(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 June 2022 (02.06.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/115714 A1

(51) International Patent Classification:
A61B 5/15 (2006.01) *A61B 10/02* (2006.01)
A61B 5/00 (2006.01) *A61M 5/00* (2006.01)
A61B 10/00 (2006.01) *A61M 37/00* (2006.01)

(21) International Application Number:
PCT/US2021/061033

(22) International Filing Date:
29 November 2021 (29.11.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/119,511 30 November 2020 (30.11.2020) US

(71) Applicant: **MINDERA CORPORATION** [US/US]; 5795 Kearny Villa Road, San Diego, California 92123 (US).

(72) Inventors: **DICKERSON, Tobin**; 5795 Kearny Villa Road, San Diego, California 92123 (US). **TAFT, Bradford**; 5795 Kearny Villa Road, San Diego, California 92123 (US). **LEE, Byung-In**; 5795 Kearny Villa Road, San Diego, California 92123 (US).

(74) Agent: **PESSIAN, Maysam**; WILSON SONSINI, 650 Page Mill Road, Palo Alto, California 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: MICRONEEDLE DEVICES AND METHODS, AND SKIN CONDITION ASSAYS

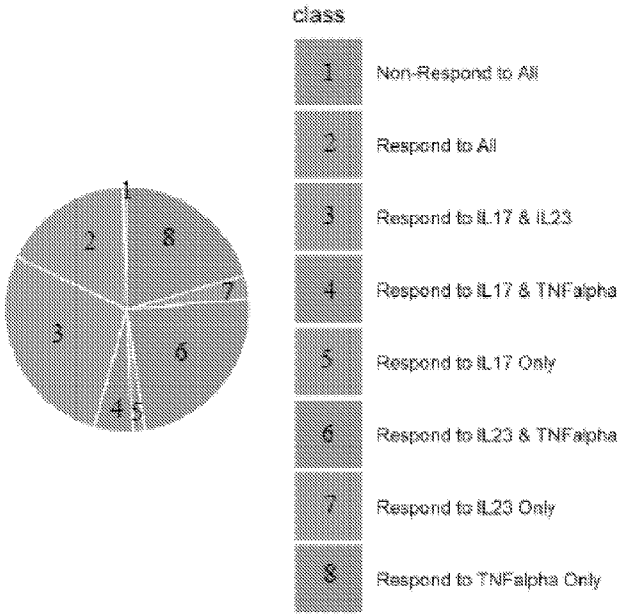


FIG. 9

(57) Abstract: The present disclosure provides microneedle devices and kits for extracting biomarkers from a tissue. The present disclosure provides methods selecting a treatment for an autoimmune disease. The present disclosure also provides methods of nucleic acid analysis to prospectively predict a patient's response to a therapeutic.

WO 2022/115714 A1

[Continued on next page]

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

MICRONEEDLE DEVICES AND METHODS, AND SKIN CONDITION ASSAYS

CROSS-REFERENCE

[0001] This application claims benefit of priority of U.S. Provisional Application No. 63/119,511, filed on November 30, 2020, the contents of which are incorporated by reference in its entirety.

BACKGROUND

[0002] Nearly 4% of the world's population is affected by one of the more than 80 known autoimmune diseases. In the United States alone, 24 million people are afflicted by an autoimmune disease. The cost of treating autoimmune diseases in the United States is estimated to be greater than 100 billion US dollars, annually. Autoimmune diseases can encompass a wide variety of different diseases and conditions that may impact a single tissue or organ, or many tissues or organs simultaneously. Some autoimmune diseases specifically target the skin. While the etiology of any particular autoimmune disease is often unknown, an imbalance in immune regulation is often involved.

[0003] For example, psoriasis is a chronic autoimmune disease characterized by raised areas of abnormal skin. It is a chronic condition that can cause thick, scaly patches, or plaques, to form on the skin. It is estimated that more than 8 million people in the United States have psoriasis. Currently, there is no cure for psoriasis, although there are treatments available to control the symptoms.

