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PRELIMINARY EVALUTION OF GENE EXPRESSION CHANGES FROM TAPE STRIPPINGS

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INTRODUCTION

The human skin is a complex system with multiple components. In order to understand changes that occur, it is important to evaluate and assess changes quickly. Unlike many other organs and cell types in the human body, the skin can be assessed visually since it is the outermost organ. However, visible inspection is not enough to know the full impact that external inputs have on the skin. Among several skin sampling techniques available to analyze additional changes in the skin, skin biopsies are the most comprehensive sampling technique to examine modulations of skin components, in spite of the highly invasive nature of collection and potential recovery problems. Depending on techniques, there are advantages and limitations with differences in detectable components [1]. The tape stripping method is less invasive than biopsies and quicker, but there are limitations on detectable components and it also lacks standard methods [1].

The skin tape stripping method has been used to collect different constituents of the stratum corneum and quantify drug penetration [2]. Even though lipids and proteins are routinely evaluated from these tape stripping samples [3], mRNAs are not one of those components that are measured regularly. Recently, there are more studies being published that demonstrate tape stripping as an alternative method to measure mRNA and long non-coding RNA expression without skin biopsies [4, 5].

Skin explants are mostly from abdominoplasty and human facial skin biopsy samples are essentially not available, and skin from different areas behaves differently due to structures in the skin. For example, there are more sebaceous glands present on the face and the scalp than the arm. We were interested in the potential to evaluate facial skin at different locations for changes that may occur with cosmetic products. We designed a preliminary study on the inner forearms of volunteers to analyze selected skin genes, before assessing the facial skin .

OBJECTIVE

To investigate the potential of a less invasive sample collection method, tape stripping, for measuring gene expression modulations by cosmetic treatments in human subjects.

METHODS

In order to evaluate the changes that would occur with different treatments, four volunteers were selected. Subjects were all females between the ages of 30 and 40. Three subjects were Caucasian and one subject was Asian. The inner forearms were used for tape stripping sites to prevent interference from other external factors that may influence gene expression, such as UV exposure. Each volunteer was following treatment instructions for each site (Figure 1A). Using the non-dominant arm as the novel treatment cleansing site, a novel device with its treatment topical was used twice daily for 30 seconds during the 4-week study. The site on the dominant arm in the similar area as where the novel treatment device was used, was chosen as the negative control site. The area on the dominant arm closer to the wrist was chosen as the acetone site. Acetone is known to cause skin dryness, which will induce changes in the skin gene expression. A cotton pad soaked with acetone (commercially available nail polish remover - 100% acetone with denatonium benzoate) was pressed for 10 seconds twice daily. Subjects were instructed not to vary any hygiene or moisturizing habits among the test sites for the duration of the study. All subjects in this study are happened to be right-handed.

GeneMarkers LLC (Kalamazoo, MI) developed a tape strip method to evaluate gene expression using their standard skin panel. The tape stripping method requires the D-Squame sampling disc (CuDerm, Dallas, TX) shown in Figure 1B, from which GeneMarkers isolated mRNAs. Tape stripping was performed and stored by a clinical lab technician based on the tape stripping instructions provided by GeneMarkers. For gene expression, the site was cleaned with alcohol wipes and the first tape was placed and marked on the skin. Each successive tape was placed on the marked location; first 10 tapes were discarded and tapes 11-15 were saved in RNAlater (Life Technologies, Grand Island, NY) for the analysis of each site. RNA was extracted using a modified protocol for the RNeasy Micro kit (Qiagen, Germantown, MD) and RNA quality was determined by Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE). cDNA was synthesized using SuperScript Vilo RT kits (Life Technologies) per the manufacturer's instructions for low sample input. Gene expression was analyzed by the QuantStudio 12K Flex instrument (Life Technologies) using a custom Taqman skin gene panel designed by GeneMarkers. Data was processed using RealTime StatMiner software v 4.2 for statistical analysis.

