Journal of Dermatological Science (2006) 44, 81—92

ELSEVIER

Journal of Dermatological SCIENCE

www.intl.elsevierhealth.com/journals/jods

Analysis of RNA recovery and gene expression in the epidermis using non-invasive tape stripping

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Received 18 May 2006; received in revised form 4 August 2006; accepted 14 August 2006

KEYWORDS
Epidermis;
Gene expression;
Stratum corneum;
Tape stripping;
Transepidermal water loss

Summary

Background: The recovery of RNA from the upper epidermis by tape stripping yields variable RNA mass but has not been evaluated for its dependence on anatomical location. Gene expression at different body locations and the origin of RNA recovered by tape stripping have not been investigated.
Objectives: To characterize the recovery of RNA from different anatomical locations by tape stripping; to correlate the recovery of RNA and removal of barrier by tape stripping, as assayed by transepidermal water loss; and to investigate gene expression in the upper epidermis at different body locations.
Methods: Twelve subjects were tape stripped at 15 body locations. RNA mass was evaluated and gene expression assayed. Subjects were tape stripped 4, 8 and 12 times on the upper back and transepidermal water loss and RNA recovery assayed.
Results: Ranked by median RNA recovery, the following order was observed: mastoid > forehead > chest > upper back > mid back > cheek > lower back > deltoid > forearm > abdomen > ventral thigh > inner arm > shin > dorsal thigh > lower leg. Expression of the housekeeping gene mRNAs is found to be uniform and reproducible while IL-8 and TNFα mRNAs are expressed in different quantities both at different body sites within an individual and between individuals at a specific anatomical site. Data show a significant and high correlation between the number of tapes used to strip a site and transepidermal water loss but no strong correlation between transepidermal water loss and RNA recovery or number of tapes used to strip a site and RNA recovery.
Conclusions: Subjects and anatomical location are shown to be significantly different for the ability to recover RNA by tape stripping. We hypothesize that RNA recovered by tape strip is not derived from corneocytes but from cells associated with the stratum corneum.

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0923-1811/$30.00
doi:10.1016/j.jdermsci.2006.08.007

1. Introduction

Tape stripping is a widely employed method in experimental dermatology. Uses for the technique include: studying the role of the stratum corneum (SC) in barrier function, *in vivo* [1—3]; as a non-invasive method of recovering SC for assessing percutaneous absorption [4—7]; and mimicking injury and irritation without the use of chemicals [8—10].

The use of tape stripping as an analytical or quantitative tool for percutaneous absorption looked initially promising [11] but the uneven nature of the SC and variable removal of SC with each tape strip created an uncertainty in the method that led to doubt as to its potential as a reliable and quantitative technique [5,12,13]. Most of the deficiencies in the use of tape stripping as a pharmacokinetic tool lay in the difficulty in defining an appropriate method of normalizing data that made drug measurements independent of the absolute mass of stratum corneum recovered with the tape.

Morhenn et al. [14] made an important innovation to the tape stripping method when they showed that RNA could be recovered from cells adherent to the tape. The authors showed that RNA recovered from normal, sodium laurel sulfate (SLS)-irritated skin (simulating irritant contact dermatitis) and nickel sulfate-treated skin (simulating allergic contact dermatitis) could be differentiated based on expression of the IL-4 and IL-8 mRNAs. Expression of these mRNAs in each sample was normalized to GAPDH, thus accounting for the variable recovery of RNA in individual samples.

Wong et al. [15] further improved tape stripping by reducing the number of tapes required to strip a site from greater than 20 to 4, and demonstrating that RNA recovered could be quantified by RT-PCR and profiled by DNA microarrays. In addition, by characterizing tape strip recovered RNA from SLS-treated, water-treated and normal skin by microarray, Wong et al. revealed hundreds of novel, differentially expressed mRNAs involved in the response to SLS-irritation. The utility of the improved technique was highlighted by the fact that of the 100 most differentially expressed mRNAs ($p < 10^{-9}$), 60% were previously identified in the literature as being involved in inflammation and wound healing, while the remaining genes were novel to contact irritant dermatitis. Thus it was clearly shown that RNA recovered by tape stripping could accurately convey the real time physiology of the skin and could be used to derive differential expression profiles of inflamed skin.

Recently, Benson et al. [16] described the use of tape stripping to sample psoriatic lesions. This group demonstrated that tape strip recovered RNA was just as effective at revealing increases in pathogenic mRNAs associated with psoriasis as biopsy captured RNA samples.

In spite of these advances in the technique, several questions linger including: (i) the reproducibility of recovering RNA at anatomically similar sites in different individuals (subject variation); (ii) the role of different anatomical sites in yielding RNA (intra-subject variation); (iii) the reproducibility of gene expression assays at different body locations and in samples with vastly different RNA mass content. In this work we describe the recovery of RNA at different body locations and the relationship of tape treatments (number of tapes used to strip a site) to RNA mass recovery and transepidermal water loss. In addition we quantify the relative expression of several housekeeping and inflammatory genes at different locations on the body.

