An Adhesive Patch-Based Skin Biopsy Device for Molecular Diagnostics and Skin Microbiome Studies

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ABSTRACT

Introduction: A number of diagnoses in clinical dermatology are currently histopathologically confirmed and this image recognition–based confirmation generally requires surgical biopsies. The increasing ability of molecular pathology to corroborate or correct a clinical diagnosis based on objective gene expression, mutation analysis, or molecular microbiome data is on the horizon and would be further supported by a tool or procedure to collect samples non-invasively. This study characterizes such a tool in form of a 'bladeless' adhesive patch-based skin biopsy device.

Methods: The performance of this device was evaluated through a variety of complementary technologies including assessment of sample biomass, electron microscopy demonstrating the harvesting of layers of epidermal tissue, and isolation of RNA and DNA from epidermal skin samples. Samples were obtained by application of adhesive patches to the anatomical area of interest.

Results: Biomass assessment demonstrated collection of approximately 0.3mg of skin tissue per adhesive patch and electron microscopy confirmed the nature of the harvested epidermal skin tissue. The obtained tissue samples are stored in a stable fashion on adhesive patches over a wide range of temperatures (-80°C to +60°C) and for extended periods of time (7 days or more). Total human RNA, human genomic DNA and microbiome DNA yields were 23.35 ± 15.75ng, 27.72 ± 20.71ng and 576.2 ± 376.8pg, respectively, in skin samples obtained from combining 4 full patches collected non-invasively from the forehead of healthy volunteers.

Discussion: The adhesive patch skin sampling procedure is well tolerated and provides robust means to obtain skin tissue, RNA, DNA, and microbiome samples without involving surgical biopsies. The non-invasively obtained skin samples can be shipped cost effectively at ambient temperature by mail or standard courier service, and are suitable for a variety of molecular analyses of the skin microbiome as well as of keratinocytes, T cells, dendritic cells, melanocytes, and other skin cells involved in the pathology of various skin conditions and conditions where the skin can serve as a surrogate target organ.

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INTRODUCTION

Frequently, obtaining high quality tissue samples to confirm clinical diagnoses is an integral part of selecting appropriate treatment options or assessing treatment outcome. While most tissue samples are still collected through surgical biopsy, there is a growing trend towards minimally invasive or ideally non-invasive approaches wherever possible without compromising the required quantity and quality of the samples obtained.[1] While micro-needle and adhesive patch based strategies appear to fit this paradigm, only the latter is truly non-invasive. This work focuses on the detailed characterization of such an adhesive patch-based device termed the Adhesive Patch-Based Skin Biopsy (APSB, DermTech, La Jolla, CA) Kit.

The APSB kit contains a simple trifold with 4 circular adhesive patches, each 19mm in diameter, as the main harvesting device. When applied to selected skin areas or skin lesions, each adhesive patch collects a thin layer of epidermal stratum corneum tissue carrying genetic information not only from keratinocytes, but also from melanocytes, basal cells, T-cells, dendritic cells, and other skin cells. The APSB device platform can be used to collect skin tissue from all anatomical locations except from palms of hands, soles of feet, and mucous membranes.[2,3,4] Total RNA of quality sufficient for a variety of molecular analyses of pigmented lesions and inflammatory skin conditions using quantitative reverse transcription-PCR (RT-qPCR), microarray-based gene expression studies, and RNA sequencing has been demonstrated.[2,5,6,7,8, and unpublished data] The entire tissue collection process with adhesive patches is completely non-invasive when 4 patches of skin tissues are collected from one skin area of interest; combining the material from the 4 patches provides sufficient tissue for most applications in dermatology including a commercial gene expression-based test supporting clinicians in their efforts to accurately diagnose melanoma.[3,6,7,9]

While previous studies focused on using the platform for RNA-based gene expression analyses, the platform also lends itself to analyses of human genomic DNA (gDNA) from skin samples and to skin microbiome analyses of co-collected samples. This study characterizes the full utility of the platform for DNA, RNA,

and microbiome analyses and provides key data on the stability of non-invasively collected RNA skin samples. This study furthermore improves our understanding of the APSB platform and the growing number of clinical applications this non-invasive skin sample collection tool can be used for.

