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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

Striae Distensae: In Vitro Study and Assessment of Combined Treatment With Sodium Ascorbate and Platelet-Rich Plasma on Fibroblasts

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Abstract

Introduction Striae distensae (SD) appear clinically as parallel striae, lying perpendicular to the tension lines of the skin. SD evolve into two clinical phases, an initial inflammatory phase in which they are called "striae rubrae" (SR) and a chronic phase in which they are called striae albae (SA). Fibroblasts seem to play a key role in the pathogenesis of stretch marks. This study was aimed at describing and analyzing stretch marks-derived fibroblasts (SMF), the differences between SR- and SA-derived fibroblasts (SRF, SAF), testing two treatments in vitro (sodium ascorbate and PrP) on SAF.

Material and Methods To characterize the SMF, the expression of alpha smooth muscle actin (alpha SMA) was investigated. Type I collagen expression was measured in SAF, before and after adding different PrP concentrations and sodium ascorbate in the culture medium. Results were processed through statistical analysis models using the Student's *t*-test.

Results A significant increase in alpha SMA ($P < 0.001$) was observed in SRF. SAF treated with PrP and sodium ascorbate showed a resumption of their metabolic activity by an increase in collagen type I production and cell proliferation. After 24 h of incubation with PrP 1% and PrP 5% + sodium ascorbate, cell viability was increased by 140% and 151% and by 156 and 178% after 48 h, respectively, compared to the control.

Conclusion Our study shows that a biologically mediated improvement in SMF metabolic activity is possible. Our promising results require further trials to be able to confirm the reproducibility of this combined treatment, particularly in vivo.

No Level Assigned This journal requires that authors assign a level of evidence to each submission to which Evidence-Based Medicine rankings are applicable.

Keywords Stretch marks · Striae distensae · Platelet-rich plasma · Ascorbic acid · Fibroblast

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Introduction

Striae distensae (SD) have two clinical phases: an initial inflammatory clinical phase (striae rubrae SR) and a chronical phase (Striae Albae, SA). SD are the consequence of lesions of the dermal connective tissue [1]. These lesions are initially associated with inflammatory phenomena that evolve eventually into scarring. What actually underlies the SD creation is a chronic quantitative and qualitative alteration of dermal collagen associated with certain metabolic anomalies of fibroblasts [2].

The trigger event seems to be an excessive cutaneous distension which causes mechanical damage of the dermal components. The initial changes found on electronic microscopy are the mastocytes degranulation and the activation of macrophages which is associated with a moderate elastolysis of the dermis. These findings suggest that the production and release of elastases by the mastocytes represent some of the most important trigger phenomena in SD pathogenesis [3–5]. When the SD start becoming clinically visible, collagen fibers show structural alterations and fibroblast anomalies are present. At this stage, the mastocytes disappear and there is an increase in dermal edema with infiltrating lymphocytes [6].

The initial phase of SR is characterized by inflammatory lesions. The SA phase is characterized by epidermal atrophy and collagen anomalies, which mimic microscopically the dermal scar tissue. The results of the studies aiming at explaining the etiology of SD vary significantly and to date there are no unanimous definitive treatments available for SD [3–6]. A very encouraging and innovative approach is based on the use of platelet-rich plasma (PrP) and microneedling [7–9].

The aim of this study is to investigate and analyze stretch marks-derived fibroblasts (SMF) and the differences between SR- and SA-derived fibroblasts (SRF, SAF) compared to normal skin-derived fibroblasts (NSF) and to test a new treatment (sodium ascorbate and PrP) on cell cultures of SAF in vitro.

Materials and Methods

The first part of the study was based on the analysis of the SMF and on the observation of the differences between SRF and SAF compared with the fibroblasts derived from the healthy skin of the same patients. On the other side, we tried to determine the effects of PrP and sodium ascorbate on cell cultures of SAF in vitro.

The rationale of using these substances exclusively on SAF depends on the fact that SR can be greatly improved through multiple techniques such as non-ablative fractioned laser resurfacing [10, 11].

Obtaining, Isolating and Growing Fibroblasts

We conducted a prospective, randomized single-blind study in sixty-nine women aged between 30 and 40 years (35 ± 5.2) old with no comorbidities and a mean BMI < 30 of 29 ± 1.8, presenting with SR in the abdominal region and an equal number of women with SA with the same characteristics. These patients had voluntarily sought a conventional abdominoplasty procedure that had been planned at least 3 months before the beginning of the study. Each operation was performed at the same plastic and reconstructive surgery center by the same surgeon.

Exclusions criteria were: ongoing treatment with corticosteroids and Cushing syndrome (both conditions alter the normal dermis metabolism) [10].

From March 2016 to July 2019, two weeks before the operation, patients had been informed of the subject and characteristics of our study and of the possibility of donating the excess skin, which was otherwise going to be discarded after the surgery. The respect of their privacy was strictly guaranteed, and an appropriate written consent was signed by all the patients.

For the purpose of randomization, hundred sealed envelopes were prepared by our medical secretary. Inside the envelopes, there was a piece of paper indicating whether their skin would be used for the study (50 envelopes) or not (50 envelopes).

Patients were asked to choose a sealed envelope, by the same blind surgeon, that didn't know what was written on the paper inside the envelopes (if the patient would be included in the study or not).

At the end of the randomization process, 23 patients were selected to be included in the study. For each abdominoplasty-derived skin sample (Fig. 1), 4 skin biopsies were performed using a 2-mm-diameter punch that was centered on areas with SD. At the same time, two healthy skin biopsies were performed using the same technique. This was to allow 4 primary cell cultures of SMF and two NSF cultures derived from the same patient. This choice has helped to minimize all inter-individual variations that could interfere with the final results.