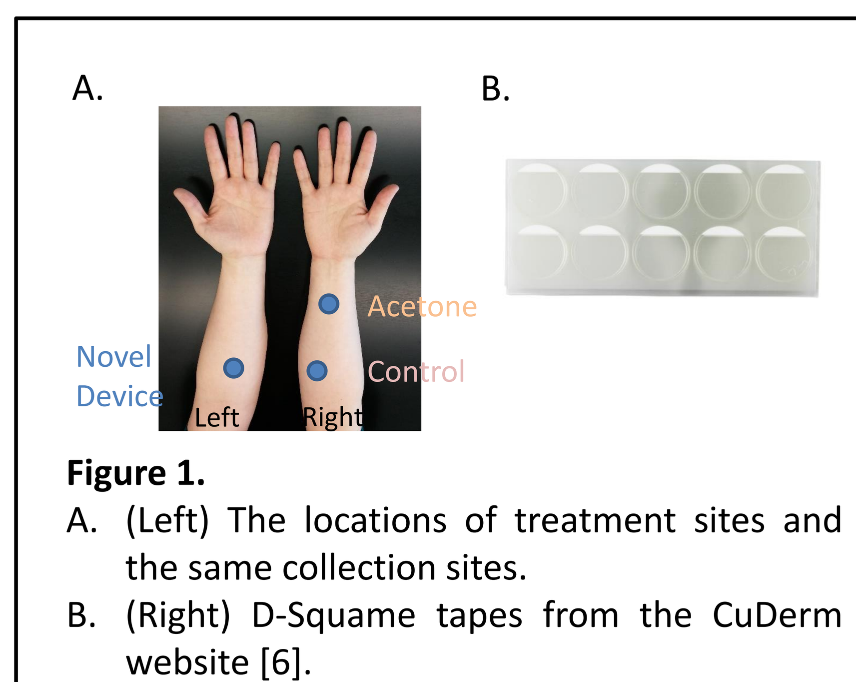

Figure 1.
A. (Left) The locations of treatment sites and the same collection sites.
B. (Right) D-Squame tapes from the CuDerm website [6].

RESULTS

There were several foreseeable challenges with this study. Two of those challenges were whether 5 tapes are sufficient to obtain the quantity of RNA needed for the study and another was whether the quality of RNA is adequate for QPCR. Prior studies by GeneMarkers suggested that 5 tapes would likely yield sufficient quantity of RNA. Therefore, the first study was to evaluate the RNA quantity and quality using the Nanodrop 2000 spectrophotometer. Four volunteers had 3 sites each on their arms for tape stripping. Table 1 shows the concentration and the ratios. As expected, the concentration of RNA was lower than other studies. A260/A280 and A260/A230 ratios demonstrate the purity of isolated RNA. Since those ratios were below standard RNA quality scores (A260/A280: 1.8-2.2, A260/A230: >1.7) [7], absorbance spectrums were visually evaluated. An example of RNA absorbance spectrum is shown in Figure 2A. This spectrum suggests low RNA quality ratios are likely due to low RNA concentrations; ratios will often decrease with low nucleic acid concentrations [7].

Subject initials	Treatment Type	Number of Tapes	ng/uL RNA	260/280	260/230
JN	No Treatment	5	13.2	1.36	0.65
	Novel Device	5	9.1	1.39	0.57
	Acetone	5	8.1	1.33	0.61
KH	No Treatment	10 *	28.1	1.40	0.27
	Novel Device	5	31.1	1.40	0.22
	Acetone	5	13.8	1.38	0.82
ES	No Treatment	5	6.8	1.50	0.42
	Novel Device	5	7.3	1.39	0.06
	Acetone	5	6.6	1.39	0.55
MN	No Treatment	5	26.0	1.38	0.71
	Novel Device	5	5.8	1.54	0.17
	Acetone	5	4.9	1.47	0.18

Table 1. RNA Quality Assessments
RNA concentration and quality were assessed using Nanodrop 2000. * indicate extra tapes added during the sample collection.

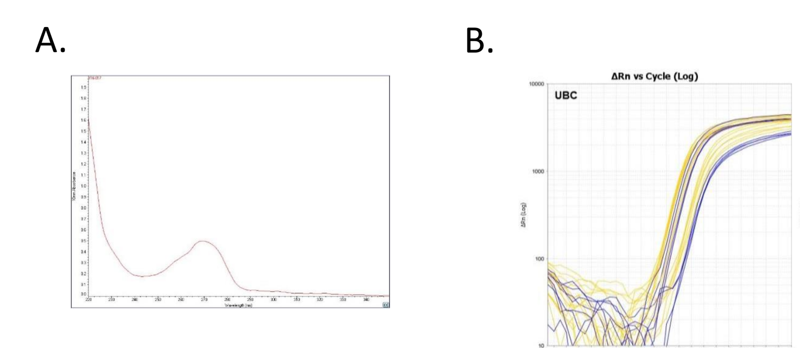

Figure 2.
A. (Left) RNA spectrum of a representative sample.
B. (Right) QPCR amplification graph for a housekeeping gene (UBC).

30ng of RNA were used in cDNA synthesis following the low sample input protocols and subsequently analyzed by QPCR. In order to select a consistent endogenous control, five different endogenous controls and two uniformly expressed genes were evaluated in different algorithms: Normfinder, Minimum Variance Median and the Coefficient of variability. UBC (Ubiquitin C) was selected with the lower stability scores. UBC amplification curves are shown in Figure 2B.

