Author's response to reviews

Title: Topically applied vitamin C increases the density of dermal papillae in aged human skin

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Author's response to reviews: see over
Dear Editor,

thank you for considering our manuscript „Topically applied vitamin C increases the density of dermal papillae in aged human skin”. We appreciate the valuable suggestions and comments of the reviewers. Please find below a point-by-point description of how we addressed their concerns.

Reviewer Professor Philippe Humbert

1. We corrected the reference specifications of the mentioned journals.
2. Since the described studies are a matter of non-invasive tests in dermatological research, using just approved and authorized instruments and methods, according to European law there is no need for an advise of an ethical committee.
3. Now, in the abstract we specify the site (inner forearm), the time (4 months) and the number of volunteers (n=33, female) of the focused study.

Reviewer Professor Salvador Gonzalez

1. We amended the manuscript according to the suggestions. Major modifications are marked in red characters.
2. We agree with the reviewer, that the parameter we referred to as papillary index is yet quite unconventional. However, in vivo microscopy offers new views into a whole variety of morphological features of the upper skin layers. This forced us to create this new parameter for description. Nevertheless the age depending of the papillary index is in good agreement with results from conventional histologic studies. Moreover, its increase due to vitamin C treatment clearly is in the range of age dependent differences. Furthermore, we never observed any indications for an inflammatory response of the skin due to topical vitamin C. The increase in the density of papillae after vitamin C treatment can not be interpreted only as a sign of epidermal hyperplasia with an enlarged area covered with basal layer and the epidermis growing down into the dermis. More likely it is linked to a restructuring of the papillary dermis, as the top of the newly formed papillae and the capillaries are localized above the average height of the plane basal layer seen predominantly in aged skin. The newly
formed blood vessels show a normal anatomical structure in confocal microscopical examination and are apparently integrated in a healthy vascular architecture. In the confocal images there are no signs of pathologic changes of the vasculature like, for example, increased diameter, parallel orientation or clew of vessels in a papillae. No perivascular infiltrations of lymphocytes could be observed during the treatment period.

3. We agree with the reviewer, that the granular cell diameter or the cross-sectional area respectively is not uniform and measuring just a few cell sizes would not be reliable. For this reason we measure at least 20 cells in different images, at least twenty and often even more. The average of these repetitive measurements seems to be a reliable parameter representing a medial cell size and it is adequate to detect changes due to aging and cosmetic treatment. As described, it is important to have trained examiners. Therefore we did trials concerning the intra- and inter-individual reproducibility, which we did not mentioned because this particular methodological aspects seemed to us not to be in the main focus of this paper.

4. Now, we specify the number of volunteers (three groups of female volunteers: n=12, ages 18 to 25, n=11, ages 40 to 50 and n=12 ages 65 to 80 years).

5. The dose of vitamin C is a result of extensive work concerning cosmetic formulations. All these examinations are described in Raschke et al.: Topical Activity of Ascorbic Acid: From in vitro Optimization to in vivo Efficacy. Skin Pharmacol Physiol. 2004 Jul-Aug;17(4):200-6. We added this publication in the references. Studies concerning a dose-effect relationship are still in progress.

6. Not all of the data of the measurements in the second study are normally distributed, so the median is not located in the centre of the boxes for all data. To avoid the impression of having a normal distribution, which would be the requirement for a valid calculation of the mean and the standard deviation, we prefer to present box whisker plots, which are showing the median, the quartiles and the extrema. Following the reviewers suggestions we added a table showing the p-values.

7. We agree with the reviewer, that the mechanism of angiogenesis up to now is just speculative. Therefore we omitted the text passages referring to angiogenesis largely and just noted it once as a possible but not proven explanation (“perhaps through the mechanism of angiogenesis”).
Again, we like to thank the reviewers for requesting us to clarify passages and thus to enhance our manuscript.

Sincerely

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