Fig. 1 Example of an abdominoplasty-derived skin sample with SA used for the study

Characterization of SMF

Viennet et al. have demonstrated that in early striae distensae, fibroblasts acquire a more contractile phenotype, corresponding to the phenotype of myofibroblasts [12].

In order to confirm this theory, the expression of the smooth muscle alpha actin (alpha SMA) in fibroblasts derived from SR and SA and healthy skin was studied.

Alpha SMA Detection—(Fluorescence Microscopy)

The cell cultures used to measure the alpha SMA expression were 2 days old.

Anti-mice IgG goat antibodies combined with rhodamine (diluted at 1:40 in PBS containing 1% BSA and 0.1% Triton) (Sigma, Saint Quentin Fallavier, France) were applied for 1 h at room temperature in a wet environment. Preparations were examined by a fluorescence microscope (Olympus IX50).

Flow Cytometry

The two-day-aged cell cultures were digested by Clostridium Histolyticum type I collagenase (0.150 U/mg) at 37°C for 1 h. Cell suspensions were fixed with 3% PFA in PBS for 10 min, permeabilized in Triton X100 at 0.4% for 15 min and colored with alpha SMA actin. Only secondary antibodies were used to control the incubated cells. All samples were analyzed on a FACScan Flow Cytometer (Beckman Coulter) with excitation at 488 nm and filter set at 610 nm for rhodamine. For all flow cytometry analysis, 20,000 cells were analyzed.

Effects of Different Treatments on SAF

For this phase of our study, adult dermal fibroblasts isolated from SA were incubated in different environments containing 1% and 5% standard PRP and the same concentrations of concentrated PrP for 24 and 48 h. We also wanted to test the effects of ascorbic acid at the same time (100 mg/M). The same measurements were made for control environments in which no molecules were tested. Each procedure was performed three times for each cell culture derived from the same donor. The products tested were: standard 1% and 5% PrP, concentrated PrP 1% and 5%, sodium ascorbate (100g/M), standard 1% and 5% PrP and sodium ascorbate (100g/M), concentrated 1% and 5% PrP and sodium ascorbate (100g /M), no added treatment (control). The control model was aimed at assessing whether fibroblasts had reacquired a "normal" phenotype thanks to the previously mentioned molecules or thanks to the cultures. The cell cultures were incubated with different treatments for 24 and 48 hours. We intended to examine and quantify type I collagen in fibroblasts in healthy skin, SA before and after the administration of different treatments.

PrP Preparation

Our 23 patients with SA who had accepted to donate the excess skin of their abdominoplasty procedures had not taken any medications, including aspirin and other non-steroidal anti-inflammatory drugs, in the 2 weeks prior to the operation and harvesting of PrP. The PrP was produced using the RegenKit®-BCT kit. For the preparation of PrP, two 8-ml tubes of venous blood were collected for each patient. Each tube was centrifuged at 1500 rpm for 5 min, resulting in 4 ml of PrP. To obtain the concentrated PrP, the resulting plasma (PRP) in the second tube was centrifuged again with a higher spin (2330 rpm, for 10 min). 2 ml of the platelet-poor plasma (PPP) was withdrawn and eliminated to obtain the concentrated PrP. A small fraction of each sample was used for hematological analysis. Platelet concentration was measured with an automated blood cell counter (XS-1000i; Sysmex Co.). Before testing, we measured the platelet concentration in the PrP.

Sodium Ascorbate

In all the experiments described, we used Sigma-Aldrich (St. Louis, MO) sodium L-ascorbate (CAS 134-03-2) prepared in pure powder form 99.0%, adapted to cell cultures (A4034).

Quantification of Type I Collagen Through ELISA Assay

The concentration of type I collagen in the supernatant of cultured cells was determined through the coliA1 dosage, made through an Enzyme-Linked Immunosorbent Assay (ELISA) technique according to the recommendations of the supplier (Biotechne). Absorption at 450 nm was determined in a microplate reader (E max; Molecular Devices, Sunnyvale, California, USA).

Real-time PCR Analysis for Type I Collagen

RNA was isolated from cultured fibroblasts. For experiments to measure type I collagen mRNA levels, a relative quantitative RT-PCR was performed. The RNAs were isolated using trizol (life technologies); then, the reverse transcriptase (Superscript First-Strand Synthesis System by Invitrogen, Villebon on Yvette, France) was used for cDNA synthesis and amplification using the SYbR green

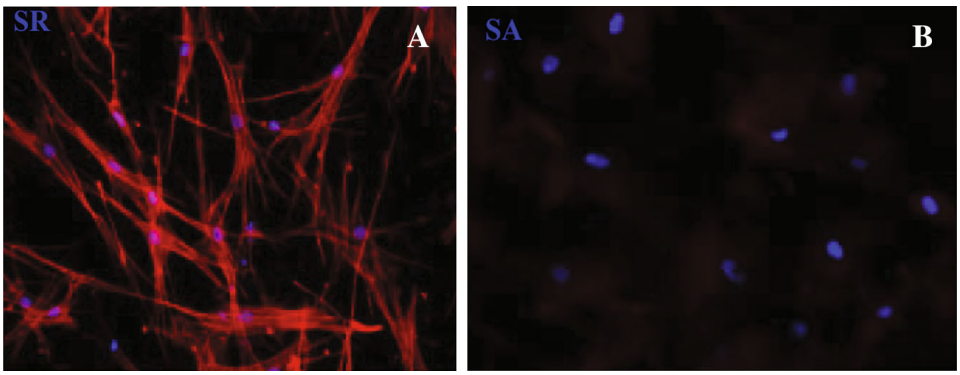

Fig. 2 Alpha SMA marking (red), fibroblasts from SR. SRF showed an important positivity for alpha SMA (**a**). Alpha SMA marking (red), fibroblasts from SA. SAF showed no alpha SMA expression (**b**).