MATERIALS AND METHODS

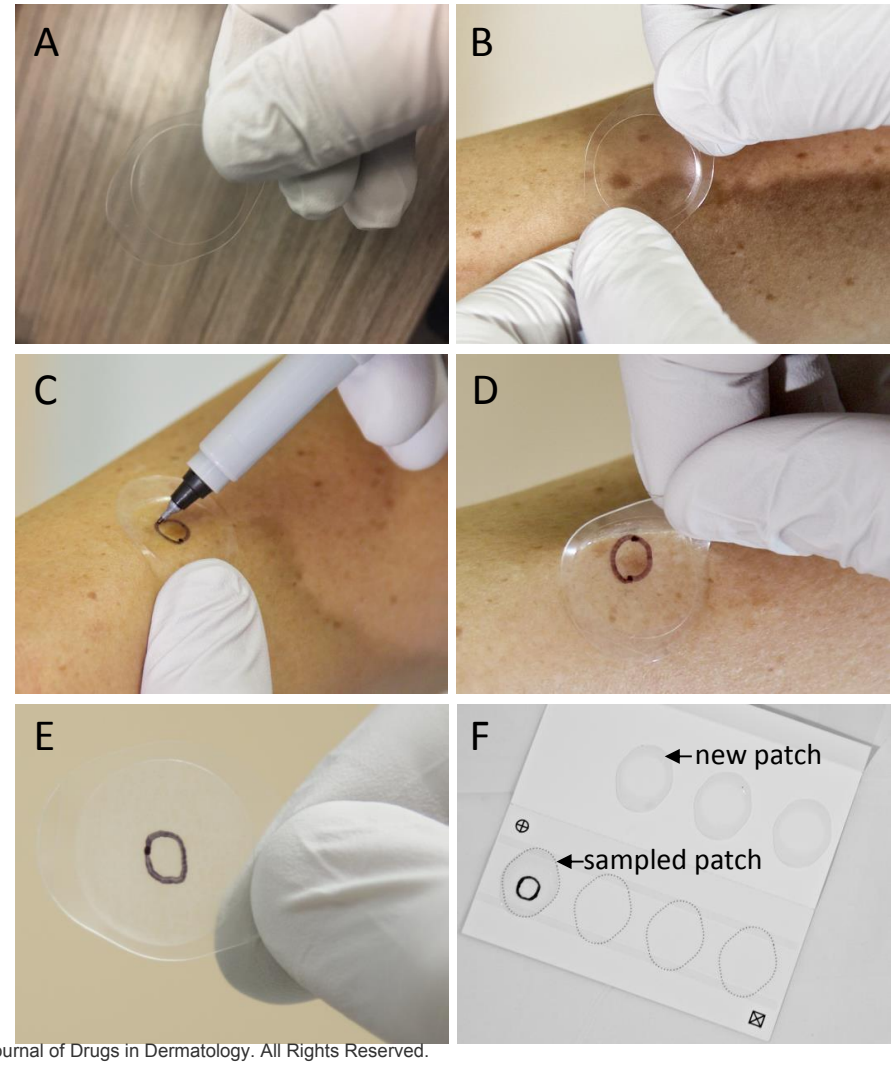
Subjects and Adhesive Patch Skin Biopsy Kit

Subjects for this study were adult males and females who met the inclusion and exclusion criteria defined in protocols approved by our Central Institutional Review Board (WCG, WIRB-Copernicus Group, Princeton, NJ). Adhesive Patch Skin Biopsy (APSB) kits were provided by DermTech (La Jolla, CA). Each APSB Kit contains a tri-fold sample collector (Figure 1) with four transparent patches (round adhesive areas 19mm in diameter) stored in a zip lock plastic bag, a 70% alcohol prep pad, a gauze pad, instructions for use (IFU), and a laboratory requisition form as well as a courier envelope if required to return skin samples to analysis laboratories without need for refrigeration.

Skin Sample Collection Procedure

Procedure for skin tissue collection using the APSB kit has been reported previously[6,8] and is shown in Figure 1. In brief, once the lesion or skin area of interest is cleaned with alcohol (and hairs if present have been removed preferably with curved scissors), an adhesive patch is placed on the alcohol-cleaned and

FIGURE 1. Adhesive patch device and procedure of non-invasive skin sample collection. As the main component, the APSB device contains 4 patches (A) with round adhesive areas of 19mm in diameter to collect one skin sample. Each adhesive patch is placed on a cleansed and dried area of skin (B) applying soft pressure via approximately 5 circular thumb motions. A lesion or area of interest can then be demarcated on the patch (C) and the patch is then removed (D, E) and placed on the sample collector trifold (F). The steps further detailed in Materials and Methods are repeated for each of the 4 patches. After the adhesive patch biopsy, the lower panel with harvested patches is folded back to the center panel then covered by the top panel to protect the harvested patches during storage and transportation.



air-dried skin lesion or area of interest (Figure 1B), followed by gently applying pressure to the patch with a thumb and about 5 circular motions to fill the adhesive with epidermal skin cells. If a skin lesion rather than non-lesional or uninvolved skin is to be investigated, the lesion is demarcated on each patch with a black marker pen (provided in the APSB kit) before each patch is peeled back from the skin area to be investigated (Figures 1C and 1D) and returned to the patch collector area on the trifold (Figures 1E and 1F). Four adhesive patches from one APSB kit are used to harvest one skin sample. The trifold with the collected patches is placed into an also provided zip lock plastic bag and shipped for analysis by courier or mail at ambient temperature with patient and sample information as required.

