pentapharm
benefiting society through science

Heiland electronic
Automatisierungs- und Messtechnik

Efficient and Simple Quantification of Stratum Corneum Protein on Tape Strippings

R Voegeli[1], J Heiland[2], S Doppler[1], AV Rawlings[3], T Schreier[1]

Introduction

Tape stripping is established as a common technique for sampling or evaluating the function of the stratum corneum in dermatological research and is used in a broad range of applications. However, a concurrent colorimetrical determination of protein content and enzyme activity on the same tape is difficult. Cutting the adhesive tapes in halves and measuring either value on each half is time consuming and prone to error. This is also the case by weighing the tapes for protein determination [1]. A promising indirect method is measuring the protein content by optical absorption. A spectrophotometer with a broad slit is needed for levelling the inhomogeneity of protein distribution on the tape strippings [2], [3]. The instruments available today are rather expensive and the handling of the sticky tapes in the measuring zone is tricky. Therefore a compact infrared densitometer was developed allowing a fast, user friendly and reliable protein determination on tape strippings.

Methods

12 healthy Caucasian subjects (6 female, 6 male, skin type II - III, age 27 - 50 y, mean 38.8 y ± 9.0) participated in the study. Before conducting the sequential tape stripping (20 times) on the ventral forearm, with men additionally being sampled on the shoulder, skin hydration and pH were measured with NOVA DPM 9003 (NOVA Technology) and Skin-pH-Meter® PH 905 (Courage & Khazaka electronic), respectively.

The absorption of the tape strippings was determined with a novel, compact infrared densitometer (Figure 1) especially designed for the application of standard D-Squame® disks (Ø 22mm) (CuDerm Corporation). This instrument is equipped with a diode emitting light with a wavelength of 850 nm which prevents the thermal denaturation of biomolecules. The diameter of the circular slit is 13 mm corresponding to 1.3 cm², covering 35% of the area of a standard D-Squame® disk. The disks can be placed easily and user-friendly, adhesive side up, onto a well accessible, round, horizontal measuring area of the densitometer. A constant absorption value is reached ten minutes after the stripping.

After solubilization of the adhered corneocytes for 60 min at 37°C in 1M NaOH, the protein content of each disk was repeatedly determined in 96-well microplates with the Micro BCA Protein Assay Reagent Kit (Pierce Biotechnology). Bovine IgG was used as the standard.

A general calibration curve demonstrating optical absorption vs. protein content was established. Moreover influences of gender, age, skin hydration rate, skin pH and different skin areas were examined.

Conclusion

The novel infrared densitometer is a suitable tool for the convenient measurement of optical absorption of stratum corneum strippings which was shown to be linearly proportional to their protein content. Although the data distribution in the different subject groups varies, the regression is always quite similar and is independent of gender, age, skin hydration rate, skin pH and varying skin areas of the tested subjects. The correlations reached are statistically highly significant. Thus the corresponding calibration curve can be used for a fast indirect protein evaluation of tape strippings by absorption measurement. Moreover the tapes can subsequently be utilized for determination of enzyme activities or any other bioassay.

Figure 1: The novel compact densitometer especially designed for a convenient measure of optical absorption of standard D-Squame® strippings (Ø 22mm).

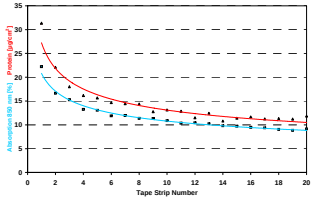

Figure 2: Mean protein and absorption values of sequential tape strippings.

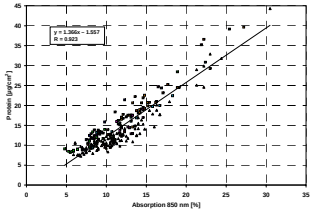

Figure 3: Overall regression of strippings on ventral forearm (males: ■, females: ▲), each color refers to another subject.

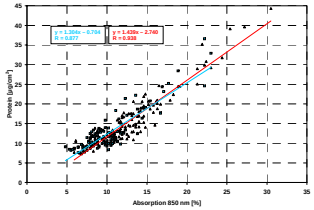

Figure 4: Strippings of subjects with more hydrated skin (■) and less hydrated skin (▲).