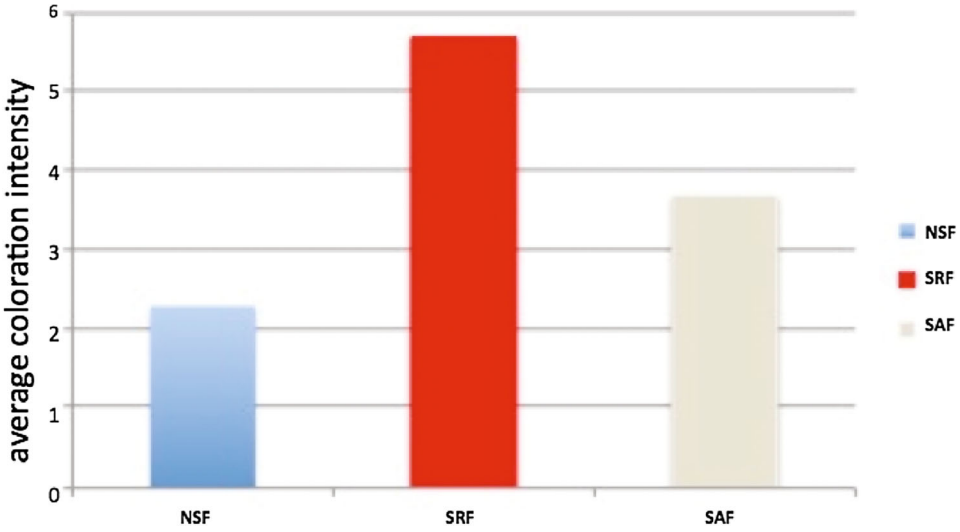

Fig. 3 Flow cytometry: fluorescence intensity (positivity for alpha SMA) detected by flow cytometry

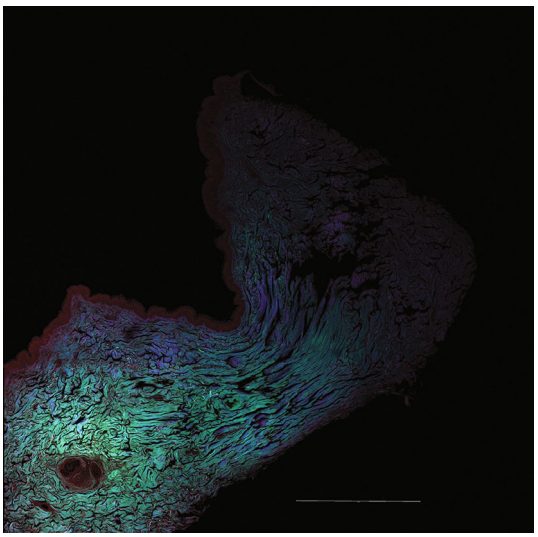

Fig. 4 Image obtained using confocal microscopy that shows SA in healthy skin. Collagen fibers appear disorganized and in reduced amounts compared to surrounding stretch marks-free skin

(PCR cycle and machine model used). The quantitative RT-PCR's were performed through a 7900 PCR real-time detection system (Applied Biosystems) using the SYBR Green qPCR Platinum SuperMix (Invitrogen). PCRs were performed using the following primers: human COL I (forward: 5'-atgatgagaaatcaaccgga-3'; reverse: 5'-ccagtagcaccatcatttcc-3') and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; forward: 5'-gtcttcaccaccatggagaaggc-3'; reverse: 5'-cggaaggccat-gccagtgagctt-3'). Type I collagen expression levels were normalized to the endogenous GAPDH levels (endogenous control). In all these experiments, SAF cultures, to which no treatment had been added, were used as a calibrator control.

Cell Proliferation

Cell proliferation was measured by a CellTiter 96 Aqueous One solution (Promega, Madison, WI, USA).

Immunocytology

Culture fibroblasts were subsequently incubated with 10g of polyclonal antibodies against type I collagen (Chemicon, Temecula, CA). The cultures were then incubated with

Table 1 Representative table of type I collagen quantification through ELISA assay

