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Innovations in translational research in dermatology: minimally invasive methods for biosample acquisition

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Abstract

Translational research has improved patient care over the last decade. In dermatology, this research often requires human tissue for laboratory analysis. The skin biopsy remains the gold standard for tissue acquisition, but the procedure comes with a small risk of bleeding and infection. It also causes scarring and anxiety in certain populations. These risks and concerns may affect participation rates in translational studies, which can require multiple biopsies. Minimally invasive procedures may mitigate these risks and concerns. We queried the PubMed database for all minimally invasive technologies studied as of May 2021. Of the 53 articles reviewed, we identified 13 unique, minimally invasive methods for tissue biosample acquisition. Herein, we describe each sampling method, biosample type analyzed, disease target, molecular application, procedure, quantity of obtained biosample, purpose, and required equipment. We organize this information into a comprehensive chart. We then synthesize this information into another table that compares the pros and cons of each intervention. We found that tape stripping, suction blistering, hair plucking, microbiopsy, and microneedle patching provide a variety of useful biosample types for laboratory analysis. In translational research, these technologies have the potential to replace more invasive methods like the punch biopsy, likely improving participation in studies.

Keywords: hair plucking, microbiopsy, microneedle patch, minimally, non-invasive, punch biopsy, skin research, suction blister, tape strip, translational

Introduction

Across the field of medicine, translational research has become key for better characterizing mechanisms of disease, producing new therapies, and personalizing treatments for patients [1]. Translational studies, especially in dermatology, require a sufficient amount and quality of tissue, containing the appropriate biosample content, for laboratory analysis. Skin biopsy is typically believed to be the gold standard for tissue acquisition. However, its invasive nature causes a small risk of bleeding and infection, with definitive scarring [2]. Some studies have also suggested biopsy can cause anxiety in certain patients [3,4]. These risks and concerns may limit participation in research studies, which can involve multiple biopsy procedures. Although collection of peripheral blood, saliva, and urine is less invasive than skin biopsies, the indirect relationship of these compartments to skin biology makes them less useful. In this review, we highlight new methods of minimally invasive skin sampling in dermatology, which have the potential to mitigate biopsy procedure risks and concerns in patients. We analyze the various modalities, the diseases in which they are studied, and the various laboratory tests that have been applied to each. Our goal is to review the data regarding minimally invasive skin sampling with the hope that researchers and clinicians may augment their current tools with these methods in future studies.

Methods

We queried PubMed in May 2021 using the following search string:

(“skin/pathology”[mesh] OR “skin/immunology”[mesh] OR “skin diseases/pathology”[mesh] OR ”hair/cytology”[mesh] OR (hair[tiab] AND cytology[tiab]) OR “Skin Diseases/diagnosis”[mesh] OR (skin[tiab] AND (immune[tiab] OR immunology[tiab] OR pathology[tiab] OR diagnosis[tiab] OR diagnostics[tiab]))) AND (sampling[tiab] OR “cell harvest”[tiab] OR “cell harvesting”[tiab] OR biopsy[tiab] OR biopsies[tiab] OR “Biopsy”[mesh] OR “skin microdialysis”[tiab] OR SMD[tiab] OR “Specimen Handling”[mesh] OR “plucked hair”[tiab] OR “tape strip”[tiab] OR “tape strips”[tiab] OR “tape stripping”[tiab]) AND (“minimally invasive”[tiab] OR “less invasive”[tiab] OR “less painful”[tiab] OR noninvasive[tiab] OR “non-invasive”[tiab]) NOT (mammary OR “lymph node” OR “sentinel node” OR neuropathy OR mastectomy OR pulmonary OR liver).

This search generated a total of 986 articles. We selected all original prospective, and retrospective studies; we also evaluated those that described techniques that demonstrated tissue acquisition capable of providing a large enough sample to run applicable laboratory tests. All review articles were excluded from our analysis. For each new modality identified, animal studies were only included if there were no studies that involved human participants. As a result, 53 articles were included in the final analysis for this review.

Results

Our research identified 13 unique minimally-invasive methods for biosample acquisition that are capable of providing sufficient samples for applicable laboratory tests in dermatology: tape stripping (23 studies [5–27]), suction blistering (3 studies [26,28,29]), hair plucking (5 studies [30–34]), microbiopsy (3 studies [35–37]), microneedle patching (3 studies [38–40]), fine needle aspiration (3 studies [41–43]), cutaneous or mucosal brushing and swabbing (5 studies [44–48]), pigmented lesion assays (2 studies [49,50]), slit aspirate, (2 studies [51,52]), filter paper impressions (1 study [53]), Flinders Technology Associates (FTA) classic card (1 study [54]), skin surface washing (1 study [55]), and ultrasonic extraction (1 study [56]). For each of the 13 modalities, we reviewed the biosamples collected (specifying whether DNA, RNA, or protein was extracted), in which disease the method was assessed, its laboratory applications, procedure, the quantity of obtained sample, its purpose, the required equipment, and, if provided, the time required to complete the procedure. These results are listed in **Table 1**. We summarize the key advantages and disadvantages of each device in **Table 2**.

Of the 13 methods reviewed, the modalities capable of providing sufficient sample to run the most laboratory tests were tape stripping, suction blistering, hair plucking, microbiopsy, and microneedle patches, which will each be discussed in further detail below.

