Tape stripping on a human nail: quantification of removal

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Background/purpose: Tape stripping is commonly used to investigate the stratum corneum (SC). This study assesses if protein quantitative tape stripping method was suitable for human nails.

Method: We used a colorimetric method to quantify proteins removed by the tape. Water barrier functions as a result of tape stripping were also observed by changes in transonychial water loss (TOWL) from the baseline.

Results: Using tape stripping, we observed no difference between nails in the protein quantity removed by tape stripping (P = 0.39). The mean TOWL before and after tape stripping were 6.9 and 9.3 g/m²/h, respectively; this was significantly increased in tape stripped nails (P < 0.0001).

Conclusion: Tape stripping seems to be an effective method to extract proteins from human nail plate and may aid the study of nail structure and function. Further studies are needed to extend our results in terms of age, gender, ethnicity and disease.

Key words: nail – tape stripping – protein – colorimetry

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The stratum corneum (SC) is recognized as a main skin barrier to solute penetration including water loss. Tape stripping is commonly used in dermatology to investigate SC structure and function (1), skin absorption (2–4) and to estimate topical bioequivalence (5, 6).

Weighing can be used to quantify SC by tape stripping. However, this method is laborious, rather time-consuming and sensitive to humidity and other factors (7). Alternative methods include protein quantification by spectrophotometry (7), spectroscopy (8) and microscopy (9, 10). Dreher and coworkers (11, 12) proposed colorimetry assessment of proteins dissolved from tape strips as a reliable method and demonstrated that this method is also feasible with 96-well microplates, which considerably shortens analysis (13).

Few studies have documented human nail permeability (14–21). Germann et al. first described tape stripping on a nail to remove nail corneocytes (22).

Transepidermal water loss (TEWL) is commonly used to assess SC integrity and is an indicator of skin water permeability. In analogy to TEWL, so-called transonychial water loss (TOWL) can also be measured (14–18, 23).

This study assessed whether nail tape stripping is also a suitable method to remove nail sequentially layers as assessed by protein extraction of human nails. Thereby, the colorimetric method described by Dreher et al. to quantify protein removed by tape stripping was used. Changes in water barrier function as a result of tape stripping were also measured by TOWL.

Materials and Methods

Nails

Five healthy volunteers (designated as volunteer A, B, C, D and E), with normal nail plates: two males, three females, 40.4 ± 19.3 years (ranging from 28 to 74 years old), participated after giving informed consent. Before the study, the volunteers washed their hands with soap. Five nails of the right hand were tape stripped and those of the left hand served as a control.

This study was approved by the University of California, San Francisco Committee of Human Research.

Stripping

Adhesive tape: D-Squame discs (CuDerm Corporation, Dallas, TX, USA) with a diameter of 2.2 cm (area of 3.8 cm²) were placed on the nails plate and immediately peeled off. Twenty strips
per nail were collected. To avoid the removal of proteins from proximal nail folds and hyponychium, a template, with a central window made with an 8-mm biopsy punch, was placed onto the nail plate before stripping. The nail surface was standardized at 2.01 cm².

**TOWL**

TOWL was measured using the Vapometer® evaporimeter (Delfin Technologies Ltd, Kuopio, Finland) following the guidelines of the Group of the European Society of Contact Dermatitis (24). To standardize the nail surface area, we used a perforated tape as a template (D-Squame® discs with an area of 0.785 cm²).

Only the investigator and the subject were present in the room while the measurement was being performed. The mean ambient air humidity was 38% and the temperature was 21°C.

TOWL was measured in triplicate before and immediately after tape stripping.

**Protein assay**

The following materials were used: sodium hydroxide (NaOH), hydrochloric acid (HCl) and water (high-performance liquid chromatography quality water) were obtained from Fischer Scientific®, Fairlawn, NJ, USA. The Bio-Rad DC protein assay® kit (Bio-Rad®, Hercules, CA, USA) included reagent A (an alkaline copper tartrate solution), reagent B (dilute Folin reagent) and reagent S (sodium dodecyl sulphate solution). Bovine serum albumin standard (BSA) was also obtained from Bio-Rad®.

**Protein quantification**

A colorimetric method (based on the Lowry assay (25)) was performed by treating the nail keratin tape strips with a 1 M NaOH solution as first described by Dreher et al. (11).

Tapes were then placed in 20 mL plastic scintillation vials (Wheaton, Millville, NJ, USA) with the adhesive side facing the screw caps. Then, 1 mL of 1 M NaOH was added in order to dissolve the nail keratin. The vials were shaken for 2 h on a Gyrotary® shaker model G76 (New Brunswick Scientific Co, Edison, NJ, USA) before the solution was neutralized with 1 mL of 1 M HCl because the assay is not compatible with alkaline conditions. The Bio-Rad DC protein microassay procedure was performed by adding 100 μL of reagent A and 800 μL of reagent B to 200 μL of sample. Spectrophotometrical analysis was performed at 750 nm on a SP-830 Plus spectrophotometer (Barnstead/Turner®, Barnstead International, Dubuque, IA, USA) using 1.5 mL quartz cuvettes (Sigma spectrophotometer cuvet®, Sigma Chemical Co, St Louis, MO, USA). Water was used as control.