Confirmation of Skin Tissue Collection

Successful collection of skin samples with adhesive patches was demonstrated through biomass measurements of the harvested skin tissue on patches, direct visualization of epidermal cells in the harvested skin tissue via transmission electron microscopy (TEM) and molecular analysis of total RNA or DNA isolated from the harvested skin tissue.

Biomass of harvested skin tissue on adhesive patches was determined through the weight changes (ΔW) of adhesive patches measured before (W_0) and after (W_s) sample collection ($\Delta W=W_s-W_0$, per patch). To prepare for TEM analysis of skin cells in harvested skin tissue on adhesive patches, the post-harvest adhesive patches were treated with methyl ethyl ketone (MEK) solution (which dissolves the adhesive to detach and release the skin tissues from the plastic base of the adhesive patches). The detached skin tissue was then collected (on a Millipore filter connected to a syringe), washed, and recovered in 3% buffered glutaraldehyde for processing via routine TEM. TEM images of the recovered skin tissue were taken at different magnifications.

Nucleic acids (DNA and RNA) isolated from the patch-harvested skin tissue provides additional evidence for successful skin tissues collection with the APSB kit. To isolate the nucleic acids, tissues from each individual patch were lysed in a modified lysis buffer from Norgen (Thorold, ON, Canada) and nucleic acids were extracted using silica-coated magnetic beads on KingFisher Duo Prime robot following a procedure developed and validated at DermTech, which isolated both total RNA and DNA.

Total human RNA in the bead eluent was quantified by RT-qPCR using human β-actin (ACTB) mRNA as a quantified marker following a procedure reported previously, [6] while total human genomic DNA (gDNA) in the same eluent was quantified using a standard gene copy number analysis qPCR using human ACTB gene as a quantified marker. Two microliter of bead eluent were used directly in each qPCR and quantities of total human gDNA in eluents were calculated from the Ct counts of ACTB from samples compared to the Ct counts of ACTB in standard curves prepared with human genomic DNA purchased from Promega (G3041; Promega, Madison, WI).

In addition to human total RNA and gDNA, microbiome DNA in the bead eluent was also analyzed with qPCR using a pan-bacterial detection assay and 16S rRNA gene (Ba04230899_s1, ThermoFisher Scientific) as a quantified marker. Quantities of microbiome DNA in the bead eluents were calculated from the Ct counts of 16S rRNA gene compared to the Ct counts of 16S rRNA gene in standard curves prepared with bacterial DNA (Ba04230899_s1, ThermoFisher Scientific). All qPCR reactions were done in the 2x TaqMan Universal Master Mix from LifeTechnologies following the manufacturer's instruction and all reactions were carried out in triplicate on 384-well plates and run on an ABI 7900 PCR system (Life Technologies, Carlsbad, CA).

Stability of RNA in Tissue Stored on Patches after Harvesting

Studies were conducted to investigate the stability of RNA in skin tissue embedded in the adhesive of patches after sample collection. Stability of RNA was assessed by changes in copy numbers of amplifiable gene transcripts recovered from freshly harvested or stored samples of the same sources. Four temperature conditions were evaluated (Table 1) and each testing engaged 5 subjects and was performed separately. Four samples were collected from the temple area, of which two were used for total RNA isolation right after collection (Fresh) and 2 were stored under a defined condition shown in Table 1 for total RNA

TABLE 1.

Experimental Design to Test the RNA Stability Under Different Storage Conditions

Test Storage Conditions	Number of Test Subjects	Total Number of Test Patches	Number of Patches for Initial Analysis (Day 0, Fresh)	Number of Patches for Final Analysis (Day 7, Stored)
25°C, 7 days	5	5x4	5x2 (Day 0)	5x2 (Day 7)
40°C, 7 days	5	5x4	5x2 (Day 0)	5x2 (Day 7)
60°C, 7 days	5	5x4	5x2 (Day 0)	5x2 (Day 7)
-80°C, 10 days	5	5x4	5x2 (Day 0)	5x2 (Day 10)
Total	20	80	40	40

FIGURE 2. Biomass of non-invasively obtained skin tissue samples from 5 anatomical areas. The sample biomass was measured as an increase in patch weight (ΔW), which is calculated as the weight of post - harvest patch (W_s) subtracted by the initial weight (W_0) of the same patch before use ($\Delta W = W_s - W_0$). Mean ± se, N=16, 4 subjects x 4 patches per subject per anatomical area.

isolation at the end of each storage condition. Total RNA was isolated and quantified from each individual patch before and after storage followed the same procedures described above and used the same β-actin mRNA as a quantified marker (compared to that in UHR through standard curve dilutions in qPCR).