2. Materials and methods

2.1. Clinical protocols

Clinical protocol #1 was compromised of 25 subjects; 13 females between the ages of 22 and 64 and 12 males between the ages of 18 and 60. Subjects were in general good health and were excluded if presenting active, clinically significant dermatitis or skin diseases or were excessively tanned at the sites to be stripped. Subjects were tape stripped with 4, 8 and 12 tapes in duplicate (left and right sides of the body with sites arranged in columns and randomized with respect to tape treatment) at the volar forearm, upper back and deltoid. Sites were cleansed with an alcohol wipe prior to tape stripping and clipped or lightly shaved to remove non-vellus hairs. Each tape was applied to the site with firm pressure and briskly rubbed with 15 circular motions covering the entire surface area of the tape. Tapes were removed from the skin and placed in a plastic envelope that was kept on ice until the procedure was competed and then stored at −80 °C until extraction. Clinical protocol #2 enrolled 12 subjects, 11 of whom had completed the study above, which had occurred approximately 6 months earlier. These subjects were tape stripped at the following 15 body locations; upper back (immediately above scapular spine; approximately 6 cm away from vertebral column); mid back (lower trapezius, approximate level of T6—T9 about 3 cm away from vertebral column); lower back (approximate level of T10—T12; about 7—8 cm away from vertebral column); deltoid (lateral upper arm at the insertion of the deltoid muscle over the deltoid tuberosity); upper inner arm; medial upper arm (slightly anterior

of the triceps); forearm (inside of forearm, volar; about 5 cm below the elbow); ventral thigh (approximately 12 cm above the patella); dorsal thigh (dorsal part of thigh, approximately 12 cm above the intercondylar fossa); shin (13 cm below the patella); lower leg (dorsal part of leg, approximately mid to upper calf between the heads of the gastrocnemius); chest (about 2.5 cm below the jugular notch and mid body to manubrium of sternum; abdomen (3 cm above the umbilicus); mastoid (directly on mastoid process); cheek (below cheek bone; approximately 2.5 cm below the eye and 2.5 cm from the nose); forehead (above the eyebrow). Sites were tape stripped in duplicate (left and right side), with four and eight tapes per site (exceptions were the cheek and forehead which were tape stripped with only two tapes) as described above. In addition, subjects were tape stripped 12 times on the upper back and transepidermal water loss (TEWL) was assayed at the 4, 8 and 12 tape strips, upper back sites (duplicate measurements, left and right). Both studies were approved by the local Institutional Review Board (Biomed IRB, San Diego, CA) and all patients signed informed consent. The studies were conducted according to the Declaration of Helsinki principles.

2.2. TEWL assay

Subjects were equilibrated for 30 min in a controlled environment of 30—50% humidity and 70 ± 3 °F. TEWL measurements were taken with a Tewameter TM30 (Courage & Khazaka, Cologne, Germany). Baseline TEWL readings were taken at the six upper back sites prior to tape stripping; TEWL positioning rings were used so that the probe did not contact the skin. After baseline measurements were taken sites were tape stripped 4, 8 or 12 times and TEWL measurements retaken.

2.3. Materials and reagents

Adhesive tape was purchased from Adhesives Research (Glen Rock, PA) in bulk rolls. These rolls were custom fabricated into small circular discs, 17 mm in diameter, by Diagnostic Laminations Engineering (Oceanside, CA). Human spleen total RNA was purchased from Ambion (catalogue # 7970; Austin, TX). RNeasy RNA extraction kit was purchased from Qiagen (Valencia, CA). Reverse transcriptase, PCR primers and probes, and TaqMan Universal Master Mix, which included all buffers and enzymes necessary for the amplification and fluorescent detection of specific cDNAs, were purchased from Applied Biosystems (Foster City, CA).

2.4. RNA isolation

RNA was extracted from tapes using pressure cycling technology (PCT [17,18]). Tapes were extracted in pairs by insertion into a PULSE^{TM} tube (Pressure Biosciences, Gaithersburg, MD) with 1.2 ml of buffer RLT (supplied in the Qiagen RNeasy kit). PULSE^{TM} tubes were inserted into the PCT-NEP2017 pressure cycler and the sample was extracted using the following parameters: room temperature; five pressure cycles of 35 kpsi with pressure held for 20 s at the top and bottom of each cycle. After pressure extraction the buffer was removed and used to process the remaining tapes used to strip a specific site; the buffer was then processed according to the standard Qiagen RNeasy protocol. The RNA from the 4, 8 or 12 tapes used to harvest a specific site was pooled to create a single sample.

2.5. Reverse transcription and amplification/detection

Five microlitres of RNA was reverse transcribed (RT) into cDNA with the MultiScribe Reverse Transcriptase kit (Applied Biosystems, Foster City, CA) using random hexamer primers in a final volume of 20 μl according to the manufacturer's directions. The reaction was diluted five-fold with sterile, nuclease-free water for use in the subsequent amplification/detection reaction. For each specific mRNA detection, three replicate RT^+ reactions and one RT^- (no reverse transcriptase; negative control) reaction were performed. Two amplification/detection reactions were done on each RT^+ reaction to yield a total of six independent determinations of the threshold value (C_t; discussed below). Amplification and detection assays were performed using TaqMan Gene Expression Assays (Applied Biosystems); specific probe and primer sets are: β-actin (human beta actin, 4326315E); GAPDH (human glyceraldehyde phosphate dehydrogenase, 4326317E); hARP (human acidic ribosomal protein RPLP0, 4326314E); ATP5I (ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit e, Hs00 273015_m1); CDH1 (E-cadherin, Hs00170423_m1); K16 (keratin 16, Hs00373910 _g1); IL-8 (interleukin 8, Hs99999034_m1); TNFα (tumor necrosis factor, TNF superfamily, member 2, Hs99999043_m1); VEGF (vascular endothelial growth factor, Hs00173626_m1). All amplification/detection assays were performed on an Applied Biosystems 7900HT Sequence Detection System as previously described [15]. All RT^- reactions were amplified using two replicates and were negative.