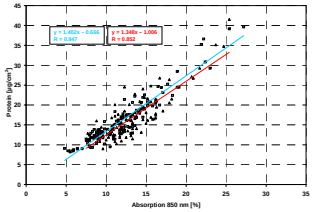

Figure 5: Strippings of different body sites of male subjects (shoulder: ■, forearm: ▲).

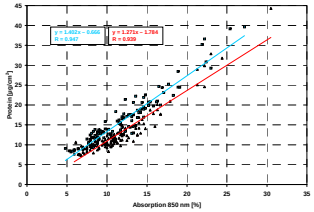

Figure 6: Strippings of males (■) and females (▲).

	Overall	Gender		Age		Hydration		pH		Skin Area Male	
Group	n.a.	female	male	younger	older	lower	higher	lower	higher	Forearm	Shoulder
mean ± SD	38.8y ± 9.0	33.8y ± 6.0	43.8y ± 9.1	31.2y ± 4.7*	46.5y ± 4.1*	96 ± 3	132 ± 59	5.49 ± 0.19*	5.73 ± 0.07*	n.a.	n.a.
Correlation	0.923	0.939	0.947	0.944	0.913	0.938	0.877	0.893	0.946	0.947	0.852
Regression	1.366x - 1.1.557	1.271x - 1.784	1.402x - 0.666	1.465x - 1.966	1.213x - 0.603	1.408x - 2.740	1.304x - 0.704	1.237x - 0.170	1.453x - 2.515	1.402x - 0.666	1.348x - 1.006

Table 1: Overall and grouped values, strippings 1 – 20 on forearm; last column on forearm and shoulder (* p < 0.05).

Strip No.	Absorption (Mean ± SD)				Protein (Mean ± SD)			
	Overall	More Hydrated Group	Less Hydrated Group	p	Overall	More Hydrated Group	Less Hydrated Group	p
1	22.2 ± 3.9		20.0 ± 2.8	24.9 ± 3.5	0.150	31.3 ± 7.5	27.4 ± 4.7	36.1 ± 5.6	0.085
2	16.6 ± 5.1	13.4 ± 3.1	19.9 ± 4.7	0.000	22.1 ± 8.3	17.2 ± 5.6	27.9 ± 8.6	0.008
3	15.3 ± 4.3	12.6 ± 2.6	17.7 ± 4.3	0.110	18.0 ± 6.2	14.3 ± 4.7	21.6 ± 5.7	0.006
4	13.2 ± 3.5	10.3 ± 2.0	15.6 ± 2.8	0.000	16.2 ± 4.6	14.0 ± 3.6	18.3 ± 4.7	0.048
5	13.1 ± 3.1	11.0 ± 2.0	15.1 ± 2.3	0.007	15.7 ± 4.4	13.6 ± 3.4	18.2 ± 3.9	0.013
6	11.9 ± 2.7	10.0 ± 2.2	13.7 ± 1.9	0.000	14.7 ± 3.6	12.7 ± 3.2	16.7 ± 2.8	0.009
7	12.0 ± 2.4	10.3 ± 1.7	13.6 ± 1.8	0.001	14.4 ± 3.4	12.7 ± 2.7	16.2 ± 3.4	0.018
8	11.4 ± 3.1	9.5 ± 2.7	13.3 ± 2.2	0.007	14.4 ± 4.4	12.7 ± 4.9	16.1 ± 3.5	0.083
9	11.3 ± 2.7	9.8 ± 1.8	13.2 ± 2.3	0.002	12.8 ± 4.0	10.3 ± 2.0	15.3 ± 3.9	0.002
10	10.9 ± 2.6	9.4 ± 1.7	12.4 ± 2.6	0.006	13.1 ± 4.1	10.2 ± 3.5	14.1 ± 4.7	0.142
11	10.3 ± 2.3	9.2 ± 2.1	11.5 ± 2.0	0.003	12.9 ± 3.6	11.5 ± 3.3	14.3 ± 3.7	0.065
12	10.4 ± 2.3	9.1 ± 1.8	11.7 ± 2.1	0.000	11.5 ± 3.1	10.1 ± 1.7	12.9 ± 3.7	0.169
13	10.2 ± 2.4	8.8 ± 2.0	11.7 ± 2.0	0.119	12.4 ± 3.2	10.5 ± 1.3	14.4 ± 3.4	0.038
14	9.8 ± 2.5	8.7 ± 2.2	10.9 ± 2.4	0.164	10.8 ± 2.1	9.9 ± 2.0	11.7 ± 1.9	0.096
15	9.7 ± 2.1	8.5 ± 1.7	11.0 ± 1.7	0.007	11.4 ± 2.5	10.3 ± 1.5	12.4 ± 3.0	0.141
16	9.5 ± 2.4	8.6 ± 2.0	10.3 ± 2.6	0.206	11.7 ± 2.7	10.2 ± 2.3	13.2 ± 2.4	0.007
17	9.4 ± 2.6	8.3 ± 2.1	10.4 ± 2.8	0.213	11.3 ± 3.4	10.4 ± 1.5	12.1 ± 4.6	0.403
18	9.0 ± 2.4	8.5 ± 1.9	9.6 ± 2.9	0.464	11.3 ± 3.3	10.1 ± 1.8	12.6 ± 4.0	0.183
19	8.8 ± 2.1	7.8 ± 1.3	9.9 ± 2.2	0.134	11.2 ± 2.2	10.0 ± 0.9	12.4 ± 2.6	0.078
20	9.2 ± 2.0	8.8 ± 1.1	9.5 ± 2.5	0.602	11.8 ± 2.7	10.9 ± 1.5	12.6 ± 3.4	0.124