Quality of the isolated RNA from both fresh and stored skin tissues of different storage conditions were further evaluated using RT-qPCR by 4 gene transcripts, including β-actin (ACTB), β-2-microglobulin (B2M), peptidylprolyl isomerase A (PPIA) and c-Maf inducing protein (CMIP), which represent genes with strong (ACTB), median (B2M), and weak (CMIP and PPIA) expression levels in human tissues. In this study, cDNA was prepared in RT reactions with a normalized input of 40pg total RNA. The resulting cDNA was diluted and used in TaqMan qPCR gene expression assays to quantify the transcripts of each of the 4 target genes. Gene expression assays of the 4 target genes were obtained from Life Technologies (ACTB Hs010606650_g1; B2M Hs00984230_m1; PPIA Hs04194521_s1; CMIP Hs00603125_m1) and qPCR followed the manufacturer's instruction. All reactions were run in duplicate.

Sanger Sequencing for Mutation Detection on Human gDNA

To demonstrate utility of DNA isolated from the patch harvested skin tissue, Sanger sequencing to detect human BRAF V600E gene mutation was conducted. PCR amplification of a 513bp length product covering human BRAF V600E mutation site was performed in a 25uL PCR reaction containing 100pg human gDNA from the above bead eluent and 200nM of both forward and reverse primers (BRAF_FTCTGGGCCTACATTGCTAAAATCTAA and BRAF_R GTTGAGACCTTCAATGACTTTCTAGT) and Invitrogen™ Platinum™ TaqGreen Hot Start DNA polymerase (ThermoFisher Scientific) following the product instructions. PCR products were first ExoSAP treated then used as templates for Sanger sequencing. Sequencing chromatogram files were examined using Chromas (version 2.01, University of Sussex, Brighton, United Kingdom).

Statistical Analysis

Statistical analyses were performed using Excel or R Tests for which the null hypothesis was no difference among procedures or conditions; analyses were performed with Student's *t*-test or

FIGURE 3. Transmission Electron Microscopy analysis of skin tissue obtained via adhesive patches. Magnifications: (A) 4,400x, (B) 20,000x and (C) 50,000x.

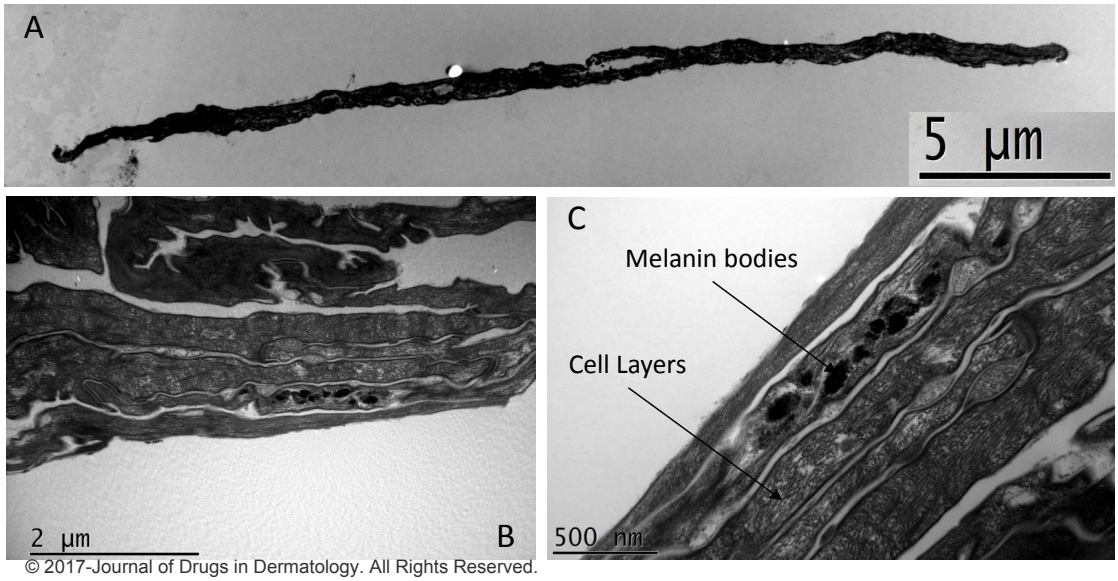
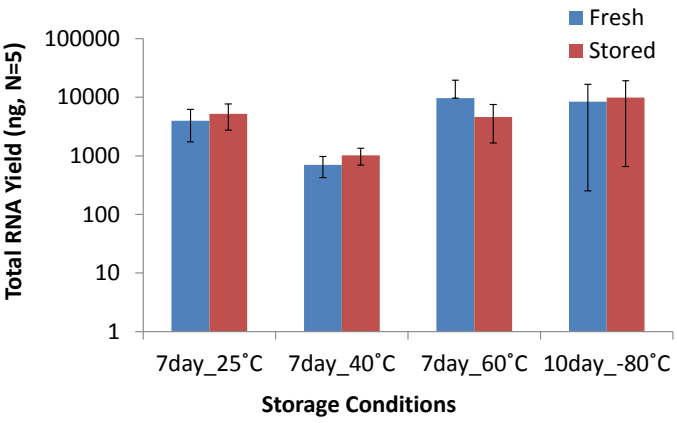