2.6. Quantitation of RNA and determination of fold-change

Quantitation of RNA mass was performed as previously described [15]. Briefly, RNA mass recovered from tapes and biopsies is determined by using quantitative RT-PCR with reference to a standard curve [19] ($C_{t,actin}$ versus log[RNA]) created from commercially purchased human spleen total RNA. We have shown that quantitation of RNA purified from skin punch biopsy by both OD_{260} and the above PCR method produce identical results suggesting that the PCR method is a good indicator of RNA recovered from tape stripping (data not shown). Amplification and detection of unknowns was accomplished using β-actin mRNA as the quantified marker. The average of six replicate $C_{t,actin}$ values was used to calculate the concentration of RNA in a sample with reference to the standard curve. The calculation of change in gene expression was determined using the comparative or $\Delta\Delta C_t$ method as described by Wong et al. [15]. Experimental data is reported as the number of PCR cycles required to achieve a threshold fluorescence for a specific cDNA and is described as the "C_t" value [19–21]. Each reported C_t is the mean of six replicate assays. Gene specific data is normalized to β-actin in each sample by determining the $\Delta C_{t,gene}$ value which is defined as $C_{t,gene} - C_{t,actin}$ (this normalization accounts for assaying differing masses of RNA in a quantitative PCR assay). Individual ΔC_t values were calculated from data captured during the same amplification/detection assay (i.e. paired gene and actin C_t values determined during same experiment). ΔC_t data for each sample is further normalized by comparison with a control sample, a process called "calibration" [19]. Calibration provides the relative fold-change of a (gene/actin) mRNA ratio in a sample of interest relative to a control sample. The value for fold-change in one sample calibrated to a control sample is given by the equation below (using the TNFα/actin mRNA ratio in the upper back relative to control as an example):

$$\frac{(\mathrm{mRNA_{TNF}}/\mathrm{mRNA_{actin}})_{\text{upper back}}}{(\mathrm{mRNA_{TNF}}/\mathrm{mRNA_{actin}})_{\text{control}}} = 2^{-\Delta\Delta C_{t,\text{upper back}}}$$

where $\Delta\Delta C_{t,\text{upper back}} = \Delta C_{t,\text{upper back}} - \Delta C_{t,\text{control}}$; and $\Delta C_{t,\text{upper back}} = (C_{t,TNF} - C_{t,actin})_{\text{upperback}}$; and $\Delta C_{t,\text{control}} = (C_{t,TNF} - C_{t,actin})_{\text{mastoid}}$; subscripts after parentheses indicate the source of the RNA sample. In this work the control sample is from the mastoid process (Table 7).

2.7. Statistical analyses of RNA mass data

Clinical protocol 1: RNA mass data was tested for normal distribution and found not to be normally distributed. We therefore defined a variable Y = RNA mass + 0.01 so that samples that yielded no RNA could be included in the analyses. The resulting log-transformed data was normally distributed. This log-transformed data was analyzed using the general linear model approach with subjects, tape treatments and body sites as class variables along with two- and three-factor interactions using SAS version 9.1. Clinical protocol 2: mass data for recovery of RNA at 15 body locations was found to be non-normally distributed; similar to Study 1 above, a new mass variable Y = RNA mass + 0.01 was defined and log-transformed data was found to be normally distributed. Analyses of the log-transformed data for homogeneity of variances (Levene's test) showed significant heterogeneity, we therefore decided to carry out non-parametric analyses using Friedman's two-way ANOVA to evaluate RNA recovery at all 15 sites. In order to perform pairwise comparisons of selected sites (Table 4) we used paired samples with the exact sign test and Monte Carlo simulations. The resulting *p*-values were arranged in decreasing order and the significance of each *p*-value calculated using Hochberg's stepwise procedure for multiple comparisons [22,23]. Analysis of upper back tape stripping treatments (4, 8 and 12 tapes) for RNA recovery and transepidermal water loss were carried out separately. Mass data was treated as described above using log-transformed data; TEWL data and its log transformation was found to be non-normally distributed, therefore that data was analyzed using Friedman's non-parametric two-way ANOVA.

2.8. Statistical evaluation of fold-change in gene expression

Wong et al. [15] have described in detail the evaluation of significance of changes in relative mRNA expression using the $\Delta\Delta C_t$ method. Briefly, to calculate whether a fold-change in the (gene/β-actin) mRNA ratio in a particular sample is significantly different than that in a calibrator sample, we calculate the 95% confidence interval for the fold-change using the $\Delta\Delta C_t$ value and its S.D. using the formula $2^{-(\Delta\Delta C_t \pm 2\text{S.D.})}$, if the resulting interval includes the value of 1, we conclude that the (gene/β-actin) mRNA ratio in that sample is not significantly different than that of the calibrator sample.

Table 1 Median and mean recovery of RNA from the forearm, deltoid and upper back in 25 subjects

Statistic[a]	RNA yield by location and number of tapes								
	Forearm			Back			Deltoid		
	4	8	12	4	8	12	4	8	12
Median	0.22	0.93	2.4	2.6	7.2	13	3	7.7	17
Mean ± S.E.M.	1.6 ± 0.79	10 ± 5	11 ± 3.6	15 ± 6	46 ± 12	91 ± 31	24 ± 7.2	58 ± 16	98 ± 36
Range	38	235	165	222	336	1110	238	564	1620

[a] Mean RNA mass ± S.E.M. is shown in nanograms; total number of samples at each site and tape stripping condition (number of tapes used per site, i.e. 4, 8, or 12) was 50 (25 subjects, left and right sides); range reports the difference between the maximum and minimum amount of RNA found in that sample set.

