

COMMENT

Comment on Choe et al “A modification for the calculation of water depth profiles in oil-treated skin by in vivo Raman microscopy”

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With interest, we have read the recent paper of Choe et al, “A modification for the calculation of water depth profiles in oil-treated skin by in vivo Raman microscopy” published in *J Biophotonics* 2020;13:e201960106.

The authors proposed an analysis of in vivo Raman spectra of skin to calculate water concentration profiles in skin, different from the original method of Caspers et al [1], claiming it corrects an error in this method. Their proposed method is meant to be applied in cases in which a product is applied to the skin, penetrates into the skin and has signal contributions in the CH-stretching spectral region used by Caspers et al [1]. This might cause an error in the water concentration calculation because such a situation was not explicitly addressed in the Caspers method.

The authors suggest a way to deal with this situation by using the Amide I band (around 1650 cm^{-1}) instead of the CH-stretching band (2910–2965 cm^{-1}) in their calculations of the water concentration profiles. They also show how to correct the measured intensity in the Amide I band for signal contributions of water and of applied product.

They continue to present the results of an experiment in which four different oils were applied to the skin of the volar forearm and show the differences between the calculated water concentration profiles according to the

classical Caspers method and their newly proposed method.

The differences in the results of the two methods are then discussed and reasoned to be due to errors in the Caspers method. The authors mention that application of the Caspers method to formulation-treated skin results in lower water concentration values than when this method is applied to untreated skin, referring to papers on oil-treated skin. According to the authors, this is not possible because oil forms an occlusive layer on the skin, which should result in a higher amount of water in the skin, referring to a 1997 paper of Filho. They blame this on the fact that oils contribute to the CH-stretching band (2910–2965 cm^{-1}).

In principle, this is justified research. The authors address the issue that the Caspers method does not *explicitly* take into account the potential effect of penetration of topically applied product(s) on the calculation of in vivo water concentration profiles.

Nevertheless, we take issue with the assumptions on which the article is based, with the data analysis that is presented and with the conclusions of the paper.

The complex method that is proposed by the authors is based on a conceptual mistake. It does not do what the authors claim it does. The proposed method calculates water mass% in the stratum corneum (SC) based on analysis of the water-to-protein ratio; that is, it only considers the intrinsic skin constituents and ignores the presence of the extrinsic material, such as oil, that has penetrated into the skin. However, the oil does have a mass too; it is

This article is a comment on C. Choe, J. Schleusener, S. Choe, J. Lademann, and M. E. Darwin's A modification for the calculation of water depth profiles in oil-treated skin by in vivo Raman microscopy [2020], DOI 10.1002/jbio.201960106.

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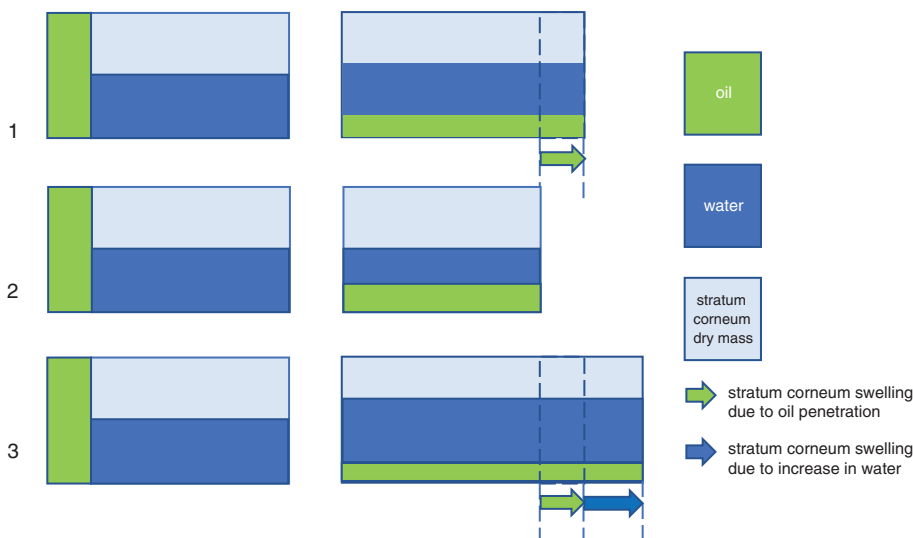


FIGURE 1 Illustration of three possible scenarios of topically applied oil penetrating into the stratum corneum

present in the skin and, therefore, must enter somewhere into the calculation of the water mass%.