Tape stripping

Tape stripping has been the most studied minimally invasive technology in recent years. It is heavily studied in patients with atopic dermatitis, especially in the pediatric population. Tape stripping involves the application of an adhesive substance to the skin, usually in an area of active disease. These adhesive substances are then removed and laboratory analyses are performed on the skin samples. Protein, RNA, and DNA can all be analyzed via various tests. In our review of studies, RNA extraction, RNA quantitation, RNA sequencing, and qRT-PCR can all be performed during analysis of RNA [5,9,11,15–19,22,24]. DNA isolation, cDNA synthesis and DNA microarrays can also be performed during DNA analysis [16,20,22]. Protein extractions, assays, and cytokine quantification can be performed to analyze protein content [6,8, 10,12–14,19,21,23]. Also, one study demonstrated that flow cytometry can be performed to characterize cell populations in diseased skin [23]. Few studies reported the yield of obtained samples, but one reported that the average mass of RNA recovered from four tape strips of normal skin is 0.92±0.35ng and 185±76ng from inflamed skin [16] and another study reported 300ng could be obtained from 30 tape strips [26]. For comparison, a 4mm punch biopsy can yield >10mg of RNA; recent studies have also shown that a 3mm punch biopsy could yield 150mg of protein [57]. Of

note, a study conducted by Simonsen et al. found no significant differences in quantities of obtained protein and mRNA between tape strips and skin biopsies [27]. Few studies reported the actual time required to perform tape stripping, but one study stated the sampling duration can take 50 minutes [26] whereas another reported it could take up to 90 minutes [17]. Patients reported little or no pain from the procedure [26] and studies did not report a need for bandaging or wound care afterwards [58]. Post procedural hyperpigmentation, a common sequalae of tape stripping, was cited to last less than a month [26]. A potential limitation of tape stripping is the depth to which biosample can be extracted from the skin. Biosample can be successfully acquired from the stratum corneum, but not to the level of the stratum granulosum [26]. Overall, tape stripping can acquire numerous biosamples and several laboratory tests can be applied allowing for extensive analysis. However, acquired samples are limited to the uppermost layers of the skin.

Suction blistering

Suction blistering obtains biosamples by attaching a dermal suction device to the skin, which applies gradually increasing suction pressure to create a fluid blister. From this newly formed blister, the interstitial fluid is removed via a needle and analyzed. This fluid contains inflammatory cells, proteins, RNA, and lipids. Suction blisters provide samples from all layers of the epidermis, but the artificial blister leads to incomplete sampling of the epidermal basement membrane [26]. One study, which evaluated T cells extracted from blisters in healthy volunteers with prior BCG vaccinations, cited the median number of cells per blister was 50,000 (range of 15,000-210,000 cells), [29]. The samples acquired by this method have been analyzed through bead arrays [28], flow cytometry [29], multiplex cytokine analysis [29], quantitative real-time reverse transcription PCR [26], SDS-PAGE [26], and western botting [26]. When compared to tape stripping, the yield of biosample (protein and RNA) was higher in suction blisters [26]. However, studies report long time requirements to create and obtain samples from suction blisters, ranging from 39 to 150 minutes [26,28]. Contrary to tape stripping, wound care may be necessary. One study reported the need for application of gauze and pressure dressings to the blister site after the procedure, which were removed after 10 days [59]. Similar to tape stripping, suction blisters cause minimal pain and only leave mild local hyperpigmentation without any scarring or long-term damage. However, the hyperpigmentation can last up to 6 months [29]. Suction blistering can procure large quantities of biosamples from all epidermal layers with minimal cosmetic damage, though sampling times can be long and wound care may be required.

Hair shaft plucking

Hair plucking was also frequently utilized as a minimally invasive technique to obtain biosamples. Typically, 4-30 plucked hairs with intact hair follicles are obtained, but the number needed is dependent on the type and location of the hair. Keratinocytes, which are present in the white sheath that surrounds hair follicles, are then used for analysis of DNA and RNA. Prior studies have demonstrated RNA extraction [30], DNA extraction [34], transcriptome and gene microarrays [30,32,33], novel HPV array [34], PCR [34], and quantitative real-time PCR [30,32] in the analysis of the white sheaths. Although the total amount of RNA extracted was not documented, the RNA quality achieved by this method, as measured by the RNA integrity number, ranged from 7.5 to 10 [30,33]. Studies have cited that it may take an experienced person about 5 minutes to obtain about 10 hairs and did not report the need for follow up wound care. One study demonstrated that keratinocytes obtained through hair follicles can provide disease-specific signatures through gene expression analysis, offering a minimally invasive technique that may be of diagnostic use for skin conditions in the future [30]. Altogether, hair shaft plucking can collect scalp keratinocytes for analysis in a time efficient manner.

Microbiopsy

Microbiopsy utilizes a novel stainless steel device, precision cut using lasers, to create a sub-millimeter skin punch biopsy tool. This microbiopsy device is pushed into the skin using a spring-loaded mechanism, which obtains a small sample of skin.

Both RNA and DNA have been evaluated by this method of sampling. Studies have documented DNA isolation [35,37], RNA isolation [35,36], whole genomic and transcriptome amplification [35], and quantitative real-time PCR [36] to be performed on samples. No studies in our review reported on the analysis of protein or cell populations from microbiopsy samples, providing a new area for research with this technique. The original microbiopsy punch was documented to obtain 5.9±3.4ng DNA and 9.0±10.1ng RNA [35]. Another method utilizing the microbiopsy, incorporated an absorbent layer within the microbiopsy device to obtain a simultaneous mixture of blood and skin. When using this alternative technique, the absorbent microbiopsy tool obtained a maximum total of 1.43±0.88ng RNA [36]. The process of the biopsy occurs within seconds, but researchers report that removal of the sample from the device can be time-consuming [36]. Compared to other traditional biopsy methods, local anesthetic is unnecessary, and the resulting wound heals without scarring. Patients report minimal pain and studies do not describe the need for post-procedure wound care [37]. The microbiopsy tool provides an additional minimally invasive technique for translational research. Further studies are needed to evaluate the capability of this device to analyze additional biosamples, including proteins and cells.

Microneedle patching

Microneedle patching is an emerging technology in dermatology research. However, the use of microneedle patches has not yet been reported in human studies. The method involves the application of a patch lined with microneedles to the skin. In some instances, these microneedles are coated with a biocompatible hydrogel to target specific antigens or antibodies. Upon application to skin, the hydrogel layer swells with the intake of interstitial fluid, then, antigen presenting cells (APCs) migrate into the matrix due to a localized inflammatory response induced by the needles. This leads to the production of chemokines and cytokine by APCs, recruiting T cells into the hydrogel matrix. Upon removal from the skin, the hydrogel layer can be dissolved and cells and other biosamples (RNA and protein) can be analyzed. Studies have demonstrated various ways microneedles can be applied; two studies specifically analyzed antigens and antibodies, performing tests like ELISA and immunoblotting [39,40]. Another study demonstrated entire cells could be extracted and analyzed through flow cytometry [38]. This study reported substantial yields for these cell populations as well. Two microneedle samples (pooled) for CD45, T, B, non-B/T, and APC cells provided 4700, 950, 900, 3000, and 1000 cells, respectively. Studies rarely described the time it took to perform an entire analysis, but one reported that the process could take two-to-three hours [39]. Microneedle patching has the capability to allow analysis of multiple biosamples, including a wide array of cell types from the skin, but further studies with human subjects are needed to assess its feasibility in patients with dermatologic diseases.