Patients: 24 h and 48 h SAF cultures	Control: no treatment	1%PRP	1% C-PRP	SA	1% PRP + SA	1% C-PRP+SA
1	499.73 ± 0.84	4354 ± 771.7	510 ± 0.57	2734 ± 339.41	4837.5 ± 1339	560 ± 3.2
2	488.61 ± 0.63	4395 ± 760.3	521 ± 0.23	2733 ± 331.21	4843.2 ± 1234	521 ± 3.1
3	494.23 ± 0.82	4377 ± 784.2	450 ± 0.11	2756 ± 323.22	4837.5 ± 1344	560 ± 2.2
4	493.12 ± 0.86	4390 ± 821.7	500 ± 0.22	2732 ± 334.32	4845.3 ± 1359	522 ± 2.4
5	495.22 ± 0.74	4399 ± 780.5	600 ± 0.65	2766 ± 421.12	4730.51 ± 1355	553 ± 4.1
6	501.10 ± 0.58	4552 ± 774.8	522 ± 0.21	2778 ± 390.32	4790 ± 1249	533 ± 3
7	490.12 ± 0.87	4532 ± 771.9	511 ± 0.33	2800 ± 321.22	4811.5 ± 1431	521 ± 2.2
8	521.73 ± 0.84	4377 ± 766.3	500 ± 0.5	2766 ± 332.45	4690.6 ± 1332	585 ± 1.9
9	478.54 ± 0.34	4390 ± 789.3	490 ± 0.7	2754 ± 190.76	4790.1 ± 1321	590 ± 3.7
10	490.34 ± 0.23	4344 ± 520.2	500 ± 0.76	2733 ± 439.11	4921.9 ± 1232	600 ± 1.8
11	552.65 ± 0.18	4310 ± 777.7	522 ± 0.32	2799 ± 336.43	4844.6 ± 1039	510 ± 2.1
12	467.45 ± 0.67	4343 ± 790.9	518 ± 0.11	2890 ± 334.76	4830.1 ± 1620	470 ± 3.2
13	497.88 ± 0.45	4355 ± 798.1	519 ± 0.89	2789 ± 332.56	4211.32 ± 1300	562 ± 4.3
14	445.98 ± 0.76	4399 ± 721.4	399 ± 0.41	2711 ± 334.43	4867.11 ± 1290	577 ± 3.9
15	511.1 ± 0.43	4514 ± 795.5	535 ± 0.44	2690 ± 339.44	5558.9 ± 1258	460 ± 3.5
16	498.43 ± 0.23	4219 ± 776.1	602 ± 0.99	2766 ± 452.67	4233.5 ± 1345	569 ± 3.1
17	499.65 ± 0.98	4322 ± 734.8	510 ± 0.23	2780 ± 344.27	4850.8 ± 1365	398 ± 3.4
18	469.34 ± 0.45	4341 ± 720.8	522 ± 0.54	2934 ± 319.54	4890.4 ± 1333	597 ± 3.8
19	514.65 ± 0.34	4366 ± 756.3	565 ± 0.66	2864 ± 509.32	4899.7 ± 1292	584 ± 3.3
20	560.89 ± 0.56	4397 ± 787.2	532 ± 0.1	2751 ± 339.51	4696.23 ± 1344	490 ± 3.8
21	499.93 ± 0.86	4401 ± 788.7	514 ± 0.23	2934 ± 379.76	4928.11 ± 1506	560 ± 3.4
22	500.56 ± 0.83	4332 ± 741.8	516 ± 0.65	2532 ± 379.83	4887.2 ± 1400	571 ± 3.1
23	496.21 ± 0.23	4400 ± 811.5	499 ± 0.32	2765 ± 329.33	5231.3 ± 1106	533 ± 3.3

Type I pro-collagen quantity after 24 and 48 h (pg/ml). Results are presented as mean values ± standard deviation. C-PRP= concentrated PrP; SA= 100 μM sodium ascorbate

secondary antibodies combined with fluorescein. The nuclei were detected with propidium iodine (Sigma, Sigma, St. Louis, MO). All cultures were then examined with a Nikon Eclipse E1000 Microscope.

Statistics

Data and Statistics Management

Our results were processed using the Prism software in its Version 5 (Graph Pad, USA). The statistical analysis was conducted using JMP 10 (SAS institute Inc. software).

In all experiments, the average values for the three SD-test sets were calculated. The results were processed through statistical analysis using the Student's *t*-test. The results were considered statistically significant when the likelihood was less than 0.05 (p.05).

Results

Expression of Alpha SMA

SRF showed an important positivity for alpha SMA. We were not able to observe alpha SMA filaments in SA and NSF (Fig. 2). Flow cytometry analyses of alpha SMA expression on NSF, SRF and SAF showed an average coloration intensity of 2,3 ± 0,6; 5,7 ± 0,4 and 3,7 ± 0,3, respectively (Fig. 3).

Compared to the fibroblasts of healthy skin, a significant increase in alpha SMA was observed for SR fibroblasts (P<0.001). There was no significant difference between SAF and NSF.

Collagen I Quantification by ELISA

The average concentration of type I collagen obtained by dosing the COLIA1 from the supernatant of NSF cultures was on average 1750 ± 390 pg/mL, 550 ± 239 pg/mL in fibroblasts extracted from Striae Albae and 1313± 247 pg/

