Sorption studies of human keratinized tissues

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Abstract. Water content is known to be the most important single parameter for keratinized tissue to remain its vital functions. In that sense, a general knowledge of the water binding properties is of great interest, and a reliable measurement setup must be found. Also, revealing the sorption properties of human keratinized tissues is vital towards a calibration of susceptance based skin hydration measurements that already is an important diagnostic tool in clinical dermatology, and we will see that any hysteresis will complicate such a calibration further. In this study we investigated the sorption properties of keratinized tissues such as human epidermal stratum corneum (SC), hair and nail. The study was performed under controlled environmental conditions with a dynamic vapor sorption (DVS) instrument, and the water uptake of the keratinized test samples was measured as the relative humidity in the ambient air was altered step-wisely. In this study, vital and characteristic water sorption properties such as the isotherm, relative water uptake, and hysteresis were investigated and will be discussed.

1. Introduction

Water content and water-holding capacity are known to be very important for the overall function of keratinized tissues such as SC, hair, and nail, and Blank showed that such tissues needed at least 10 per cent water content in order to remain its vital functions [1]. Electrical measurements as a tool for estimating the hydration state of keratinized tissues have been studied and continuously improved over the last 30 years [2, 3, 4, 5, 6, 7], and a low frequency susceptance method has been found to be the most suitable [8]. Water content has been shown to be the most significant parameter governing electrical susceptance of human tissue [9]. The basic knowledge of how absorbed water effects the conductivity of keratinized tissues rests upon insight in the binding mechanisms of water, and therefore it is of general interest to investigate the sorption properties, including any differences, of SC, hair, and nail.

The main target in this study was to investigate the absorption and desorption properties of human keratinized tissues. The findings in this manner will have direct implications on the progress of calibration of susceptance based hydration measurements that, among others, are aiming to better estimate absolute water content of e.g. human skin. Such attempts have been carried out in vitro [10] and in vivo [11] based on absorption data of SC from the heel. However, any hysteresis, that is differences in hydration level between absorption and desorption at similar environmental conditions, will necessarily influence the final result depending on whether the calibration uses absorption or desorption data, and thus prevents the calibration from being unique.
Although the general structure and properties of SC, nail and hair, all consisting of dead cells tightly cemented together by a group of proteins called keratins, are quite similar, they show different sorption properties, especially at high relative humidities (RH). Also the amount of hysteresis for keratinized tissues may be a measure of structural changes that occur during a full sorption cycle. The differences in water uptake between SC, nail and hair also reveal differences in composition and structure, and our results suggest that hair and nail are similar in structure, however deviating more from epidermal SC.

2. Methods
The keratin samples were taken from healthy and male volunteer test subjects. The two SC pieces were taken from the heel by an Aesculap dermatome (Braun, Tuttingen Germany), and the skin was gently abraded before the dermatome was used in order to get a smooth surface. The sample pieces were about 1 by 1 cm in size and with a thickness of 0.2 mm which is the smallest adjustment possible by the dermatome. The two samples of toenail were cut in full thickness, and the sample sizes were approximately 3 by 3 mm. Finally, one hair sample was prepared so that both of the two ends, the follicle and the tip, were removed, leaving only the center part as the sample. The water sorption properties of each sample were measured separately by means of a dynamic vapor sorption (DVS) from the Surface Measurement Systems, U.K. The DVS setup provides very delicate and sensitive gravimetric measurements provided by a Cahn microbalance system and ensures a resolution of 0.1 µg. The samples were placed in a closed chamber where both temperature and relative humidity is controlled. Furthermore, the DVS was pre-programmed to increase RH in steps of 10 %, and allowed each of the samples to reach thermodynamical equilibrium with its ambient surroundings in the measurement chamber before a new step in RH was initiated. Starting from completely dry conditions at 0 % the RH was increased in steps up to 90 % and then back to 0 %. The constraint for the sample to be considered in its equilibrium state was that the mass rate was less than 20 ppm per minute over a time period of minimum 10 min. All samples were investigated one by one, and each measurement run lasted from 5 to 7 days.

3. Results
Figure 1 shows the time course for absorption (water gain) and desorption (water loss) for hair. The time courses of the two other sample types showed the same form. The alteration in sample weight corresponds to the water content relative to the dry weight. We note that the change in weight, initiated by an instantaneous step in RH, followed closely an exponential time course, indicating that the mass changed at a rate proportional to the difference between the final, equilibrium mass and the actual mass at the time of interest.

The relative water gains for the various samples as a function of RH in the DVS chamber are given in figure 2. The dotted lines correspond to absorption data, and the continuous lines to desorption data. We note the distinct differences between the different sample types: SC gained nearly no amount of water until 40 % RH was reached, thereafter gaining weight with an increasing rate as RH increased. Nail followed a similar curve as SC until RH was 50 %, but had a much lower water uptake at high RH. Hair, however, gained water quite rapidly at low RH, and had a more or less constant gain rate over the entire span of RH. Nail and hair had similarities in curve form, only with the hair data more elevated. Hysteresis was substantial for hair and nail, but for SC there were only little signs, if any at all, of hysteresis at RH lower than 30 %.

4. Discussion
In this paper we have found the sorption properties of SC, hair, and nail to be quite different, and also that there may be a substantial hysteresis. The hysteresis is large for hair and nail,
but very little for SC where it totally vanishes when RH is larger than about 30 %. This means that calibration of susceptance based hydration measurements, as described by Martinsen et al. [10], will end up with the same result for estimated absolute SC water content independently of whether absorption or desorption data are used as long as the RH is greater than roughly 30 %. For hair and nail, however, the large amount of hysteresis, that is the large gap between absorption and desorption data, will necessarily make such a calibration method ambiguous. The SC hysteresis data are in contradiction to similar results found by Polefka et al. who reported on significant hysteresis in porcine SC [12]. However, the porcine data were gathered using a larger value of the DVS mass rate that is used to establish the steady state sorption data at a given RH, and therefore the samples have less time to reach their thermodynamical equilibrium state before a step in the RH is initiated, which finally can be expected to result in substantial hysteresis.
The much lower water uptake of hair and nail compared to the SC is best interpreted as differences in the structure and water binding mechanisms of the constituents as well as the amount of lipids. Hair and nail cells are formed by restrictions imposed by the shape of their anatomical site, whereas SC cells do not suffer any restrictions at formation and are therefore free to swell [13, 14, 15].

Further knowledge on the water binding mechanisms and structure may be gathered from the time constants, that is the time needed for water to enter and leave the keratinized samples following a step in the RH. This should be a topic for future studies, and would be of interest for among others the differentiation process which is the overall description of the production process of keratinized tissues in the human integument, as well as for equivalent modeling of electrical properties of SC, hair, and nail.

5. Conclusions
This paper shows that hysteresis may be a complicating factor in calibrations of susceptance based hydration measurements of keratinized tissues. Also, SC, hair, and nail show very different sorption properties, which again may be expected to induce different electrical properties. The differences in sorption properties of the various keratinized sample types may be related to structural differences resulting in differences in water holding capacities.

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