FIGURE 4. Comparison of total RNA yields from freshly harvested or stored patches (both harvested at the same time from the same subjects) under each test condition. Four test conditions were studied independently and each test involved 5 subjects (mean ± se).



analysis of variance, and *P*-values less than 0.05 were considered significant.

RESULTS

Figure 1 depicts the procedure of skin tissue collection using adhesive patches from an APSB kit; technical details are provided in Materials and Methods. Depending on the anatomical location from which skin samples were obtained, the skin biomass harvested per patch varied between 0.20-0.35mg. Figure 2 shows the average biomass per patch from 5 anatomical sites from 4 test subjects (values are mean±SE, 4 patches per test site from 4 subjects, N=16 measurements). Biomass analysis confirms the collection of skin tissue via adhesive patches that can be subjected to extraction procedures enabling nucleic acid, exosome, protein, lipid, and microbiome analyses within the obtained tissue. This study, as mentioned above, focuses on gene expression, genomic DNA analyses, and microbiome DNA analyses within the obtained skin samples. To further corroborate the nature of the obtained skin samples, the samples were visualized at high magnification. Figure 3 shows the TEM pictures of a representative section of skin tissue collected via adhesive patch methods as viewed at low (4,400x, Figure 3A), medium (20,000x, Figure 3B), and high (50,000x, Figure 3C) levels of magnification. At medium and high magnification, layers of intact skin cells (primarily keratinocytes) and intracellular structures such as melanin bodies were observed (Figure 3B and 3C). These observations confirm the successful collection of epidermal skin tissue comparable to a very superficial shave biopsy procedure.

Figure 4 shows the total yield of RNA recovered from adhesive patches from an RNA stability study. Values in blue bars ('Fresh') represent averaged total RNA yields from freshly harvested tissues while values in red bars ('Stored') represent averaged total RNA yields from tissues stored on adhesive patches after harvesting. Four storage conditions were independently investigated. Though the total RNA yield varied among the

FIGURE 5. Threshold cycle (Ct) values of qPCR analysis of 4 genes on RNA from both the freshly harvested and stored patches, under each of 4 test storage conditions: (A) 7 days at 25°C; (B) 7 days at 40°C; (C) 7 days at 60°C and (D) 10 days at -80°C.