3. Results

3.1. RNA recovery at different anatomical locations

Previous work comparing RNA recovered by tape stripping and biopsy showed that tape stripping recovered variable amounts of RNA from the upper back of different individuals [15]. In addition, our unpublished data have shown that RNA recovery from tape strips is variable from tape to tape at a single site and from immediately adjacent sites on the same individual. To more thoroughly assess the recovery of RNA at similar locations on different individuals we tape stripped 25 subjects on the ventral forearm, upper back and deltoid muscle using 4, 8 or 12 tapes at each location.

Table 1 shows the median and average yield of RNA from 25 subjects using 4, 8 and 12 tapes at 2 symmetrical sites on the left and right side of the body. The data show a large inconsistency between the median and mean RNA recovery. Furthermore, there is no consistent relationship between the number of tapes used to strip a site and the recovery of RNA. Statistical analysis (two-way ANOVA with replication) of log-transformed data from each anatomical site showed that subjects, sites and tape treatments were significantly different from each other ($p < 0.0001$ in all comparisons). Pairwise comparisons of tape treatments and sites show that there were significant differences in RNA recovery between tape treatments and that significantly less RNA was recovered from the forearm compared to the back or deltoid (Table 2; recovery from the back and deltoid was not significantly different).

The data in Tables 3 and 4 demonstrate that subjects and at least two anatomical locations (deltoid and back versus forearm) are significantly different in terms of RNA recovery by tape stripping. To more fully investigate these differences, a subset of the subjects in this study were tape stripped again (6 months later) at 15 body locations using 4 and 8 tape strips.

Table 3 shows data for the recovery of RNA from 12 subjects at 15 body locations using 4 tape strips (exceptions were the forehead and cheek, which were stripped with 2 tapes; the 8 tape strip data are not shown). The data again reveal a considerable discrepancy between the median and average amounts of RNA recovered. The variability in mass recovery is also revealed in the range of RNA mass recovered from each site, this range is typically an order of magnitude larger than the average RNA recovery. The table shows that there are clear differences in median RNA recovery between different body locations. A statistic that most clearly defines differences between sites is the number of samples that contained no RNA. At 6 body locations, 29–58% of the 24 samples from each location did not contain detectable RNA (Table 3).

The median RNA recovery for the 15 sites shown in Table 3 was tested for significance with exact Friedman's test using Monte Carlo simulations. The result showed that some sites are significantly different than others ($p < 0.0001$). Rather than performing the exhaustive pairwise comparisons of all sites in Table 3 we chose to focus our analysis on the site that provided the highest median RNA yield (the mastoid) and compare that site with eight other sites. Those pairwise analyses are shown in Table 4. The analysis reveals that the mastoid is a

Table 2 Pairwise comparisons of tape treatments and anatomical locations for RNA mass recovery from 25 subjects at 3 different body sites

Variable	Comparison	*p*-Value[a]
4 tape strips	4 vs. 8	<0.00001
	4 vs. 12	<0.00001
8 tape strips	8 vs. 12	0.001
Forearm	Forearm vs. deltoid	<0.00001
	Forearm vs. back	<0.00001
Upper back	Back vs. deltoid	0.769

[a] Statistical methods are described in Section 2.

Table 3 Recovery of RNA from different body locations using tape stripping

Statistic[a]	RNA mass[b] and anatomical location							
	Mastoid	Forehead	Chest	Upper back	Mid back	Cheek	Lower back	Deltoid
Median	18.3	3.88	2.55	1.8	1.4	1.38	1.1	0.73
Average	46 ± 21	7.4 ± 1.8	68 ± 53.6	14 ± 7.7	2.5 ± 0.98	7.6 ± 3.5	1.9 ± 0.39	2.3 ± 0.66
$Y = 0$	0	0	0	1	2	1	0	3
Range	502	37.1	1270	179	24	77.6	7.34	12.1

	Forearm	Abdomen	Ventral thigh	Upper inner arm	Shin	Dorsal thigh	Lower leg
Median	0.47	0.42	0.21	0.09	0.075	0.02	0
Average	7.4 ± 3.8	2.7 ± 1.35	3.2 ± 1.4	0.59 ± 0.23	3.2 ± 1.5	2.6 ± 1.9	3.3 ± 1.7
$Y = 0$	7	3	8	11	11	12	14
Range	74.2	31.3	27.1	4.47	29.6	44.7	36.

[a] Median and average ± S.E.M. values are calculated from 24 samples (12 subjects, left and right sides); the statistic "Y = 0" reports the number of samples that had no RNA; range reports the difference between the maximum and minimum amount of RNA recovered at that site.

[b] RNA mass is reported in nanograms of total RNA; data is for the use of 4 tape strips at each site (exceptions were the cheek and forehead, which were stripped with 2 tapes).

significantly better source of RNA than six of those locations and likely better than the lower back as well. We could not demonstrate that the chest or mastoid sites were significantly better than one another. Because the yield of RNA from the mastoid is significantly higher than five of the other locations tested, it is clearly higher than the remaining locations in Table 3, which have less than 1 ng median RNA recovery and high numbers of samples with no RNA recovery. The fact that a specific site can be shown with confidence to yield more or less RNA than another site suggests that the very upper epidermis of those sites have intrinsically different RNA content.