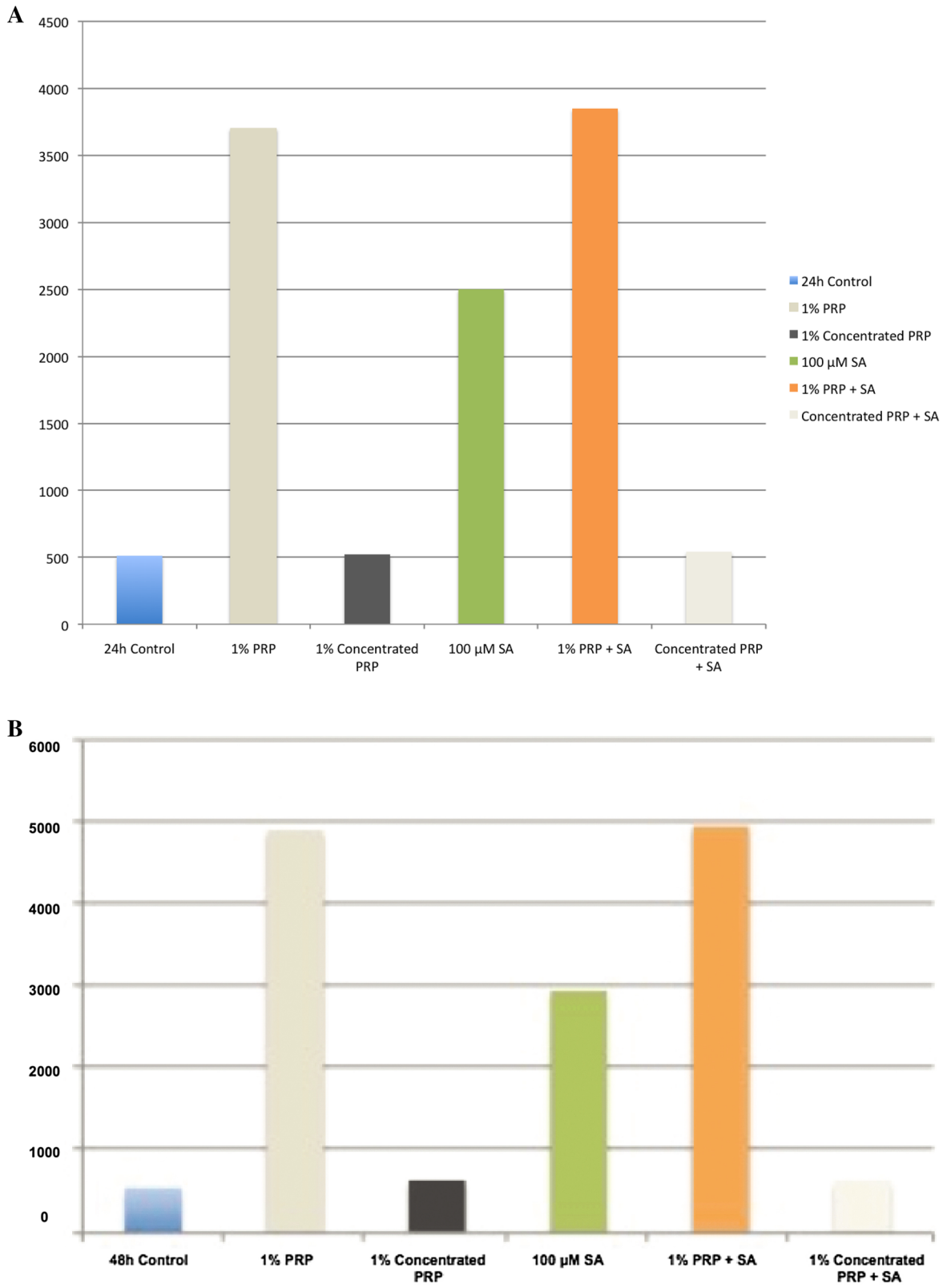

Fig. 5 Quantification of type I collagen through ELISA assay. X= type I pro-collagen quantity after 24 (**a**) and 48 h (**b**); Y=Concentration pg/ml

mL in fibroblasts extracted from the skin with Striae Rubrae. Statistically significant differences ($p<0.05$) were observed only between the collagen production of fibroblasts from SA and healthy skin.

These results were confirmed observing SA samples at confocal microscope (Fig. 4). Confocal microscopy, which is based on tissue self-fluorescence, was used to get more reliable observations and to avoid the biases that are often associated with immunomarking.

PrP Characterization

After 24 h of serum deprivation, fibroblasts derived from SA were grown in a culture medium without serum and incubated for 24 and 48 h with different concentrations (1–5%) of "standard" PrP or "concentrated" PrP (cPrP) combined or not with 100 μM sodium ascorbate. The outcomes were compared to the control cultures.

The average platelet concentration ($\times 10^4$ μL) was 22 .4±4.59 (whole blood) before the first cycle of centrifugation was 62.3 ±13.8 for the PrP and 319.6 ± 31.8 for the concentrated PrP.

Type I Collagen Quantification by ELISA After Treatment administration

Collagen biosynthesis was measured in dermal fibroblasts from SA treated with 1% and 5% PRP, 1% and 5% concentrated PrP combined or not with sodium ascorbate (Table 1). As shown (Fig. 5), PrP 1% and 5% with sodium ascorbate contributed after 24 and 48 h of incubation to the increase in collagen biosynthesis significantly compared to the control. A greater effect was observed for cell cultures that had received a combination of Sodium PrP-Ascorbate treatment ($p< 0$ .0001). Concentrated PrP showed a non-significant effect on collagen production ($p = 0.123$).

Rt-PCR for Type I Collagen Quantification

Similar results were observed with RT-PCR. The expression of type I collagen mRNA was significantly increased for fibroblast cultures from SA treated with 1% and 5% PrP for 24 and 48 h. The PrP/Ascorbate sodium combination showed an increase in the mRNA expression of type I collagen 3,2 times greater than the control after 24 and 48 h of incubation. Concentrated PrP showed a non-significant effect on the mRNA expression of type I collagen (Fig. 6).

Cell Proliferation

After 24 h of incubation with PrP 1% and PrP 5% and sodium Ascorbate, cellular viability was increased by 140% and 151% and by 156 and 178% after 48 h, respectively, compared to the buffer. These results were statistically significant ($p < 0.05$).

Surprisingly, concentrated PrP had a small or even negative effect on cellular viability in all experiments.

Immunocytology

The cytological immunoanalysis confirms our previous observations. SAF produce less type I collagen compared to healthy skin fibroblasts in the same patient. The combination of PrP and sodium ascorbate has been proved as the most effective treatment for the induction of collagen production in vitro (Figs. 7, 8).