Other minimally invasive methods

Fine needle aspiration is a viable tool to procure biosamples for laboratory tests such as PCR, though its use has been limited in dermatology to the diagnosis of certain infectious diseases. Cutaneous brush swabbing has been used for numerous laboratory applications, although acquisition of biosamples has been limited to mucosal surfaces, scabs, and crusts of the skin. Pigmented lesion assay can provide sufficient biosample for a variety of laboratory tests and it has been demonstrated to be an effective tool in the diagnosis of melanoma. Slit aspirate specimens, filter paper impressions, and FTA classic cards, similar to brush swabs, rely on acquiring biosamples from the uppermost surface of lesional skin to diagnose certain infectious diseases. Skin surface washing involves attaching a well to the skin and washing the skin with buffered solution to acquire biosamples. Its prognostic and diagnostic use has been explored in psoriasis and atopic dermatitis. Similarly, ultrasonic extraction involves attaching a chamber to the skin of the patient. The chamber is filled with ultrasonic extraction medium and a sonicator is used to isolate biosamples from lesional skin.

Discussion

Skin biopsy is the standard procedure for biosample acquisition in translational research studies. A

drawback is that the procedure leads to scarring and comes with small risks of bleeding and infection. The procedure may also cause anxiety in certain patient populations. Translational research studies can require multiple biopsies and the risks and concerns associated with biopsy may limit participation. However, acquiring sufficient variety and quantity of biosample is critical for translational research and skin biopsy remains the gold standard for these studies.

We thoroughly reviewed all new minimally invasive technologies to date. Tape stripping, suction blistering, hair plucking, microbiopsy, and microneedle patching were capable of providing sufficient sample to run the most laboratory tests.

Tape stripping is the most studied technology to date. Studies have demonstrated this technique can acquire all biosample types, including DNA, RNA, protein, and cells. Numerous laboratory tests can be run on samples and patients experience minimal side effects. However, multiple tape strips are needed to acquire sufficient sample, sampling time can be long (50-90 minutes), and cells have only been acquired from the stratum corneum, the topmost layer of the epidermis. Suction blistering can provide biosamples to run multiple tests and yield higher amounts of RNA and protein than tape stripping. However, it is also limited by a long sampling time (39-150 minutes) and the need for wound care. Microbiopsy has been applied to a variety of skin diseases and the amount of DNA and RNA acquired has been quantified in studies. However, further reports on its sampling time and procedure are needed. Microneedle patching is a technology capable of acquiring multiple biosamples, including specific cell populations beyond the stratum corneum. The obtained cell populations have been characterized and quantified in studies. However, further studies with human participants are necessary.

Additional studies using standard skin biopsy as a comparator, with quantities of biosample totaled, would be helpful to evaluate the feasibility of replacing standard skin biopsy for the purpose of translational research in dermatology.

Conclusion

Minimally invasive technologies for biosample acquisition are an area of ongoing research. These technologies have the potential to replace more invasive methods of tissue acquisition, including the punch biopsy, thereby mitigating risks of scarring, bleeding, infection, and anxiety associated with the procedure, likely improving participation in translational research studies.

Potential conflicts of interest

Dr. Tina Bhutani has received research funding from Abbvie, Celgene, Galderma, Janssen, Pfizer, Regeneron, and Sun. She has served as an advisor for Abbvie, Boehringer-Ingelheim, Bristol Myers Squibb, Pfizer, Leo, Lilly, and Novartis. Dr. Wilson Liao has received research grant funding from Abbvie, Amgen, Janssen, Leo, Novartis, Pfizer, Regeneron, and TRex Bio. Edward Hadeler, Megan Mosca, Julie Hong, and Dr. Nicholas Brownstone have nothing to disclose.

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Table 1. *Studies assessing the use of non-invasive modalities of biosamples acquisition.*

-	Biosample description: skin depth and biosample type (DNA/RNA/ protein/cells)	Diseases (e.g. atopic dermatitis, psoriasis)	Molecular application (RNA sequencing, flow cytometry, etc.)	Procedure	Quantity (amount of biosamples, number of cells collected, etc.) Mean (SD)	Purpose (diagnostic, prognostic, etc.)	Materials required	Comments
Tape Stripping								
Simonsen, 2021 [27]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation, and barrier biomarkers (-/+/+/-)	Atopic dermatitis	qRT-PCR	8 consecutive tape strips applied to lesional site on the volar side of forearms or popliteal fossa in atopic patients, strips were evaluated for cytokine levels before and after topical treatment	Unknown	Prognostic (characterizing disease response to treatment)	D-Squame tape strip (CuDerm Corp)	
Kim, 2019 [5]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation, and barrier biomarkers (-/+/+/-)	Atopic dermatitis	qRT-PCR; hematoxylin and eosin staining; immunostaining	20 tape strips applied to lesional and nonlesional skin (5cm from lesion) in atopic patients, strips were evaluated by hematoxylin and eosin staining, immunostaining, and gene expression.	Unknown	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	
Koppes, 2016 [6]	Stratum corneum with evaluation for inflammatory mediators (-/-/+/-)	Atopic dermatitis	Cytokine evaluation	8 consecutive tape strips applied to lesional site on the volar side of forearms or popliteal fossa in atopic patients, strips were evaluated for cytokine levels before and after topical treatment	Unknown; 50 microliters were required for analysis	Prognostic (characterizing disease response to treatment)	D-Squame tape strip (CuDerm Corp)	
He, 2020 [7]	Stratum corneum with evaluation for immune markers, chemokines, and proteins related to	Atopic dermatitis	Olink proteomic assay	30 consecutive tape strips collected from representative lesions and nonlesional skin on the upper or lower extremities.	Unknown	Prognostic (characterizing disease response to treatment)	D-Squame tape strip (CuDerm Corp)	Measured inflammatory markers in AD patients undergoing