3.2. TEWL, tape treatments and RNA yield

The recovery of RNA from the skin by tape stripping yields variable amounts of RNA from different areas of the body and similar anatomical locations yield differing amounts of RNA from individual to individual. If one assumes that tape stripping removed a generally consistent amount of stratum corneum with each tape application, the observation of highly variable RNA recovery implies that RNA is not a uniform component of the stratum corneum. We further questioned if tape treatment (number of tapes used to strip a site) were correlated with the amount of stratum corneum removed or with the mass of RNA recovered. As a proxy for the amount of stratum corneum removed by tape stripping we assayed transepidermal water loss (TEWL) after 4, 8 and 12 tape strippings of the upper back. Our hypothesis was that there would be a high and significant correlation between tape treatments, the amount of transepidermal water loss and RNA recovery.

Table 4 Pairwise comparisons of median RNA recovery at the mastoid process and select anatomical sites

Pairwise comparison	p-Value[a]	Significance[b]
Mastoid vs. abdomen	0.0002	Significant
Mastoid vs. deltoid	0.0005	Significant
Mastoid vs. forehead	0.0054	Significant
Mastoid vs. cheek	0.0056	Significant
Mastoid vs. mid back	0.0058	Significant
Mastoid vs. upper back	0.0066	Significant
Mastoid vs. lower back	0.0392	Not significant
Mastoid vs. chest	0.3923	Not significant

[a] The p-value is calculated using paired samples and the exact sign test with Monte Carlo methods.

[b] Significance of the p-value was determined using Hochberg's stepwise procedure for multiple comparisons [22,23].

Table 5 shows data for TEWL and number of tapes used to strip a site on the upper back. As expected, average TEWL and average RNA yield increase with the number of tape strips used at a site. Analysis of the number of tapes used to strip a site and TEWL showed that tape treatments were significantly different in their effects on TEWL ($p < 0.001$). The results of correlation tests between RNA yield versus TEWL, RNA yield versus tape treatments, and TEWL versus tape treatments are shown in Table 6. The table reveals a high and significant correlation between the number of tapes used to strip a site and TEWL from the same site. Table 6 also shows that the correlation between tape treatments and RNA recovery was poor, as was the correlation between TEWL and RNA recovery.

We interpret the high and significant correlation between the number of tapes used to strip a site and TEWL to imply that tape stripping removes a

Table 5 RNA mass recovery and transepidermal water loss resulting from 4, 8 or 12 tape strippings on the upper back

Statistic	RNA mass[a] and tape treatment			TEWL[b] and tape treatment		
	4	8	12	4	8	12
Median	2.32	11.4	13.2	6.7	34.4	62.5
Average	14.9 ± 8.39	16.3 ± 4.14	56.8 ± 19.6	9.17 ± 1.86	34.7 ± 3.71	58.8 ± 3.85

[a] RNA mass is reported in nanograms ± S.E.M. for 4, 8 and 12 tapes applied per site. Values are the average or median of 22 assays (11 subjects and duplicate stripping for each condition).
[b] The units of TEWL are given in g m^{-2} h^{-1}. Each value is the average or median of 22 assays per tape stripping condition (11 subjects, duplicate assays for each condition). Statistics are given for baseline subtracted readings; the average baseline reading was 16.0 ± 0.029 (N = 66).

relatively uniform amount of stratum corneum with each application. The observation that neither TEWL nor tape treatment are correlated with RNA recovery suggests that the RNA recovered by tape stripping is not a uniformly distributed component of the stratum corneum. From this data we conclude that the RNA recovered by tape stripping may not be derived from corneocytes but from other cells associated with the stratum corneum.

3.3. Gene expression at different body locations

If tape stripping is to be a useful method for recovering RNA from the skin, the reproducibility of gene expression assays must be defined in these samples, both with respect to intra- and inter-subject variation. In addition, because tape strip RNA samples can have wide ranges of RNA content, it is important to test that internal normalization to an unchanging housekeeping gene is indeed adequate and effective. In order to address these questions we assayed mRNA expression for several "housekeeping" genes as well as genes involved in the inflammatory response at 7 of the 15 locations for which we quantified RNA recovery.

We first consider expression of genes that would not be expected to vary over the surface of the body, i.e. "housekeeping" genes. These genes are GAPDH, hARP, ATP5I, CDH1 and K16. Table 7 shows the (gene/actin) mRNA ratio at a specific location relative to the mastoid process. This value for relative fold-expression would be equal to "1" (as it is for the mastoid sample, which is relative to itself) for all sites if gene expression were unchanged relative to the mastoid. The first row for each mRNA in Table 7 shows the average relative expression of the (gene/actin) mRNA ratio at a specific site calibrated to the mastoid process (calibration described in Section 2). The table shows that for these five mRNAs, most values of relative expression are close to 1 and not significantly different than expression at the mastoid.

An additional measure of variation can be determined by inspection of the width of the 95% confidence interval for relative fold-expression. The width of the 95% CI for fold-expression can be determined from the S.E.M.s of average ΔC_t values (Appendix 1, Table A1). That width can be expressed as a fold-expression with the lower limit of the interval expressed as "1", and the upper limit becoming the width of the interval. The higher the value of the width of the 95% CI, the more variability there is between individual (gene/actin) mRNA ratios. Inspection of the 95% CI width for the five housekeeping genes shows that there is relatively little variation in gene expression between different body locations. Thus, we conclude that RNA recovered by tape stripping can accurately and reproducibly report gene expression at different body locations.