To make this clear, let us assume oil is applied to the skin, which penetrates into the SC, such that $X\%$ of the SC mass is now oil.

What the new method of the authors calculates is the water mass % in the intrinsic SC mass, which constitutes $(100-X)\%$ of the total SC mass when oil is present.

This is obviously not the same as the water mass % in the SC in the presence of oil (ie, taking the mass of the oil into consideration), which is what the Caspers method implicitly calculates, by taking into account the signal contributions of both intrinsic skin constituents and the extrinsic material that has penetrated. The method of Caspers uses the signal intensity in the CH-stretching region as a measure of the solid material in the skin and the part of the OH-stretching region not overlapping with protein NH-str vibrations to determine water concentration in the skin.

Because, apart from water, protein is the main constituent of SC, the Caspers method was calibrated using a range of protein solutions in water. As the authors show, oil that has penetrated into the SC will also contribute to signal intensity in the CH-stretching region.

A point of criticism of the Caspers method might be that it has not been calibrated for oils; therefore, the results might not be exactly right when much oil is present.

Comparing the results of the newly proposed method and the Caspers method, as the authors do in their paper, is comparing apples and oranges. One should not expect the results to be the same. Dismissing the Caspers method based on such a comparison is wrong.

After application of oil to the skin, which penetrates into the SC, there are basically three scenarios (illustrated in Figure 1), none of which the authors seem to take into consideration when analyzing and comparing the results of the two methods:

- 1 Oil penetrates into the SC without any effect on the *amount of water* in the SC. In this case, because the penetrating oil will take up a certain volume, the SC must swell. This means that the same amount of water is now divided over a larger volume. Therefore, due to the presence of the oil, the actual *water mass percentage* would be lower.
- 2 Oil penetrates into the SC without any swelling of the SC. Because the penetrating oil will take up a certain volume in the SC, this must mean it displaces intrinsic skin constituents. Water should be the likely candidate because the two do not mix very well. Therefore, again, due to the presence of the oil, the actual *water mass percentage* would be lower.
- 3 Oil penetrates into the SC, and because of the occlusive effect of the oil, preventing evaporation of water from the skin, the *amount of water* in the SC also increases. In comparison with scenario 1, this must lead to an even further swelling of the SC because both the oil and the additional water take up a certain volume. Whether or not the actual *water mass percentage* increases will depend on how much oil penetrates and on how much extra water accumulates in the SC.

One might wonder why the authors did not take the swelling of the stratum corneum into consideration in their analysis. Well, they could not have because, in their analysis, normalization of the SC thickness is applied; that is, SC thickness is always set at 100%, and results are discussed in terms of % SC thickness (paragraph 2.2.6).

The swelling of the SC as a result of oil penetration and/or water accumulation due to occlusion has been completely left out of the equation due to this normalization. As a result, the opportunity to determine the

amount of water in the SC is lost, as well as the possibility to compare the amounts of water in the SC before and after treatment.

The amount of water in the SC (per unit skin surface) is the integral of the water concentration over the thickness of the SC. Therefore, application of oil may lead to both more water overall in the SC and a lower water concentration in the SC due to the presence of oil in the SC. However, due to the normalization of SC thickness that has been applied, the authors have not considered this.

Therefore, the main conclusion of the authors is wrong: it is based on a conceptual mistake, leading to a method that does not calculate the water mass percentage in the SC, in contrast to what the Caspers method does. The method proposed by the authors is based on a wrong interpretation of their measured Raman profiles due to the unfortunate normalization on SC thickness. The Caspers method for the calculation of water concentration in oil-treated skin must not be dismissed based on the results presented by the authors. It enables a more transparent interpretation, and it comes much closer to the truth than the method proposed by the authors.

CONFLICTS OF INTEREST

Gerwin Puppels, Peter Caspers and Claudio Nico are employees of RiverD International B.V., which sells in vivo skin analyzers based on Raman spectroscopy and software for analysis of in vivo skin spectra. Gerwin Puppels is managing director of RiverD International and holds an equity interest in the company.

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How to cite this article: Puppels G, Caspers P, Nico C. Comment on Choe et al “A modification for the calculation of water depth profiles in oil-treated skin by in vivo Raman microscopy”. *J. Biophotonics*. 2020;e202000043. <https://doi.org/10.1002/jbio.202000043>