	atherosclerosis and cardiovascular risk. (-/-/+/-)							treatment with Dupixent.
Clausen, 2013 [8]	Stratum corneum with evaluation for barrier biomarkers (-/-/+/-)	Atopic dermatitis	ELISA; protein assay	16 tape strips applied to both lesional and nonlesional skin.	Protein concentration μg/ml (lesional=18, nonlesional=10)	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	Quantities were extrapolated from graphs provided in paper
Sølberg, 2020 [9]	Stratum corneum with evaluation for cellular biomarker and biomarkers of immune activation (-/+/+/-)	Atopic dermatitis	RNA extraction; RNA-seq	Tape samples were collected from both lesional and non-lesional skin areas. 2 sampling discs were applied consecutively to the skin area. Note: biopsy was collected from lesional skin areas as well (locations included dorsal hands, back, and arms)	Unknown	Prognostic (characterize disease activity at baseline)	Tape strip	Notable findings: Atopic dermatitis relevant pathways can be assessed by both tape strip and epidermal biopsy samples; skin relevant molecules can be detected and assessed by tape stripping
Yamane, 2009 [10]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (-/-/+/-)	Atopic dermatitis	Protein extraction; Immunoblotting; SDS-PAGE	2 consecutive tape strips applied to the lesional skin on patient's forearm.	60-150μg of total protein recovered in the 100μL extract from skin site	Prognostic	Skin tapes (HL Checker, Asahi Med, Tokyo, Japan)	
Guttman-Yassky, 2019 [11]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation, and barrier biomarkers (-/+/+/-)	Atopic dermatitis in pediatrics	qRT-PCR; immunostaining	16 consecutive tape strips applied to lesional skin in the antecubital fossa and nonlesional skin nearby on the same arm, strips are then evaluated with epidermal genetic information retrieval (EGIR™; DermTech International, La Jolla, CA, U.S.A.) for cellular biomarkers, mRNA expression (immune activation), barrier	Unknown; however, 500pg of total RNA was used for qRT-PCR	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	

				biomarkers (ex. FLG), and proteins (cytokines).				
Hulshof, 2019 [12]	Stratum corneum with evaluation for immunomodulatory mediators, natural moisturizing factors, and corneocyte morphology (-/-/+/-)	Atopic dermatitis in pediatrics	Protein assays (immunomodulatory mediator levels); UV high-performance liquid chromatography (natural moisturizing factors); Dermal texture index (corneocyte morphology)	6 consecutive tape strips applied to lesional site of the forearm, strips evaluated for corneocyte morphology, natural moisturizing factors, and immunomodulatory mediators.	Unknown	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	
McAleer, 2019 [13]	Stratum corneum with evaluation for cytokines, chemokines and natural moisturizing factors (-/-/+/-)	Atopic dermatitis in pediatrics	Natural moisturizing factor analysis; Cytokine analysis (immunoassay); Cytokine quantification	8 consecutive tape strips applied to nonlesional skin 2cm away from viable eczematous areas, strips evaluated for cytokine type and quantification and natural moisturizing factor levels.	Unknown	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	
Lyubchenko, 2020 [14]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (-/-/+/-)	Atopic dermatitis in pediatrics	Protein extraction; quantification; multiplex assay for cytokine analysis	20 consecutive tape strips collected from lesional skin over the forearm of patients utilizing a pressure instrument for skin sample and stored in glossy storage cards.	Atopic dermatitis skin=0.15mg/mL; Normal skin=0.11mg/mL	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp); Squame D500 pressure instrument	
Pavel, 2020 [15]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation,	Atopic dermatitis in pediatrics	RNA extraction; RNA-seq	16 large tape strips applied to extremities (antecubital or popliteal fossa) and from nonlesional skin on the same extremity, far from lesions.	Unknown	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	

	and barrier biomarkers (-/+/-/-)							
Wong, 2004 [16]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (+/+/-/-)	Irritant contact dermatitis; Allergic contact dermatitis	RT-PCR; DNA microarray	Irritant solution 1% SLS or water (vehicle control) applied to mid back of patient, 4 tapes then applied for cell recovery.	Average mass of RNA recovered from normal skin site=0.92 (0.35)ng. Average mass of RNA recovered from water occluded skin=0.69 (0.27)ng. Average mass of RNA recovered from SLS inflamed skin=185 (76)ng	Diagnostic	Irritant occlusive patch; Skin tape	
Morhenn, 1999 [17]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (-/+/-/-)	Irritant contact dermatitis; Allergic contact dermatitis	RNA extraction; RNase protection assay (RPA) analysis	Irritant solution 0.5% sodium lauryl sulfate (SLS) applied for 72 hours to induce ICD and dibutyl squarate (2%) in acetone applied for 48 hours and reapplied 14 days later under occlusion to induce allergic contact dermatitis reactions. Tape strips applied up to 23 times to sites.	Unknown	Diagnostic	D-Squame tape strip (CuDerm Corp)	Time involved: 90 minutes
Wachsman, 2011 [18]	Stratum corneum with evaluation for genetic markers (-/+/-/-)	Melanoma	RNA isolation and quantification (qRT-PCR); RNA amplification and array hybridization; Gene expression analysis	Tape strips applied to suspicious pigmented lesions then evaluated with epidermal genetic information retrieval (EGIR™; DermTech International, La Jolla, CA, U.S.A.)	Unknown	Diagnostic	Adhesives Research tape strips	