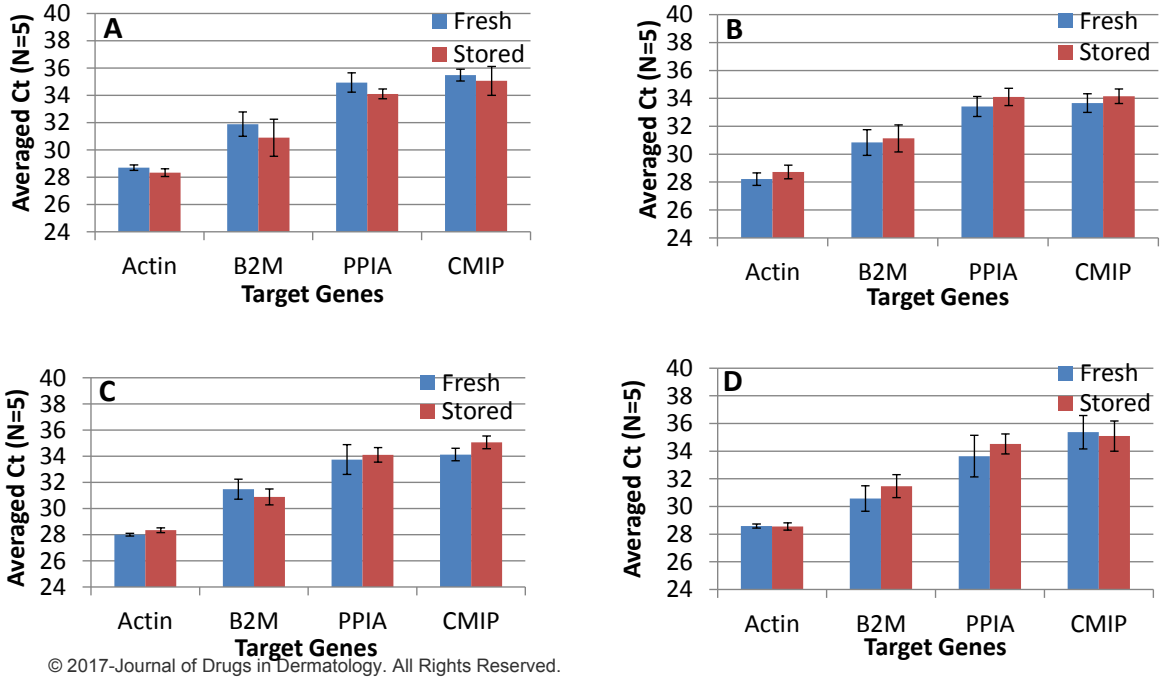
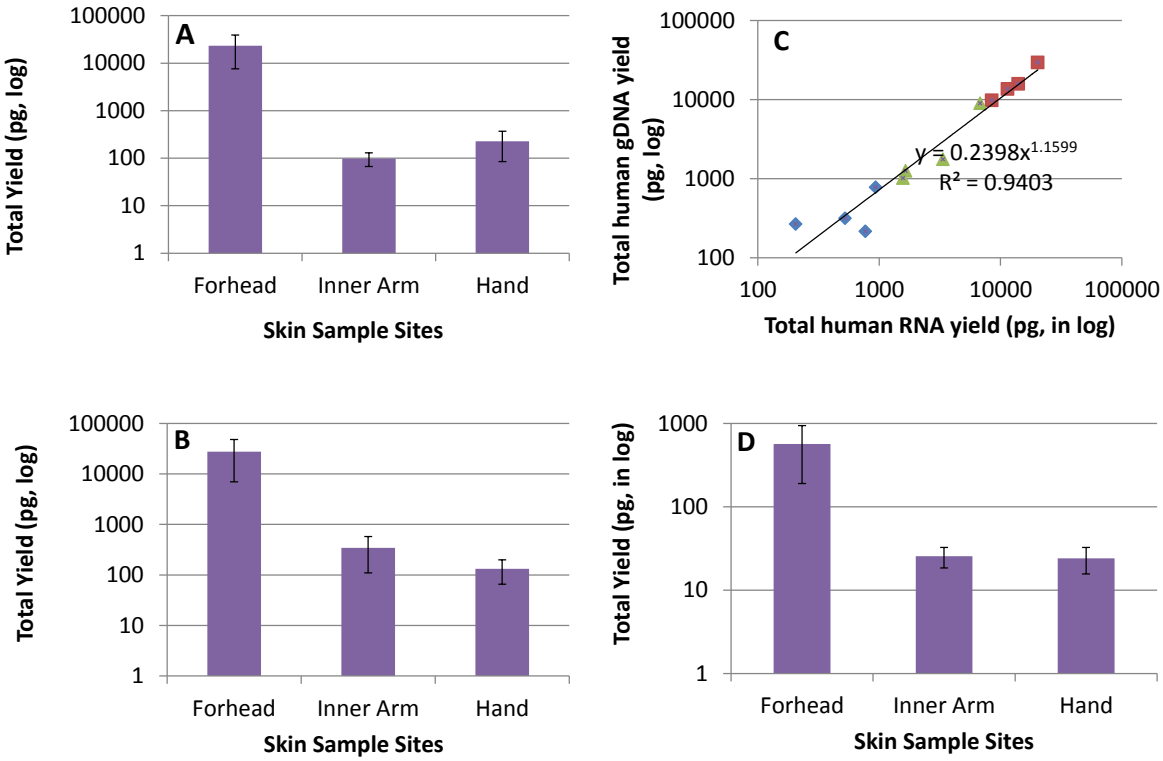


FIGURE 6. Total yields of human RNA (A) and human genomic DNA (B) co-extracted from the same skin samples non-invasively collected with the adhesive patches from 3 anatomical locations of 4 subjects. Yields are shown as mean ± se. The correlation of human RNA yield versus human gDNA yield is depicted in (C). Total yield of microbiome DNA co-isolated from the same skin samples harvested on adhesive patches is shown in (D).

