




# Striae Distensae: In Vitro Study and Assessment of a Combined Treatment with Sodium Ascorbate and Platelet Rich Plasma on Fibroblasts



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Dear Sir:

We really appreciate your interest in our paper that validated the use of a combined stimulation with PRP and sodium ascorbate on SA in vitro to increase type I collagen in fibroblasts ( $p < 0.001$ ) [1].

Concerning Alpha SMA detection – (fluorescence microscopy):

(1) 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 hour at room temperature in a wet environment. Preparations were examined by a fluorescence

microscope (Olympus IX50). SRF showed an important positivity for alpha SMA (red staining in figure 2).

Concerning the Immunocytology:

Culture fibroblasts were subsequently incubated with 10 g of polyclonal antibodies against type I collagen (Chemicon, Temecula, CA). The cultures were then incubated with 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. We used green immunomarking for collagen type I and red for fibroblasts.

We agree and believe that levels of extracellular matrix deposition could be assessed in further studies, including TGF- $\beta$ 3, type III collagen, Col1A1, and Col3A1.

We chose to evaluate only the changes of type I collagen contents since it has been demonstrated that it is the most implicated in SM pathogenesis [1].

Figure 4 is a representative image obtained using confocal microscopy to show SA in healthy skin. Collagen fibers appear disorganized and in reduced amounts compared to surrounding stretchmarks-free skin.

In Figure 4, collagen fibers may not appear poorly organized in the SA area. However, tissue architecture may be affected by the amount of pressure used to prepare the microscopy sample.

Figure 4 was intended to outline that the total amount of type 1 collagen in SAFs was decreased, compared to the surrounding healthy skin.

The resumption of SAF cellular metabolic activity is shown and clearly demonstrated with the significant increase deposition of type I collagen following treatments administration.

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Our research preludes to further clinical applications and studies on skin regeneration where fibroblasts stimulation with combined PrP-Sodium Ascorbate [2–5] can be beneficial.

#### Declarations

**Conflict of interest** The authors declare that they do not have any conflicts of interest.

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

**Informed consent** For this type of study informed consent is not required.

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