He, 2020 [19]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation, and barrier biomarkers (-/+/-/-)	Atopic dermatitis; Psoriasis	RNA extraction; RNA-seq; qRT-PCR	20 consecutive tape strips applied to lesional and nonlesional skin in the extremities (in close proximity to but >10cm away from lesions) of patients.	39.7 (56.6)ng; required 500pg of RNA for qRT-PCR	Prognostic (characterize disease activity at baseline)	D-Squame tape strip (CuDerm Corp)	Time involved: Describes time-consuming RNA isolation as a limitation of tape stripping
Taslimi, 2017 [20]	Stratum corneum with sampling and isolation of DNA for specific diagnosis (+/-/-/-)	Leishmaniasis	DNA isolation; PCR amplification	Tape strips applied to infected and non-infected areas for DNA sampling and analysis.	19.5ng/μL of DNA from lesions; 1.9ng/μL isolated from control skin sites	Diagnostic	D-Squame tape strip (CuDerm Corp)	
Mehul, 2019 [21]	Stratum corneum with evaluation for immune markers, natural moisturizing factor (NMF) and protein analysis (-/-/+/-)	Cutaneous T-cell lymphoma (CTCL); Psoriasis	Proteome analysis (mass spectrometry, Luminex assay); NMF analysis (Posneg)	Tape strips applied to chest and forearm of T-cell lymphoma patients and forearms of psoriasis patients, samples were evaluated using quantitative mass spectrometry and Luminex assays including Posneg NMF.	For CTCL stratum corneum lesional (pg/mg), quantities of CXCL10, CCL27, CXCL9, TNF, CXCL8, sICAM-1, VEGF-A, MPO, IL3, MIF, and IL1A collected were 637, 811, 424, 29, 1164, 31281, 281, 2329, 42, 585, and 640 for lesional skin, respectively. For non-lesional skin: 43, 88, 50, 4, 245, 6919, 93, 787, 112, 330, 2403, respectively.	Diagnostic and prognostic	Tape strip	
Tam, 2020 [22]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation,	Allergic contact dermatitis; Irritant	RNA extraction; purified RNA electrophoresis; cDNA	20 consecutive tape strips applied to same site	Unknown	Diagnostic	D-Squame tape strip (CuDerm Corp)	

	and barrier biomarkers (+/+/-/-)	contact dermatitis	synthesis; qPCR; immunohisto chemistry					
Berekmeri, 2018 [23]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (-/-/+/+)	Psoriasis; Atopic dermatitis	Protein extraction; ELISA; Immunoassay ; Flow cytometry	11 tape strips applied to lesional and nonlesional skin (ventral forearm).	Unknown	Diagnostic	D-Squame tape strip (CuDerm Corp)	
Benson, 2006 [24]	Stratum corneum with evaluation for cellular biomarkers and biomarkers of immune activation (-/+/-/-)	Psoriasis	RNA extraction; reverse transcription and amplification; RNA quantitation	12 tape strips applied to nonlesional skin and 4 tape strips applied to lesional skin; nonlesional sample sites included deltoid and bilateral back at level of mid trapezius.	116 (19.5)ng of RNA (lesional), 83ng of RNA recovered from 3 control sites.	Diagnostic	Skin tapes (Adhesives Research, PN 90068-991, Glen Rock, PA)	Researchers note that 90068 adhesive outperformed other acrylic adhesives (including Cu Derm, Dallas, TX) for amount of RNA extracted
Merola, 2021 [25]	Stratum corneum with evaluation for biomarkers (-/+/-/-)	Cutaneous lupus erythema-tosus	RNA extraction; qPCR	4 adhesive tape strips were used to collect each patient sample.	4 tape strips averaged 42.58ng of RNA	Diagnostic	DermTech tape strips	
Suction Blister								
Clark, 2015 [28]	Interstitial fluid with inflammatory cytokines, chemokines, and growth factors (-/-/+/-)	Systemic sclerosis	Fluid profiled by Luminex® (Life Technologies, Paisley, UK) bead array	Uses a dermal suction machine left on for several hours at a time (2.5 hours in some cases) which applies suction pressure with small stepwise increases to the skin. Interstitial fluid is taken from the newly created blister and analyzed.	Concentrations of factors were obtained	Prognostic	Suction chamber	Median concentration obtained for : EGF, epidermal growth factor; FGF, fibroblast growth factor; Flt, FMS-like tyrosine kinase; GCSF, granulocyte colony-stimulating

								factor; GMCSF, granulocyte–macrophage colony-stimulating factor; GRO, growth regulated oncogene; IFN, interferon; IL, interleukin; IL1RA, interleukin-1 receptor antagonist; IP-10, Interferon gamma induced protein 10; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normal T cell expressed and secreted; SSc, systemic sclerosis; TGF-α, transforming growth factor alpha; TNF-α, tumor necrosis factor alpha; VEGF, vascular

								endothelial cell growth factor. Time involved: 2.5 hours
Holm, 2018 [29]	Interstitial fluid with cells (T cells), cytokines, and other proteins representative of the skin microenvironment (-/- /+/+)	Healthy volunteers with prior BCG vaccination evaluating T cells after PPD inoculation	Flow cytometry and multiplex cytokine analysis	Uses a dermal suction machine left on for several hours at a time (2.5 hours in some cases) which applies suction pressure with small stepwise increases to the skin. Interstitial fluid is taken from the newly created blister and analyzed.	Median number of cells per blister was 50,000 (range: 15,000 - 210,000 cells). Number of cells within blister fluid correlated with the clinical response to recent tuberculin skin test (amount of induration)	Investigational	Suction chamber	Collapsed suction blisters heal without scarring, but some degree of hypopigmentation may occur. Infections are rare.
Tape stripping; suction blistering								
Svoboda, 2017 [26]	Stratum corneum with evaluation for cellular biomarkers, biomarkers of immune activation, and barrier biomarkers (-/+/+/-)	N/A healthy volunteers	Protein extraction; RNA extraction; RNA amplification; qRT-PCR; SDS-PAGE and western blotting; immunohistochemistry; high performance thin layer chromatography (HPTLC) of lipids	Suction blistering (SB) performed on left volar forearm of the donors; Tape stripping (TS) was also performed on left volar forearm, 30 consecutive discs consecutively applied onto the same spot and removed.	Weight in mg (TS=2mg, SB= 6mg P<0.0001); protein yield µg/mg (TS=20, SB=95 P<0.0001), total RNA yield ng/mg (TS=100, TS amplified=600, SB=1400 P<0.0001)	Investigational	D-Squame tape strip (CuDerm Corp); Suction chamber	Little to no pain reported for each technique, reported by the volunteers. The skin of volunteers developed local hyperpigmentation after SB and TS, less than a month in TS and over a 6-month period in case of SB, no permanent damage in either technique; Quantities were extrapolated from graphs