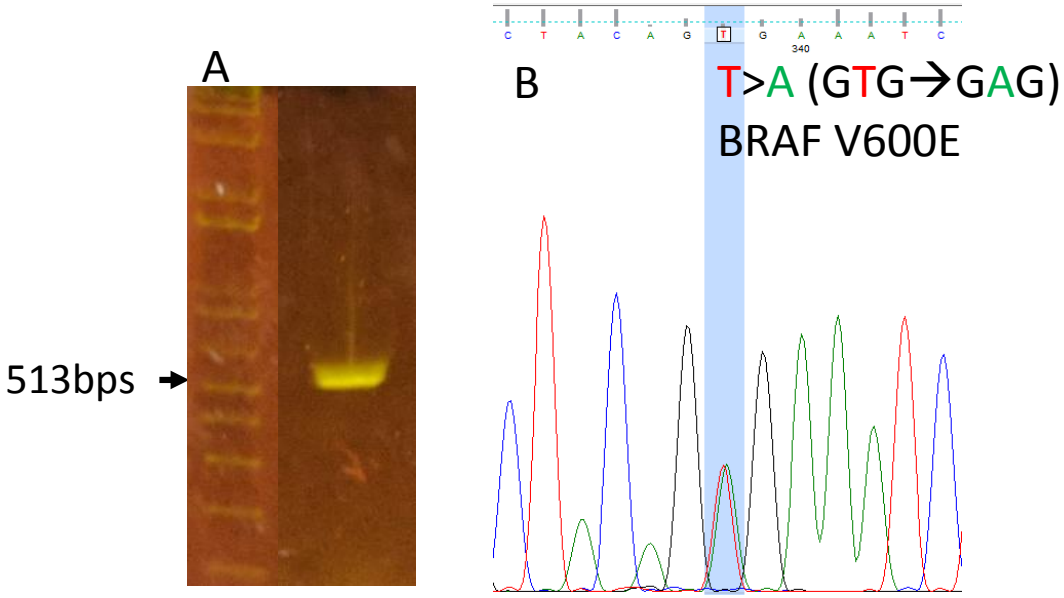


different storage conditions, no statistically significant difference (P<0.05) was seen between the fresh and stored samples in any of the storage conditions tested. To demonstrate that the obtained RNA was suitable for further evaluation, gene transcripts were analyzed. Figure 5 shows the transcript analysis of 4 genes in the isolated total RNA from fresh and stored skin tissue samples from 4 storage conditions. As Ct counts in qPCR correlate to the copy numbers of amplifiable transcripts in total RNA, a similar Ct count in total RNA from fresh (Blue) and stored (Red) tissues suggest a similar copy number of the amplifiable transcripts in fresh versus stored samples. Ct values from none of the 4 genes showed statistically significantly different (P>0.05) data between fresh and stored samples in any of the 4 temperature conditions, even with storage temperatures as high as 60°C for 7 days. These results confirm the observations shown in Figure 4 and indicate stability of RNA molecules in skin samples on adhesive patches.

To expand the spectrum of molecular tests adhesive patch skin samples can be subjected to, we also evaluated gDNA and DNA-based analyses within the samples. Figure 6 shows results from skin samples collected from the forehead, the inner arm, and the back of the hand of 4 test subjects for total nucleic acid extraction and quantification. Though nucleic acid yield varied among skin from different anatomical locations, both total human RNA and human gDNA could be reliably isolated from various anatomical locations via the adhesive patch method (Figure 6A, 6B). The yield of total human RNA and human gDNA was 23.35±15.75ng, 27.72±20.71ng, respectively, and was correlated linearly in each sample (Figure 6C). Besides human host RNA and gDNA, microbiome DNA was also detected in the same eluent from the skin tissue samples collected via adhesive patches (Figure 6D). This indicates that the APSB kit can also be used for simultaneously obtaining skin microbiome samples. Microbiome DNA yield did not linearly correlate to the yield of human nucleic acids (RNA and gDNA) from the same sample collections. Total microbiome DNA yield was 576.2±376.8pg.

We used a short product of ACTB PCR for human gDNA quantification. Figure 7A shows the isolated gDNA can also be used to successfully amplify longer PCR products such as the 513bp human BRAF gene exon. Sanger sequencing on this 513bp PCR product reliably detects, again as a representative example, BRAF V600E mutations (Figure 7B) within adhesive patch skin samples. These results demonstrate that human gDNA of appropriate quality for a variety of genetic analysis can be

FIGURE 7. PCR amplification of the human BRAF gene target exon from isolated gDNA (A) for Sanger sequencing and a chromatogram of the target exon sequence of a mutated sample (B).



obtained via adhesive patch biopsy significantly expanding potential uses of the APSB platform.