Table 7 also shows the relative expression of mRNAs for the growth factor VEGF and the inflammatory cytokines IL-8 and TNFα. In contrast to the housekeeping gene data, the data for IL-8 and TNFα are strikingly different. Inspection of the (IL-8/actin) mRNA ratio reveals numbers ranging from a 2.8-fold decrease to an 8-fold increase relative to the mastoid samples. Inspection of the width of the 95% confidence interval for individual body locations shows high variation with the lowest interval being 7.5-fold range of expression and the highest being 110-fold. These data are in contrast to the housekeeping mRNA data which show values close to 1 for

Table 6 Correlation coefficients between TEWL, RNA yield and tape treatment for the upper back

	R[a]	t[b]	p[c]
TEWL vs. RNA	0.209	1.71	>0.2
TEWL vs. no. of tapes	0.804	10.8	<0.005
RNA vs. no. of tapes	0.283	2.36	<0.05

[a] Correlation coefficient.
[b] A t-statistic with $N-2$ degrees of freedom (N = 66) has been calculated to test the significance of the observed correlation coefficient using the formula: $T_{(N-2)} = R(N-2)^{0.5}/(1-R^2)^{0.5}$.
[c] Significance of R drawn from tables for two-tailed t-test.

Table 7 Relative expression of housekeeping and other genes at six anatomical locations relative to expression at the mastoid process

mRNA	Statistic[a]	Relative fold-expression and anatomical location[b]						
		Mastoid	Cheek	Forehead	Upper back	Deltoid	Forearm	Ventral thigh
GAPDH	Rel. exp.	1	1.1	1.2	1.1	1.5	1.4	1.3
	95% CI	0.76–1.3	0.8–1.5	0.92–1.7	0.78–1.6	0.99–2.2	1–1.9	0.82–2.1
	Width CI	1.8	1.9	1.8	2	2.2	1.9	2.5
	N	23	15	21	19	14	13	8
hARP	Rel. exp.	1	0.56	0.76	1.1	0.85	1.2	2.2
	95% CI	0.69–1.4	0.36–0.88	0.53–1.1	0.68–1.6	0.51–1.4	0.65–2.2	1.4–3.4
	Width CI	2.1	2.5	2.1	2.4	2.8	3.4	2.5
	N	24	17	22	21	16	13	8
ATP5I	Rel. exp.	1	0.86	1.1	1.4	1.2	1.1	1.1
	95% CI	0.83–1.2	0.67–1.1	0.89–1.3	1.1–1.8	0.96–1.6	0.9–1.4	0.72–1.6
	Width CI	1.3	1.6	1.5	1.7	1.7	1.6	2.2
	N	22	7	12	19	6	7	4
CDH1	Rel. exp.	1	0.9	0.9	1.1	1.4	1	0.7
	95% CI	0.84–1.2	0.58–1.4	0.63–1.3	0.89–1.4	1.1–1.9	0.72–1.5	0.33–1.5
	Width CI	1.4	2.4	2	1.5	1.8	2.1	4.4
	N	21	8	12	20	9	6	4
K16	Rel. exp.	1	1.2	1.4	1.1	1	1.1	0.99
	95% CI	0.64–1.6	0.81–1.8	0.84–2.3	0.7–1.6	0.49–2.1	0.66–1.9	0.45–2.2
	Width CI	2.4	2.2	2.7	2.3	4.3	2.9	4.9
	N	22	12	12	22	11	9	7
IL-8	Rel. exp.	1	6.8	2.1	0.46	8	0.51	0.35
	95% CI	0.29–3.4	1.1–41	0.32–15	0.17–1.3	1.8–34	0.049–5.2	0.11–1.1
	Width CI	12	36	46	7.5	19	110	10
	N	20	11	11	2	7	4	2
TNFα	Rel. exp.	1	2.8	2.8	1.5	13	0.59	–
	95% CI	0.53–1.9	1.4–5.7	0.53–15	0.33–6.8	–	0.22–1.6	–
	Width CI	3.6	4.2	28	21	–	7.6	–
	N	16	4	4	3	1	2	0
VEGF	Rel. exp.	1	1.8	2.7	1	0.72	0.59	0.5
	95% CI	0.64–1.6	0.69–4.5	1.5–4.9	0.6–1.8	0.34–1.5	0.34–1.1	0.3–0.86
	Width CI	2.5	6.6	3.2	3	4.5	3.14	2.9
	N	21	8	11	16	4	5	3

[a] Relative expression is calculated by calibration of anatomical locations to the mastoid process (which is defined as 1 as it is relative to itself) as described in Section 2 and using the data in Appendix 1, Table A1; the 95% confidence interval for relative fold-expression is calculated from the $\Delta\Delta C_t$ value and S.E.M. as described in Section 2; the width of the 95% CI is calculated from the formula $2^{4S.E.M.}$ where the S.E.M. is for the $\Delta\Delta C_t$ value; *N* is the number of samples in which the specific mRNA could be assayed (out of a total of 24 samples at each location).

[b] All data is from four tape strip samples with the exception of the cheek and forehead (two tape strip samples).

relative expression and rarely exceed three-fold for the width of the 95% confidence interval.

