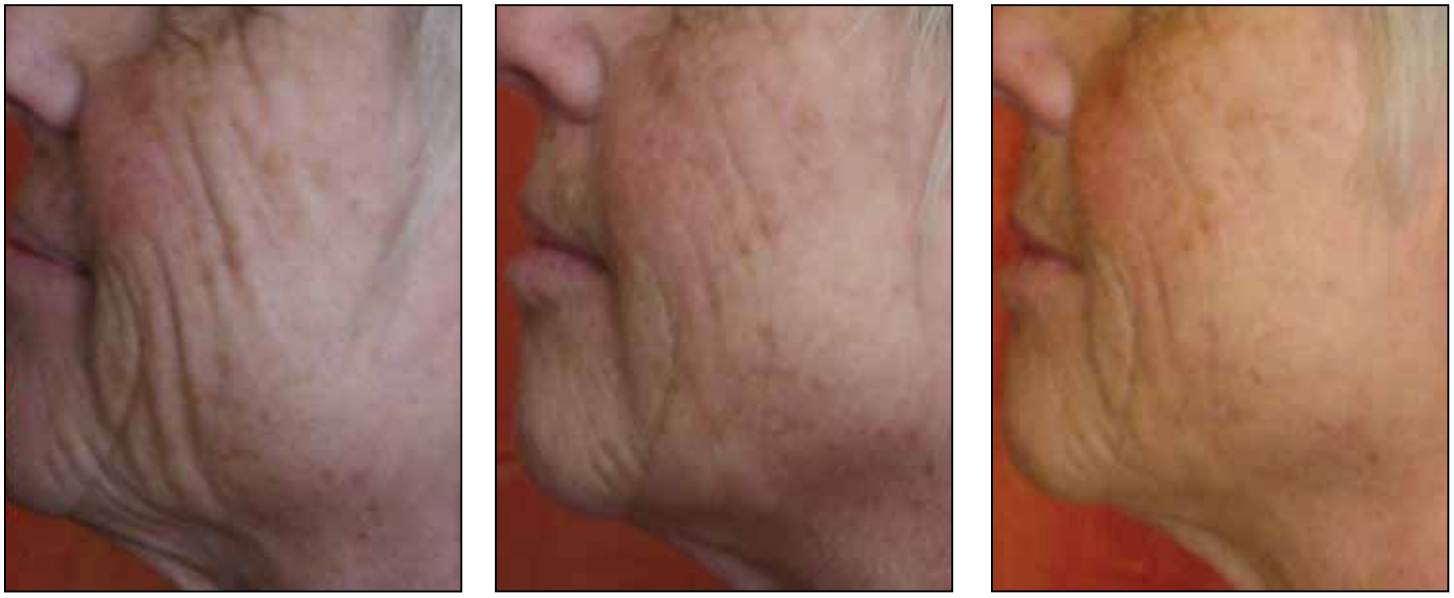


Figure 12 Clinical aspect of treatment: (A) before, (B) after, and (C) 3 months later

lasted for less than 1 hour. No nodular reactions, infections or pigmentation anomalies were reported.

Although the subject of this study is exclusively the biometric evaluation, *Figure 12* provides an idea of clinical aspect. It would be profitable to extend this study to more numerous cases. As it is merely an assessment of bio metrological parameters, it ought to be completed by other research bearing on the comparative analysis of pictures as well as biologic and histological aspects.

Conclusions

The study tracked 37 patients for a period of 3 weeks following PRP treatment, and 27 for an additional 10 weeks post-PRP treatment. A good anti-ageing effect was assessed by measured anisotropy values of -24.1% and -16.9%., respectively. Additionally, a cross-analysis involving the initial anisotropy readings demonstrated further improvement. The anisotropy correlates were -33% and -39.7%, respectively, if the treatment is provided to patients who would mostly benefit from it (anisotropy > 30 %). The study also demonstrated that the effects of one PRP treatment could last for up to 10 months. An additional predictor for a PRP treatment was found to be the initial hydration status of the skin. Namely, post-treatment skin hydration was inversely proportional to the initial hydration.

The objective criteria used in the study underscored the 'repair' and 'rejuvenation' ability of the PRP. Therefore, it can be concluded that PRP repairs more effectively provided there is something to repair. Those not in need of repair may use the treatment as an anti-ageing prophylactic.

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