Discussion

Several mechanisms appear to be the basis for the appearance and chronicization of the Striae Distensae [7]. An alteration of the fibroblast phenotype taking place at some stage between the initial and old lesion seems to be the determining event. These cells seem to react to the mechanical stress that occurs in the skin, first turning into myofibroblasts (alpha SMA+) and then into quiescent fibroblasts (alpha SMA−).

The mechanical forces generated by SMA + cells have a very important biological role in cells (mechanical transduction, vasoregulation) and tissues (maintaining tone). These resistances can be disrupted and be the cause of multiple anomalies, causing sometimes a loss of function [13, 14]. Our results highlight the different mechanical behavior of fibroblasts in vitro depending on the origin of the cells. Based on their mechanical properties, SMF appear as a different population from healthy fibroblasts. This observation is consistent with the idea of fibroblast heterogeneity [15]. This observation also explains a key moment in the appearance of SDs, the mechanical stress that skin undergoes during pregnancy or every weight change in an individual. The alpha SMA+ phenotype that characterizes the SRF is significantly reduced in SAF. Our results confirm and provide additional information to the works of Viennet et al. [12]. It is currently believed that SAF are quiescent and produce inferior quantities of collagen compared to NSF [2–6]. It has been demonstrated that PrP (platelet-rich plasma) and ascorbic acid can increase the synthesis of collagen in normal fibroblasts [13]. There are no studies in the current literature testing and proving the effects of the combined in vitro stimulation of PrP and ascorbic acid on SD fibroblasts, and especially about the preparation protocol of PrP and the standard platelet concentration (which represent paramount elements for the value and reproducibility of the results) [16–27]. The platelets, and in particular the platelets-

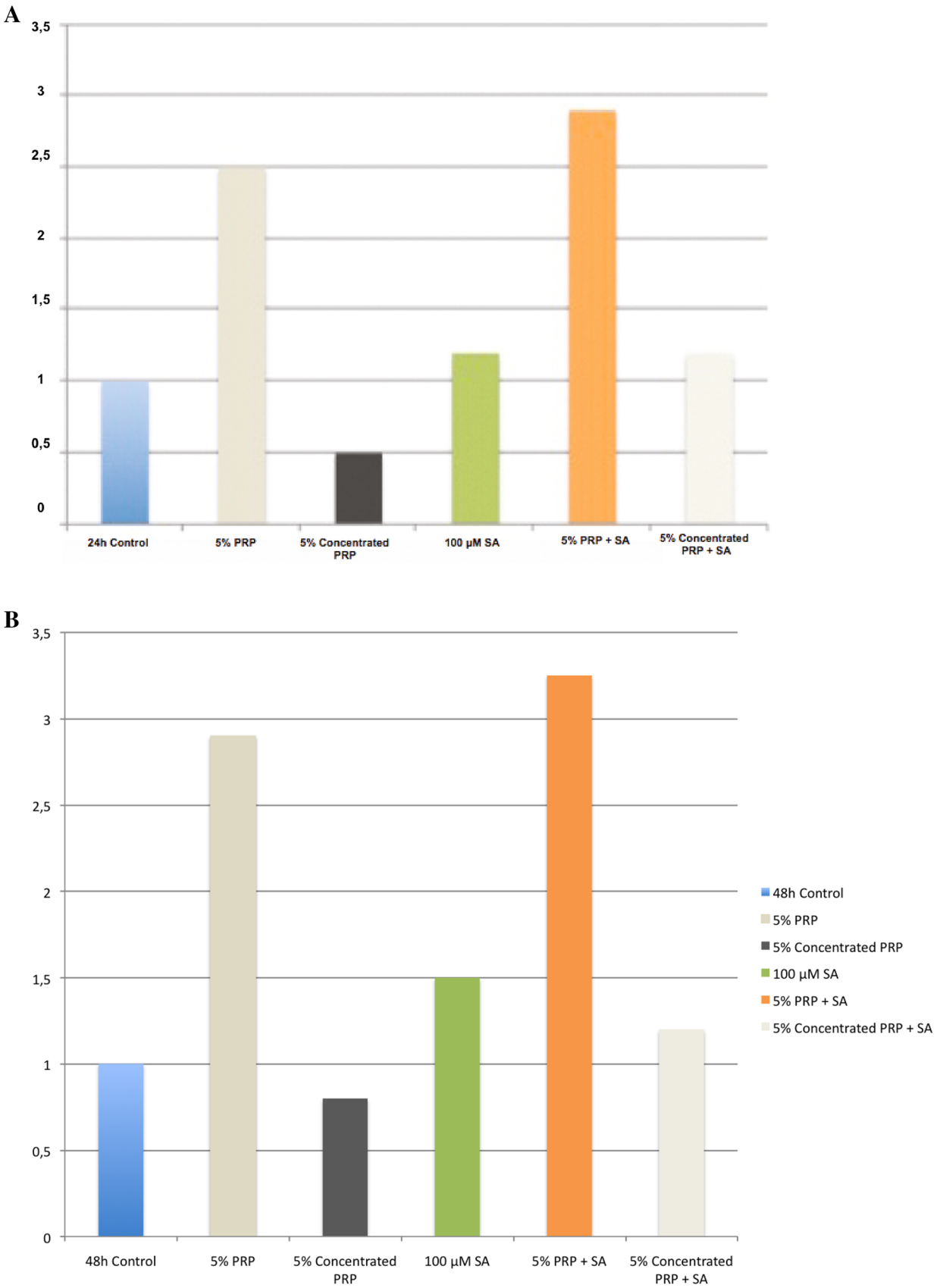

Fig. 6 Real-time PCR Analysis (type I collagen): the expression of type I collagen mRNA after **a** 24 and **b** 48 h (PrP 5%)