Further highlighting the variability in the (IL-8/actin) mRNA ratio, inspection of individual $\Delta C_{t,IL\text{-}8}$ values (data not shown), either within a subject (comparing different locations) or between subjects (comparing the same locations) reveals large differences. For instance Subject 1's forearm sample $\Delta C_{t,IL\text{-}8} = 1.48$ while the forehead $\Delta C_{t,IL\text{-}8} = 8.08$. The calculation of the fold-expression of the forehead (IL-8/actin) mRNA ratio relative to the forearm is $2^{-(8.08\ -\ 1.48)}$ or 97-fold more expression in the forearm compared to the forehead. Similarly, subject 9's forearm $\Delta C_{t,IL\text{-}8} = 8.56$, which equates to 135-fold more expression of IL-8 in subject 1's forearm compared to subject 9's forearm. Because these changes in the (IL-8/actin) mRNA ratio occur in a background of unchanging housekeeping gene expression there is no possibility of these changes being artifacts due to changes in β-actin mRNA expression (to which all mRNA expression is normalized; see Section 2). Thus, we conclude that IL-8 gene expression is highly variable in the upper epidermis.

The expression data for TNFα mRNA is similar to that of IL-8, although we judge TNFα mRNA expression to be slightly less variable. This variability is again most easily appreciated by inspection of the width of the 95% confidence intervals at different anatomical sites (Table 7). These confidence interval widths span values of 3.6—28, not nearly as high as the IL-8 data but still an indication of more regional and subject variation in TNFα mRNA expression than in housekeeping gene expression.

Table 7 also shows data for variation of the (VEGF/actin) mRNA ratio at different body locations. The fold-expression of VEGF at most locations relative to the mastoid is very close to 1. For the two regions with the most samples, the mastoid and upper back, the width of the 95% CI is 2.5 and 3, suggesting that VEGF expression is fairly uniform at these locations.

4. Discussion

In this work we describe the variation in RNA recovery at different body locations and between individuals, the relationship of tape stripping, RNA recovery and invasiveness as assayed by transepidermal water loss and the reproducibility of gene expression at seven different anatomical locations.

In a study with 25 subjects, each tape stripped 4, 8 and 12 times on the upper back, forearm and deltoid, we observed large and significant variations in RNA recovery between subjects and locations. Our data show high variances in RNA recovery at symmetrically located sites on the body axis as well as a wide range of values between individuals. We have also observed similar variability in RNA recovery from immediately adjacent sites (data not shown).

Further investigations into the recovery of RNA from different anatomical locations demonstrated that anatomical locations are significantly different with respect to the recovery of RNA by tape stripping (Tables 3 and 4). By classification of body sites by median RNA recovery we found the following order (highest to lowest): mastoid > forehead > chest > upper back > mid back > cheek > lower back > deltoid > forearm > abdomen > ventral thigh > inner arm > shin > dorsal thigh > lower leg. These data suggest that, when normal skin is being tape stripped for RNA recovery, certain locations should be avoided.

It was of interest to us to understand the source of RNA recovered by tape stripping. It is certain that our tape treatments recover mostly stratum corneum and do not extend into the viable epidermis. Therefore, candidates for the source of RNA are: (i) corneocytes, the primary component of the stratum corneum or (ii) specialized keratinocytes or other cells that dwell within ducts and line hair follicles that traverse the SC (i.e. components of adnexal structures).

In order to differentiate between these two possibilities we examined the correlation of removal of SC with RNA recovery. Our hypothesis was that if corneocytes were the primary source of tape strip recovered RNA then RNA recovery should be highly correlated with removal of SC and hence the number of tapes used to strip a site. As an additional proxy for SC removal we assayed TEWL after tape stripping with 4, 8 and 12 tapes per site. Although weighing tapes before and after stripping has been a traditional method of determining mass of skin removed, the method is extremely time consuming, labor intensive and subject to weighing artifacts related to hydroscopic characteristics of adhesive and SC [3,12] and has been abandoned in favor of methods that directly assay protein recovered from tape [24]. Because we could not use methods that sacrifice the nucleic acid on tapes we chose TEWL as an alternative indicator of barrier removal.

Our data clearly revealed a significant and high correlation (Table 6) between tape treatments and TEWL and the absence of such correlation between RNA recovery and tape treatments or RNA recovery and TEWL. A similar experiment was performed on the forearm, the results of which yielded virtually identical correlation coefficients (data not shown). We conclude that corneocytes are not the primary source of RNA recovered by tape stripping.

Our hypothesis that RNA recovered by tape is not primarily from corneocytes is consistent with our data demonstrating different recovery at differing anatomical locations. If corneocytes were the principal contribution to RNA then the vastly different yields from some body sites would be perplexing. However, if tape strip recovered RNA is primarily from structures traversing the SC then regional variation is to be expected. In support of this conclusion we note that inter- and intra-individual differences in stratum corneum composition, permeability and physical properties are well documented in the literatures [25,26].

We also assayed gene expression at different locations, demonstrating that housekeeping gene expression is constant while IL-8 and TNFα gene expression is variable between different anatomical locations within an individual and amongst individuals at the same location (Table 7). We have eliminated possible artifacts by showing that expression of five housekeeping genes remains unchanged within and between individuals and anatomical sites.