DISCUSSION

While significant progress has been made over the last years in introducing gene expression and mutation analyses in a variety of areas including oncology, dermato-oncology and dermatopathology,[10-13] progress in using objective DNA and RNA - based information in clinical dermatology has been hampered by the lack of suitable tools. The non-invasive adhesive patch skin biopsy device, distinct from tape stripping, appears to meet key criteria for such a tool. It can provide actionable objective skin sample gene expression[6] and mutation information to clinicians in the absence of surgical biopsies and with potential advantages over the current gold standard and the added benefit to also investigate the microbiome from the same sample. The APSB Kit non-invasively samples skin lesions of interest in their entirety while leaving the lesion intact for additional diagnostic procedures if needed or desired. Different from surgical strategies where a lesion is no longer available after the procedure, the APSB Kit allows for repeat sampling and disease monitoring. Potential prognostic information and treatment guidance options will also continue to emerge. This study provides several levels of evidence that the APSB Kit harvests epidermal skin samples in a reliable, non-invasive fashion.

It extends our previous investigations to focus on the stability of RNA in skin tissue after collection on adhesive patches and further uses of the APSB kit. We further characterized sample collection through biomass measurements and electron microscopy analysis of skin tissue samples collected via adhesive patches. Both results confirmed that the APSB kit harvests epidermal skin tissue suitable for further analysis. Four patches placed on the same skin area were used to obtain 1 tissue sample with a biomass of about 1mg from which about 1000-10,000pg of total RNA were recovered. These numbers are similar to the ones reported previously.[3,6] Because of biomass variations related to inter-subject variability and different yields in different anatomical locations, the use of four patches to obtain one sample, especially for studies that focus just on lesional skin from the demarcated areas on each patch, appears important for assuring sufficient biomass collection. This strategy also keeps the process non-invasive as confirmed through transepidermal water loss studies.[3]

Depending on intended application and underlying skin disease or condition, the samples from one site can be pooled or used separately. In inflammatory and immune mediated skin conditions including psoriasis, atopic dermatitis, lupus, vitiligo, and alopecia areata, where lesion areas often exceed the diameter of the patch, samples from full patches have been used in most cases.[5 and unpublished data] For skin cancer in general and primary pigmented lesions in particular, where the size of the patch frequently exceeds the size of the lesion, samples from demarcated areas on patches shown in Figure 1 are usually macro-dissected from full patches to minimize the target signal dilution.[6 and unpublished data]

Especially the stability of RNA in skin tissue samples after patch collection is an important consideration that determines the

utility of the APSB kit for gene expression applications. As previous exploratory and validation studies were conducted under conditions where RNA was isolated from either freshly collected samples or samples stored at -80ºC, the impact of other conditions on RNA integrity was unknown. This study demonstrated for the first time that RNA molecules are stable in skin samples that remained on adhesive patches for at least 1 week over a broad range of temperatures up to 60ºC. The dry nature of patch-collected skin samples likely contributes to RNA stability that enables sample shipment by regular mail or standard courier service at room temperature without need for refrigeration or freezing. This is a clear advantage over surgically obtained biopsy specimens where surgically obtained samples for molecular analyses are frequently frozen, stored at -80ºC and shipped on dry ice to maintain sample quality.

In addition, this study also demonstrates successful co-isolation of genomic DNA from the same patch-collected tissues with silica-coated magnetic beads. A linear correlation of total human RNA yield and human gDNA yield in patch-collected tissue samples confirms the same tissue origin. Successful PCR amplification of long products (>500 bps) from the isolated gDNA and gene mutation detection with amplicons through Sanger Sequencing methods (using the BRAF V600E mutation as an example) suggests sufficient quality of the obtained gDNA for DNA-based genetic analyses. The quality of the obtained RNA is sufficient for PCR and microarray as well as RNA seq applications.[6,7,14 and unpublished data] With the co-isolated RNA and DNA, we can now study both the gene expression changes and genetic alterations in a variety of skin conditions.

Furthermore, the APSB kit can also be used to simultaneously harvest microbiome skin samples for prokaryotic DNA analyses. The lack of a linear correlation of the microbiome DNA with human nucleic acids confirms the different cell origin of these procaryotic molecules. The successful co-isolation of microbiome DNA offers opportunities to study the direct impact of microorganisms on the skin diseases including in inflammatory diseases such as psoriasis or atopic dermatitis.

This study systematically characterizes the performance of a widely usable Class I epidermal skin biopsy / general skin sampling device that is non-invasive and well tolerated.

DISCLOSURES

Zuxu Yao, Talisha Allen, and Burkhard Jansen are employed by DermTech. Ronald Moy declares no potential conflict of interest or financial relationship with DermTech.

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