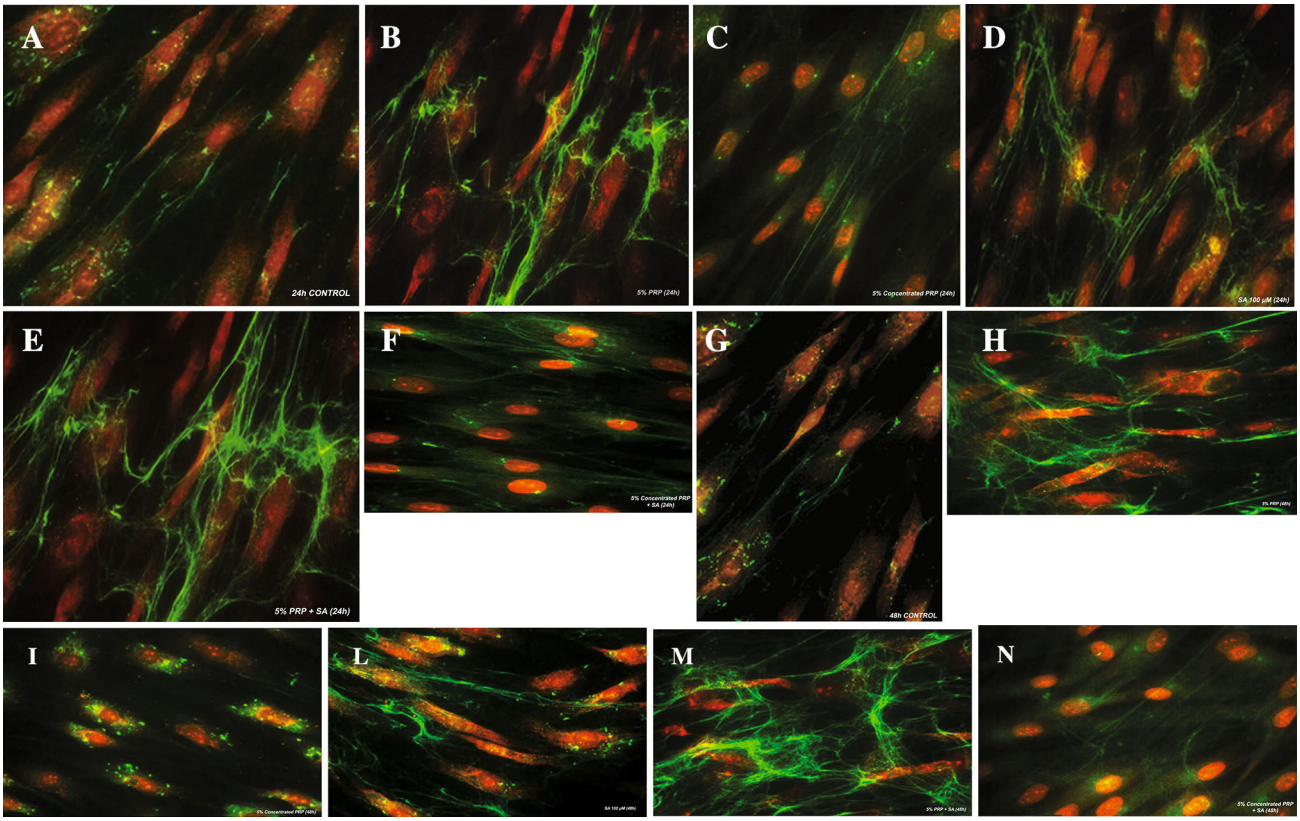

Fig. 7 Immunocytology. Fibroblasts from SA; immunomarking for collagen type I (green) and fibroblasts (red); Fibroblasts from SA 24h (**a**–**f**) and 48 h (**g**–**n**) after addition of treatments to the cultures. The combination of PrP and Sodium Ascorbate has been proven as the most effective treatment for the induction of collagen production. Immunomarking collagen type I (green) and fibroblasts (red)

derived growth factors contained in the alpha granules, are responsible for the biological effects of PrP. The interaction between these GFs and the surface receptors on target cells activates intracellular signaling pathways that trigger the expression of genes responsible for regeneration processes such as cell proliferation and extracellular matrix formation [17–28]. It seems clear to us that the effectiveness of this process depends on how PrP is prepared. However, despite the numerous benefits associated with PrP and the promising results reported proving its therapeutic potential, the clinical results are heterogeneous and sometimes contradictory. These controversial results are mainly due to the lack of standardization of the PrP preparation procedures. L-ascorbic acid (vitamin C) is a natural hydrophilic and anti-oxidant acid that has been used in cosmetics as a preservative, to adjust pH, and/or as an active compound. This molecule has been shown to promote collagen synthesis in the human skin fibroblasts in vitro. In particular Sodium Ascorbate appears to be the most effective form in stimulating collagen production [29]. The concentrations of sodium ascorbate, tested in a recent study, necessary to increase the collagen secretion in healthy human fibroblast cultures in vitro were between 50 and 200 µM [30]. Higher concentrations (400 µM) were not found to be more efficacious and even higher concentrations (800 µM) had an inhibitory effect on collagen synthesis [31]. This is the first study testing the combined use of sodium ascorbate and PrP on SMF [32]. The combined stimulation with standard PrP and sodium ascorbate of SAF triggered the resumption of cellular metabolic activity, increased the production of type I collagen and stimulated cell proliferation.