								provided in paper Time involved: Sampling durations: SB=62 minutes (range 39-105 min); TS=50 minutes (range 47-55 min), depends on number of strips used.
Hair shaft plucking								
Shalbaf, 2019 [30]	Hair follicles with keratinocytes, in which gene analysis was performed (-/+/-/-)	Chronic discoid lupus erythematosus (CDLE), psoriasis and healthy controls	Microarray and quantitative real-time PCR	Four to five plucked hairs were obtained from the scalp and only hairs with fully visible hair follicle were used. Hairs with incompletely plucked follicles or telogen hairs are not included. The hair shaft is then cut off and only the white sheath is used for analysis.	At least 100ng per person (as this was the amount used in the microarray)	Diagnostic	None	RNA integrity number (RIN) number for RNA samples ranged between 7.5 and 10, with the majority of samples between 9 and 10. Time involved: Takes an experienced person 5 minutes to obtain about 10 hairs for the appropriate yield
Hung, 2015 [31]	Hair follicles with hair-derived keratinocytes to generate integration-free human induced pluripotent stem cells	Not specified	Not specified	Five to ten plucked hairs were obtained from the scalp and only hairs with fully visible hair follicle were used. Hairs with incompletely plucked follicles or telogen hairs are not included.	Unknown	Investigational	None	

	(-/-/-/+)			The hair shaft is then cut off and only the white sheath is used for analysis.				
Akashi, 2010 [32]	Hair follicle with keratinocytes, in which in which gene analysis was performed (-/+/-/-)	Abnormali-ties with the circadian clock	DNA microarray analysis, quantitative real-time PCR	Number needed to pluck was dependent on hair type. Plucked hairs were taken from both the scalp and the chin. Hairs with incompletely plucked follicles or telogen hairs are not included. The hair shaft is then cut off and only the white sheath is used for analysis.	Unknown	Investigational	None	The required number of hairs to reduce the experimental error was dependent on hair type, where 5 thick head hairs were sufficient for reproducible detection and 20 thin hairs were required for reliable detection.
Vogt, 2017 [33]	Hair follicle with keratinocytes (-/+/-/-)	Male androgenic alopecia	Microarray	A group of 30 hair follicles were plucked. Plucked hairs were taken from both the scalp and the chin. Hairs with incompletely plucked follicles or telogen hairs are not included. The hair shaft is then cut off and only the white sheath is used for analysis.	At least 100ng per person (as this was the amount used in the linear T7-based amplification)	Diagnostic	None	The quality of total RNA was checked, all RNA samples revealed RIN values between 8.6 and 10.
Rollison, 2008 [34]	Hair follicle with keratinocytes (+/-/-/-)	HPV	DNA extraction, PCR, and novel HPV assay	Six to eight eyebrow hairs were plucked with tweezers. The hairs with the intact follicles were snap frozen in liquid nitrogen until analysis.	Unknown	Diagnostic	None	
Microbiopsy								
Lin, 2013 [35]	Skin sample for RNA and DNA isolation (+/+/-/-)	Healthy volunteers and AK samples	DNA and RNA isolation and quantification, whole genomic amplification, and whole	Novel microbiopsy device was fabricated by laser cutting plates of stainless steel resulting in a sub-millimeter skin punch biopsy. Microbiopsy device was pushed into the arm using a	One microbiopsy punch obtains 5.9 (3.4)ng DNA and 9.0 (10.1)ng RNA	Investigational	Microbiopsy tool (0.15mm channel)	Local anesthetic is not needed

			transcriptome amplification	spring-loaded mechanism and removed to obtain a sample.				
Lei, 2019 [36]	Simultaneous sampling of skin and blood for RNA isolation (-/+/-/-)	Healthy volunteers	RNA isolation, cDNA synthesis, RT-qPCR	Microbiopsy device was laser cut from stainless steel sheets and an absorbent layer placed between the layers when assembled. Novel microneedle-based diagnostic platform, absorbent microbiopsy, was pushed into the arm, left for 10s seconds and removed, obtaining a small amount of skin mixed with blood.	1.43 (0.88)ng RNA	Investigational	Microbiopsy tool	Local anesthetic is not needed, and the application site does not cause scarring Time involved: "within seconds" for the biopsy, but the main limitation of is the time-consuming nature of the removal of the microbiopsy from the device"
Churiso, 2020 [37]	Skin sample for DNA analysis (+/-/-/-)	Cutaneous leishmaniasis	DNA extraction and real-time PCR	Novel microbiopsy device was fabricated by laser cutting plates of stainless steel resulting in a sub-millimeter skin punch biopsy. Skin was anesthetized with topical cream and microbiopsy device was pushed into the arm using a spring-loaded mechanism. Device was removed to obtain a sample.	Unknown	Diagnostic	Microbiopsy tool	Does not require local anesthesia. Microbiopsy device has a chamber volumetric size of 0.003mm 3 that is more than 6000 times smaller than a conventional 3mm punch biopsy and more than 5 times smaller than an 18GA syringe needle