Table 2: Absorption at 850 nm [%] and protein content [µg/cm²] on forearm overall and of more hydrated and less hydrated group, yellow lighted: p < 0.05.

Results

The progression of absorption and protein curves runs parallel, descending with increasing tape strip number (Figure 2). The overall correlation between absorption and protein content of forearm measurements is 0.923 and the corresponding overall regression is 1.336x - 1.557 (Figure 3).

Table 1 summarizes the data of strippings 1-20 of the different subject groups. Interestingly the only statistically significant differences between groups in context to the tape strip number and the absorption and protein values respectively could be seen in the groups with more and less skin hydration. Most of the first ten strippings were statistically significant different (Table 2). Although both groups showed a similar regression, the data distribution was different. The strippings of subjects with less hydrated skin tend to show more protein and a higher absorption compared to subjects with more hydrated skin (Figure 4).

Under the chosen test conditions skin pH, age (figures not shown) and different skin areas (forearm, shoulder) (Figure 5) do not influence either the regression or the distribution results of absorption and protein values. However, a clustering is obvious comparing the distribution of strippings on female and male forarms. At comparable protein concentrations females tend towards higher absorption values (Figure 6).

References

1. F Dreher, BS Modjtahedi, SP Modjtahedi, HI Maibach: Quantification of Stratum Corneum Removal by Adhesive Tape Stripping by Total Protein Assay in 96-Well Microplates Skin Research and Technology 11:97-101, 2005
2. HJ Weigmann, U Lindemann, C Antoniou, GN Tsikrikas, AI Stratigos, A Katsambas, W Sterry, J Lademann: UV/VIS Absorbance Allows Rapid, Accurate, and Reproducible Mass Determination of Corneocytes Removed by Tape Stripping Skin Pharmacology and Applied Skin Physiology 16:217-227, 2003
3. A Bornkessel, M Flach, M Arens-Corell, P Elsner, JW Fluhr: Functional Assessment of a Washing Emulsion for Sensitive Skin: Mild Impairment of Stratum Corneum Hydration, pH, Barrier Function, Lipid Content, Integrity and Cohesion in a Controlled Washing Test Skin Research and Technology 11:53-60, 2005

[1] Pentapharm Ltd., Basel, Switzerland
[2] Heiland Electronic GmbH, Wetzlar, Germany
[3] AVR Consulting Ltd., Northwich, UK

AVR Consulting Ltd
Independent Consultant For The Personal Care Industry