Clinical outcomes of the combined stimulation with PRP and sodium ascorbate on SD and healthy skin are to be investigated with *in vivo* studies, as there are no published studies reporting such experience. PRP has been used to improve skin elasticity and firmness [33]. According to Hersant et al., three sessions of PRP injection at 1-month intervals result in significant improvement in skin texture and smoothness parameters [33, 34]. More recently, Zasada et al. have demonstrated beneficial effects of L-ascorbic acid on the healthy skin, with a significant reduction in the signs of aging and an improvement in the skin firmness elasticity and hydration [35]. Both sodium ascorbate and

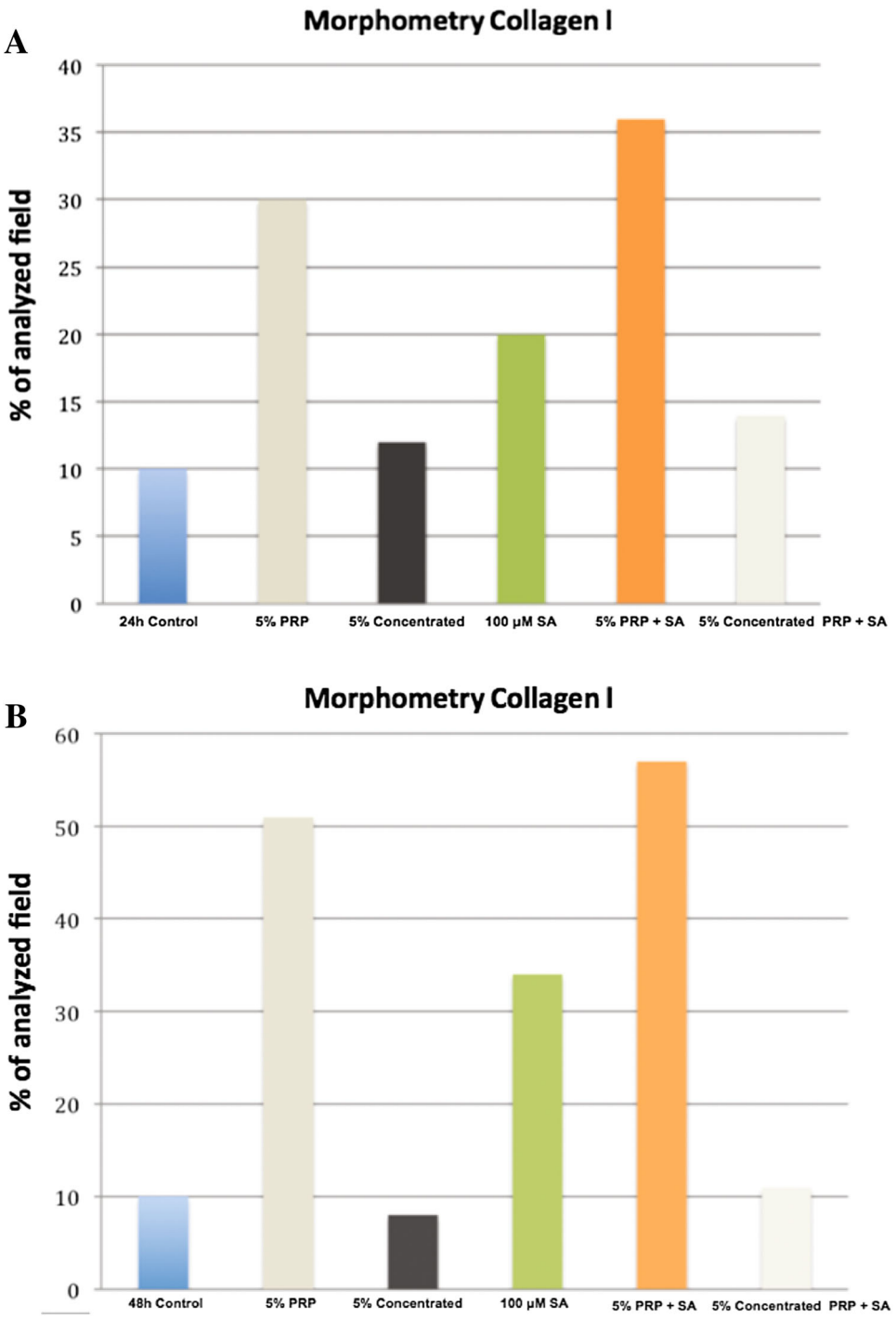

Fig. 8 Results of the morphometric evaluation and quantification of type I collagen at 24 h (**a**) and 48 h (**b**) after addition of treatments to the cultures

PRP have been used for the treatment of SD with promising results [7, 36]. Ibrahim et al. performed a prospective study on 68 patients, complaining of striae distensae (albae and rubrae). Each patient underwent two to six PRP injection sessions at 2-week interval. Authors observed a clinical improvement in SD in all the patients with an increase in dermis collagen and elastic fibers at the end of treatment. Casabona et al. [8] have demonstrated that sodium ascorbate stimulates collagen production in the dermis by increasing fibroblast proliferation, improving the atrophic appearance of stretch marks. To date, the clinical effects of the simultaneous use of sodium ascorbate and PrP have not been assessed yet. The encouraging results of our study may require further testing to confirm their reproducibility before being used in clinical practice. Our study preludes to further clinical applications and studies

on skin regeneration where fibroblasts stimulation can be beneficial.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval Ethical approval was given, by FRENCH institutional committee, and the relevant judgement's reference number is 2020-A01250-39.

Human and Animal Participants All procedures in the study involving human participants have been performed in accordance with the ethical standards of institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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