								Time involved: "rapid biopsy time"
Microneedle patches								
Mandal, 2018 [38]	Viable cells and interstitial fluid (ISF) acquired from skin, incorporation of antigen and adjuvant carrying nanoparticles into sampling layer can enrich for memory cells and TRMs. (-/+/-/+)	N/A	Flow cytometry	Application of square array of pyramidal solid polymer microneedles (each 250µm width, 600µm height) coated with biocompatible hydrogel layer embedded with adjuvants and lipid nanocapsules containing antigens of interest. Upon application to skin, hydrogel layer swells with intake of ISF, APCs migrate to matrix in response to localized inflammatory response induced by microneedle penetration, APCs uptake nanocapsule antigens, produce cytokines and chemokines which recruit T cells into gel coating. After removal from skin, hydrogel layer is dissolved, releasing cells for analysis, subject to immunological tools for phenotyping and immune profiling.	Number of cells per two microneedle samples (pooled) for CD45, T, B, non B/T, and APC cells was 4700, 950, 900, 3000, and 1000, respectively.	Investigational	Microneedle patch with hydrogel layer	Provides cell quantities obtained for various antigen specific microneedle patches at different time points. All quantities presented in this table were extrapolated from graphs. Studies occurred in mouse models. Time involved: Variable, samples were collected at 12-, 24-, and 48-hour intervals to identify optimum times for cell collection
Wooi Ng, 2015 [39]	Interstitial fluid containing proteins including inflammatory antibodies and antigens. (-/-/+/-)	N/A	Antigen acquisition; Antibody immobilization; ELISA; Immuno-blotting	Polylactic acid (PLA) microneedle devices were firmly applied to hairless skin, eventually washed and incubated in solution to protect non-specific antigen binding sites.	Detection limit of microneedle device is ≤10pg/mL	Investigational	Polylactic acid (PLA) microneedle devices	Time involved: 2-3 hours

Coffey, 2018 [40]	Interstitial fluid containing proteins including inflammatory antibodies. (-/-/+/-)	N/A	Antibody acquisition	Microprojection arrays (MPAs) and microneedle arrays applied to skin to access the dermal layer of skin.	Unknown	Investigational	Micro-projection array (MPA)	
Fine-Needle Aspiration								
Eddyani, 2009 [41]	Aspiration of cells and fluids (+/-/-/-)	Nonulcerative Buruli ulcer	PCR	Up to 6 aspirates were taken from each lesion using fine-gauge needles (23GA by 25mm) attached to 10ml syringes, the needle was advanced through the skin without applying suction, for nonulcerative forms the middle of the lesion is sampled, for ulcerative the edges are sampled. Suction is applied while moving the needle back and forth repeatedly through the lesion in multiple directions.	Unknown	Diagnostic	Fine-gauge needles and suction	
Phillips, 2009 [42]	Aspiration of cells and fluids (+/-/-/-)	Nonulcerative Buruli ulcer	PCR	Aspirates were taken from each lesion using fine-gauge needles (21GA) attached to 5ml syringes, the needle was advanced through the skin without applying suction, for nonulcerative forms the middle of the lesion is sampled. Suction is applied while moving the needle back and forth repeatedly through the lesion in multiple directions.	Unknown	Diagnostic	Fine-gauge needles and suction	
Cassisa, 2010 [43]	Aspiration of cells and fluids (+/-/-/-)	*Mycobacterium ulcerans*	PCR	Aspirates were taken from each lesion using fine-gauge needles (20GA, 250mm) attached to 5ml syringes, the needle was advanced through the skin without applying suction, for nonulcerative forms the middle	Unknown	Diagnostic	Fine-gauge needles and suction	

				of the lesion is sampled. Suction is applied while moving the needle back and forth repeatedly through the lesion in multiple directions.				
Cutaneous or mucosal brushing/swab								
Daoui, 2020 [44]	Cutaneous samples (+/-/-/-)	Cutaneous leishmania-sis	PCR and nested PCR amplification of cDNA	A cotton swab was gently rubbed over the ulcer 10 times around the border of an ulcer. For non-ulcerated lesions such as papules and nodules, swab samples were taken from the incision made while obtaining the skin scrapings.	Unknown	Diagnostic	Cotton swab	
Blaizot, 2020 [45]	Cutaneous samples (+/-/-/-)	Cutaneous leishmania-sis	Real time PCR	After removing overlying scabs or crusts, a cotton swab was pressed and rotated 360 degrees at the center of the ulcerated lesions	Unknown	Diagnostic	Cotton swab	
Hamizan, 2019 [46]	Nasal mucosal brushing analyzed for specific biomarkers (spIgE) (-/-/+/-)	Allergic rhinitis	Immunoassay	Nasal brushing of both nasal cavities was performed using a cytology brush.	Unknown	Diagnostic	Cytology brush	
Schmohl, 2012 [47]	Wound exudate with evaluation of inflammatory mediators, chemokines, and matric metalloproteases (-/-/+/-)	Diabetic ulcers	LDS-PAGE and multiplexed bead-based sandwich immunoassay s	Nylon-flocked swabs and film dressings were used to collect wound fluid from foot ulcers of diabetic patients.	Using the swabbing technique, a median sample volume of 40μL (2–120μL) was obtained.	Prognostic	Nylon-flocked swabs	
Boni, 2017 [48]	Cutaneous samples (+/-/-/-)	Leishmania-sis	PCR and qPCR	After removing overlying scabs or crusts with a moist gauze, a cotton swab is rubbed over the surface of the lesion.	Unknown	Diagnostic	Cotton swab	
Pigmented lesion assay								
Gerami, 2014 [49]	Adhesive patch applied to the skin to	Melanoma	mRNA extraction;	Adhesive patch applied to pigmented lesion and briskly	Unknown	Diagnostic	Adhesive patch,	Results later validated in the

	obtain mRNA and gene analysis. (+/+/-/-)		cDNA synthesis, qRT-PCR; gene expression quantitation	rubbed 15 times in a circular motion with a 1cm diameter rounded device before patch was removed, a total of 4 patches per lesion.			pigmented lesion assay (DermTech, Inc)	following study: Gerami, 2017,(60) identified LINC00518 and PRAME gene expression as sensitive markers for melanoma detection.
Ferris, 2019 [50]	Adhesive patch applied to the skin to obtain mRNA and gene analysis. (+/+/-/-)	Melanoma	mRNA extraction; cDNA synthesis, qRT-PCR; gene expression quantitation	Adhesive patch applied to pigmented lesion and later removed for analysis	Unknown	Diagnostic	Adhesive patch, pigmented lesion assay (DermTech, Inc)	Follow up study, Ferris, 2017,(61), a study that demonstrates the utility of this tool in the clinical setting, improving biopsy specificity while maintaining or improving sensitivity. Ferris, 2019,(62) reviews registry data demonstrating 99.9% of PLA(-) lesions were clinically monitored, and 96.5% of all PLA(+) lesions were appropriately biopsied.
Slit aspirate specimen								
Verma, 2012 [51]	Lesional skin for DNA analysis	Post-kala-azar dermal	qPCR	The chosen lesion, preferably an indurated one, was cleaned with spirit and allowed to dry. The	Unknown	Diagnostic	Scalpel and NET buffer	