**Calibration curve**

A calibration curve relating keratin protein concentration to optical density at 750 nm was established using eight dilutions of BSA protein standard from 0 μg/mL (tape alone) to 200 μg/mL. Dilutions were assayed using the protein assay as described above. The calibration curve was made in triplicate.

An analysis of variance of the regression was performed.

Protein removed by tape stripping of nail was based on using the following calibration curve:

\[ \mu g \text{ protein/mL} = (OD_{750} - 0.00624)/0.002. \]

**Statistical analysis**

Statistical analysis was performed using Sigmastat® (SPSS Science, Chicago, IL, USA) with the Mann–Whitney test to analyse the evolution of the TOWL before and after tape stripping, and one-way ANOVA for protein analysis.

**Results**

**Calibration curve**

Figure 1 shows calibration curves for BSA with and without tape. Analysis of variance of the regression showed no significant difference between both curves. Table 1 summarizes the slopes, intercepts and 99% confidence interval of the linear best fits for both calibration curves. A slope (0.0021 and 0.0020 for BSA and BSA with tape respectively) and determination coefficient higher than 0.99 appear to be appropriate for this experiment.

**Protein removal**

Table 2 summarizes the protein mass removed from each volunteer’s nail and the TOWL difference before and after tape stripping and Fig. 2...
represents protein removed by tape stripping (mg/cm²) from each nail. More protein was removed from volunteer D but the difference was not statistically significant (P < 0.39), possibly due to the small number of subjects. Except for the fifth finger, the tape stripping did not remove any protein from volunteer E.

Figure 3 represents the total protein removed from all nails (we excluded volunteer E because no proteins were removed from four of five of his nails). We observed no difference between nails in the quantity of protein removed by tape stripping (P = 0.39).

TOWL
The mean TOWL ± standard deviation (all volunteers, all right-hand nails data) before and after tape stripping was 6.9 ± 2.7 and 9.3 ± 3.2 g/m²/h, respectively. The TOWL immediately after tape stripping (Fig. 4) was significantly increased in tape-striped nails (P < 0.0001). There was no significant difference before and after tape stripping for the control nails (P = 0.67).

Discussion
The human nail plate, composed of approximately 25 layers of flattened, keratinized cells with a typical thickness of 0.5–1.0 mm, resembles the SC but is approximately 100-fold thicker (26). Few studies have documented nail function due to the difficulty of working with a relatively rigid membrane.

The use of tape stripping led to removal of proteins from the nail plate of four of five volunteers’ nails. We lack an explanation regarding the absence of protein removal from four of five fingers of volunteer E. There are no universally accepted methods for tape stripping, and the influence of procedure on tape stripping has been well described (27, 28). There remains a need for an internationally standardized method. As the nail plate proteins are specialized keratin (23), the calibration curve might utilize this keratin rather than the BSA utilized here.

Germann observed significantly higher corneocytes in the nails of aged subjects (22); this might be the case for nails as volunteer D was the oldest subject.

The mean TOWL (all volunteers, all right-hand nails data) before tape stripping was 6.9 g/m²/h, a result lower than others studies. Jemec’s median TOWL was 19.4 g/m²/h in normal nails (15) and Spruit found a mean TOWL of 13 g/m²/h (16). Working on diseased nails (eczema, psoriasis and onychomycosis), Krönauer estimated a mean TOWL of 12.9 g/m²/h (14). Burch and Windsor (17) found lower values but also important flux variations (1.6–53 g/m²/h) depending on the temperature and humidity.
A standard TOWL calibration, not currently available, would make such a comparison more meaningful.

The TOWL right after tape stripping was significantly higher ($P < 0.0001$). As we removed proteins, this result was expected. Our measurements were made immediately after the tape stripping; perhaps the increase may be greater if repeated later.

**Conclusion**

Tape stripping seems to be an effective and practical method to quantify extract protein nail...
layers via the protein method. Further studies with larger numbers are needed to extend our results in terms of age, gender, ethnicity and disease.

Onychomycosis topical therapy has been minimally effective, possibly due to the poor penetration of substances through the nail plate. Tape stripping could be a useful adjunct of drug penetration through nails – in that more permeable nails might allow a greater flux, as may be the case for human skin (29).

References
stripping in combination with optical spectroscopy in the visible range as prerequisite to quantify percutaneous absorption. Skin Pharmacol Appl Skin Physiol 1999; 12: 34–45.


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