Table A1 Average ΔC_t values for housekeeping and inflammatory mRNAs at different body locations

mRNA	Statistic	ΔC_t and anatomical location[a]						
		Mastoid	Cheek	Forehead	Upper back	Deltoid	Forearm	Ventral thigh
GAPDH	Median	3.51.	3.53	3.05	3.52	2.88	3.21	3.27
	Mean ± S.E.M.	3.52 ± 0.14	3.37 ± 0.19	3.21 ± 0.16	3.68 ± 0.21	2.97 ± 0.24	3.04 ± 0.19	3.13 ± 0.3
	N	23	15	21	19	14	13	8
hARP	Median	−0.03	0.69	0.47	0.06	−0.075	−0.46	−1.45
	Mean ± S.E.M.	0.006 ± 0.189	0.845 ± 0.268	0.40 ± 0.18	−0.07 ± 0.26	0.237 ± 0.32	−0.241 ± 0.397	−1.1 ± 0.28
	N	24	17	22	21	16	13	8
ATP5I	Median	3.43	3.54	3.20	2.88	3.1	3.15	3.32
	Mean ± S.E.M.	3.39 ± 0.09	3.61 ± 0.15	3.26 ± 0.12	2.9 ± 0.17	3.07 ± 0.17	3.2 ± 0.15	3.3 ± 0.27
	N	22	7	12	19	6	7	4
CDH1	Median	1.67	1.75	1.74	1.57	1.48	1.42	1.91
	Mean ± S.E.M.	1.73 ± 0.09	1.88 ± 0.30	1.88 ± 0.24	1.58 ± 0.13	1.21 ± 0.20	1.68 ± 0.24	2.25 ± 0.53
	N	21	8	12	20	9	6	4
K16	Median	−0.98	−1.59	−1.99	−1.46	−1.29	−1.24	−1.07
	Mean ± S.E.M.	−1.23 ± 0.23	−1.50 ± 0.18	−1.69 ± 0.28	−1.31 ± 0.19	−1.25 ± 0.48	−1.41 ± 0.31	−1.21 ± 0.53
	N	22	12	12	22	11	9	7
IL-8	Median	5.01	2.41	4.51	6.1	1.3	6.9	6.48
	Mean ± S.E.M.	4.97 ± 0.63	2.2 ± 1.13	3.87 ± 1.23	6.1 ± 0.37	1.98 ± 0.85	5.96 ± 1.57	6.48 ± 0.56
	N	20	11	11	2	7	4	2
TNFα	Median	8.94	7.05	6.76	8.58	5.21	9.62	–
	Mean ± S.E.M.	8.86 ± 0.32	7.39 ± 0.40	7.36 ± 1.16	8.29 ± 1.05	5.21	9.62 ± 0.65	–
	N	16	4	4	3	1	2	0
VEGF	Median	4.68	3.32	2.81	4.34	4.81	5.56	5.73
	Mean ± S.E.M.	4.54 ± 0.23	3.76 ± 0.64	3.12 ± 0.35	4.54 ± 0.32	5.06 ± 0.49	5.32 ± 0.34	5.56 ± 0.31
	N	21	8	11	16	4	5	3

[a] ΔC_t values are defined as the threshold value for the gene of interest minus that of β-actin as explained in Section 2.

We show that on average, IL-8 mRNA expression can vary at different locations by factors of 0.35—8-fold relative to the mastoid. Furthermore, the width of the 95% CI, an indication of variation amongst individuals, can be as high as 110-fold (see forearm; Table 7). Similar data was seen for TNFα mRNA and similar observations for these and other inflammatory mRNAs are described by Grangsjo et al. [27] for the gluteal area. These observations add to those of others describing the heterogeneity of the skin in the response to barrier disruption and percutaneous penetration of chemicals [11,25,28].

The irregular distribution of high quantities of mRNA for these inflammatory mediators may be linked to the variability in irritant skin responses between and within individuals. Takeuchi et al. [29] have observed variability in the response in IL-8 mRNA expression after minimal trauma to the skin (rubbing with a pencil eraser for 2 min; a stimulus designed to be clinically and histologically benign) and hypothesized that minimally traumatic events may predispose the skin to future inflammatory responses at those areas. One possible contribution to the variation in response could be different normal baseline cytokine gene expression (such as observed here), with regions of high baseline expression more susceptible to inflammation.

Previous quantitative applications of tape stripping such as for drug recovery and percutaneous absorption studies have suffered for lack of adequate normalization and the uneven surface presented by the SC [5,13]. We have shown here that internal normalization employing several different housekeeping gene mRNAs accounts for different RNA masses in our assays. Thus our application of tape stripping to study gene expression in the skin does not suffer from previous deficiencies.

We have shown that IL-8 and TNFα gene expression can be quite different at distinct anatomical sites. This raises the question of how does one establish a baseline or ''normal'' level of gene expression for these mRNAs? One possible solution is to assay many subjects to better define ''normal'' levels of an mRNA at a specific location and to combine samples from predefined locations to create an average value for mRNA expression. We have used such a strategy in a separate study by assaying uninvolved skin in psoriatic patients. We have assayed over 200 patients at uninvolved skin sites where three sites are combined to produce one control sample. This method has shown that that normal and abnormal (lesional psoriatic) mRNA levels can be unambiguously established [16].

In conclusion, we have characterized the recovery of RNA with tape at 15 anatomical locations and compared gene expression between 7 of these locations. While RNA recovery is reproducibly variable between individuals at similar sites and within subjects at different anatomical sites, housekeeping gene expression is uniform. We hypothesize that the variability of RNA recovery using tape stripping is linked to differences in stratum corneum and general epidermal cellular anatomy at different body locations and between individuals. Despite this variability in RNA recovery we have shown here and elsewhere [15,16] that valuable gene expression data can be extracted non-invasively with tape stripping.

Acknowledgement

The authors would like to thank Vera Morhenn for reviewing, critiquing and otherwise improving the manuscript.

Appendix A

Average ΔC_t values are shown in Table A1.

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