	(+/-/-/-)	Leishmania-sis		lesion was gently pinched between index finger and thumb for 1–2 min, exerting enough pressure to blanch it. A clean cut about 5 mm long and 3mm deep was made with a sterile scalpel to reach the infiltrated layer of dermis. The blade of the scalpel was turned 90°, and using the blunt side of blade, the sides of the cut were scraped two or three times to obtain tissue pulp. This material (approximately 5µl) was then transferred from scalpel blade to the tube containing 250µl NET buffer. DNA from slit aspirate was extracted in 25µl distilled water using QIAamp DNA Tissue kit according to the manufacturer's instructions.				
Kamal, 2010 [52]	*M. leprae* protein (+/-/-/-)	Pediatric leprosy	In situ PCR on slit-skin smears	Slit-skin smears were taken from lesion site for AFB staining (Ziehl-Neelsen method) and in situ PCR.	530bp fragments of gene encoding the 36kD protein of *M. leprae*	Diagnostic	Scalpel	
Filter paper impressions								
Boggild, 2010 [53]	Filter paper sampling of ulcer fluid. (+/-/-/-)	Cutaneous leishmania-sis	PCR	Filter paper gently pressed onto moist ulcer base, allowing fluid to be wicked onto the filter paper, which is then air-dried, stored in ethanol for PCR testing.	Unknown	Diagnostic	Filter paper	
Flinders Technology Associates (FTA) classic card								
Kato, 2010 [54]	Leishmaniasis DNA, nucleic acids (+/+/-/-)	Leishmania-sis	Genomic DNA stored on FTA Cards at room temperature for over 17 years (and counting) has	Tissue materials were taken by aspirating or scraping the margins of active lesions of a patient and then spotted onto an FTA classic card (Whatman, Newton Center, MA) and stored at room temperature. 2mm-	Four sample areas for application of up to 500µL whole blood	Diagnostic	FTA card	Time involved: “Minimal time involvement for sample collection”

			been successfully amplified by PCR. RNA, being chemically less stable than DNA, is best analyzed upon return of samples to the laboratory. Frozen storage is helpful for RNA preservation.	diameter disks were punched out from each filter paper and washed three times with FTA purification reagent (Whatman) and once with Tris-EDTA buffer. The disks were air dried and subjected directly to PCR amplification.				
Skin surface wash sampling								
Portugal-Cohen, 2012 [55]	Solution containing inflammatory cytokine (TNFα, IL1α and IL6) levels, total antioxidant scavenging capacity, uric acid content, and fluorescent emission spectra of tryptophan moieties, collagen cross-links and elastin cross-links (-/-/+/-)	Psoriasis, atopic dermatitis, and healthy volunteers	Oxygen radical absorbance capacity (ORAC) assay, HLPC system, ELISA	A well is placed on the skin and attached with parafilm foil. One ml of phosphate-buffered saline (PBS) injected into the well through a small opening and incubated for a 30min period. The opening is covered using parafilm foil during incubation. After, the solution is extracted by a syringe and stored.	Unknown	Diagnostic and prognostic	Skin well	Time involved: At least 30 minutes
Ultrasonic ultrasound								

Ogura, 2012 [56]	Extracted several classes of biomolecules such as proteins, lipids, and nucleic acids, enabling creation of skin's unique "biomolecular signature" (+/+/+/-)	Atopic dermatitis, psoriasis	RNA and DNA were sequentially isolated with TRIzol LS (Invitrogen, Carlsbad, CA)., SDS-PAGE analysis, western blotting, Nucleic acids were quantified with UV spectroscopy	Chamber was glued to the shaven area with a minimal amount of cyanoacrylate adhesive. The chamber was filled with 1.8ml of ultrasonic extraction medium. Extractions were performed with a 600 Watt sonicator (Sonics and Materials, Newtown, CT) operating at a frequency of 20kHz. The ultrasound transducer was placed at a distance of 5mm from the skin surface for 5min.	Unknown	Prognostic	Sonicator	Time involved: 5 minutes for sonicator process

Table 2. *Advantages and disadvantages of minimally invasive devices.*

Minimally invasive device	Advantages	Disadvantages
Tape strips	Can acquire all biosample types (DNA/RNA/Protein/Cells) Multiple laboratory tests can be run on samples Minimal side effects (hyperpigmentation for 1 month)	Requires multiple tape strips to acquire sufficient sample Long sampling time (50-90 minutes) Can only acquire cells from stratum corneum
Suction blister	Multiple laboratory tests can be run on samples Higher yield than tape stripping (RNA/protein)	Long sampling time (39-150 minutes) Longer side effects (hyperpigmentation up to 6 months) Subjects require wound care
Hair shaft plucking	Multiple laboratory tests can be run on samples Short sampling time (5-10 minutes)	Can only analyze scalp keratinocytes
Microbiopsy	Multiple laboratory tests can be run on samples	Studies report challenges with removing sample from device
Microneedle patches	Multiple laboratory tests can be run on samples Can acquire multiple cell populations	Lack of data on human subjects
Fine needle aspiration	Good diagnostic capabilities	Limited evaluation of use in dermatology studies Few laboratory tests have been evaluated with samples
Cutaneous brush swab	Multiple laboratory tests can be run on samples	Most studies have evaluated its use in diagnosing infectious diseases only
Pigmented lesion assay	Multiple laboratory tests can be run on samples	Aside from its use in diagnosing melanoma, has not been studied in other conditions
Slit aspirate specimen	Minimal equipment required	Use has only been studied in infectious disease on the skin
Filter paper impressions	Minimal equipment required	Use has only been studied in infectious disease on the skin
FTA classic card	Low sampling time reported	Use has only been studied in infectious disease on the skin
Skin surface washing	Can evaluate numerous proteins for diagnostic and prognostic purposes	Long sampling time (at least 30 minutes)
Ultrasonic ultrasound	Multiple laboratory tests can run on samples Low sampling time reported	Only one study on device Lack of data on human subjects