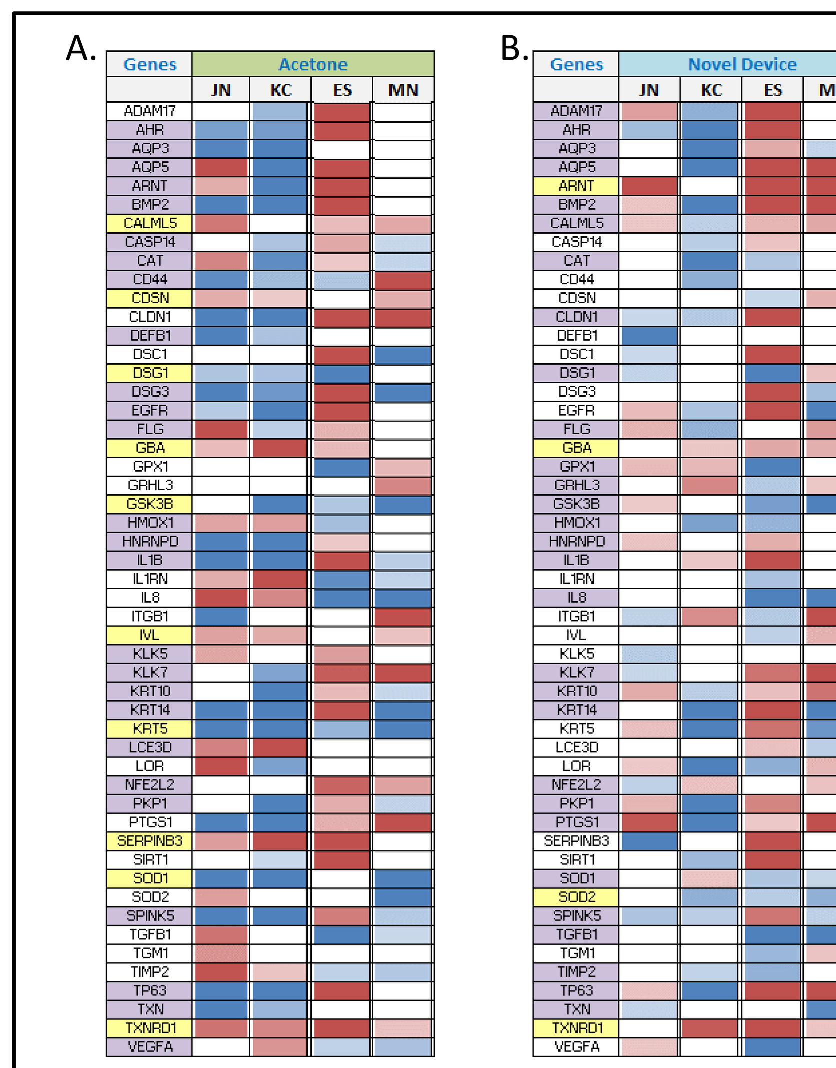

Figure 3. QPCR Data

A. (Left) Results obtained from acetone treated sites.
B. (Right) Results obtained from the novel device treated sites.

Names of the target genes are shown on the left column. Yellow gene names demonstrate consistent results across 3 to 4 subjects. Purple indicates the result from one subject was in conflict with the others. White cell indicates no consistent results among 4 subjects. Because several target genes were modulated over 100-fold, directional modulations are shown only with colors without numbers in the columns under the subject initials. If the cell is red, the treatment stimulated the gene expression. Darkest red cells indicate stimulation of 5-fold or more compared to the endogenous control, UBC. If the cell is blue, the treatment suppressed the expression. Darkest blue cells indicate inhibition of 5-fold or more. If the cell is white, then there were either no amplification or no significant changes with the treatments.

Figure 3 shows global overview of QPCR results from acetone treated (3A) or the novel device treated (3B). Overall, acetone treated sites had more modulations than the novel device treated sites. Acetone treated sites had more barrier and keratinocyte cohesion target genes modulated and the novel device treated sites modulated oxidative stress, detoxification and anti-inflammation associated targets genes. Subjects did not complain of pain or discomfort associated with tape stripping, although one Asian subject eventually developed post-inflammatory hyperpigmentation on the tape stripped sites. Two Caucasian subjects were known "stingers", who react more readily to cosmetics. Overall, a 15-tape stripping was well-tolerated by subjects with QPCR data demonstrating the potential uses on the future product evaluations.

CONCLUSIONS

- Gene expression analysis from tape stripped samples demonstrated potential usage on facial skin without skin biopsies.
- Some limitations include target genes from non-proliferating keratinocytes only, low RNA quality, consistency of sample collection and skin sensitivities.

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