[0004] Microneedle devices comprising arrays of relatively small structures, sometimes referred to as microneedles or micro-pins, can be used in connection with the delivery of therapeutic agents and other substances through the skin and other surfaces. While treatments exist for autoimmune diseases, patient response is variable. There is a need for new strategies for determining a likelihood that a subject with an autoimmune disease will respond to a therapeutic drug.

[0005] To treat a subject's autoimmune disease, a physician may prescribe a therapeutic aimed at inhibiting or blocking a particular immune system biochemical pathway to manage symptoms. Unfortunately, due to the genetic variability of subjects with autoimmune diseases, a single therapeutic may not be an ideal candidate for two subjects with similar autoimmune diseases. This variability between subjects' genetic profiles leads to a trial and error methodology for prescribing therapeutics, often requiring several months of administering a therapeutic that may prove to be ineffective in treating a given subject. This approach is both costly to insurance

payers and an ineffective route of managing an autoimmune disease that may have substantial impact on quality of life for the subject.

SUMMARY

[0006] An aspect of the present disclosure provides a microneedle device comprising: 1) a microneedle region comprising (i) a microneedle base substrate comprising a first base substrate surface and a second base substrate surface, wherein the first base substrate surface and the second base substrate surface are positioned on opposite sides of the microneedle base substrate; and (ii) a plurality of microneedles protruding from the first base substrate surface; and b) a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate and comprising a support substrate depth, wherein the support substrate depth is greater than the minimum distance between the first base substrate surface and the second base substrate surface.

[0007] Another aspect of the present disclosure provides a microneedle device comprising: 1) a microneedle region comprising a microneedle base substrate comprising a first base substrate surface with a plurality of microneedles protruding from the first base substrate surface, the microneedle base substrate also comprising a second base substrate surface on a side opposite from the first base substrate surface, the second base substrate surface comprising a recess aligned with at least a portion of the plurality of microneedles; and b) a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate.

[0008] In some embodiments, the minimum distance between the first base substrate surface and the second base substrate surface of the microneedle base substrate is between about 1 μm to about 500 μm less than the depth of the support substrate. In some embodiments, the minimum distance between the first base substrate surface and the second base substrate surface is between 150 μm to about 350 μm. In some embodiments, the ratio between (a) the minimum distance between the first base substrate surface and the second base substrate surface, and (b) the support substrate depth is at least 1:5. In some embodiments, the microneedle region comprises a perimeter and the support substrate are adjacent to at least half of the perimeter. In some embodiments, the support substrate comprises a first support substrate surface proximal to the plurality of microneedles (at times, referred to herein as "the front surface of the support substrate surface") and a second support substrate surface distal to the plurality of microneedles (at times, referred to herein as "the back surface of the support substrate surface") and positioned opposite to the first support substrate surface, and wherein the second base substrate surface is not coplanar with the second support substrate surface distal to the plurality of microneedles. In

some embodiments, the first base substrate surface is not coplanar with a surface of the support substrate. In some embodiments, the plurality of microneedles is plasma treated. In some embodiments, a plurality of probes is coupled to a microneedle of the plurality of microneedles. In some embodiments, the plurality of probes comprises a negative charge. In some embodiments, the plurality of microneedles comprises a polyolefine resin. In some embodiments, the polyolefine resin comprises one or both of Zeonor 1020R or Zeonor 690R. In some embodiments, a microneedle of the plurality of microneedles is non-dissolvable. In some embodiments, a microneedle of the plurality of microneedles is pyramidal. In some embodiments, a microneedle of the plurality of microneedles is solid. In some embodiments, an angle between a base of the microneedle and the microneedle base substrate is between about 60° and about 90°. In some embodiments, the recess aligned with at least a portion of the plurality of microneedles comprises a width that is greater than a width of a mechanical applicator.

[0009] Another aspect of the present disclosure provides a kit comprising microneedle device comprising: 1) a microneedle region comprising a microneedle base substrate comprising a first base substrate surface with a plurality of microneedles protruding from the first base substrate surface, the microneedle base substrate also comprising a second base substrate surface on a side opposite from the first base substrate surface, the second base substrate surface comprising a recess aligned with at least a portion of the plurality of microneedles; and b) a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate, and (c) a mechanical applicator that fits within the recess aligned with at least a portion of the plurality of microneedles.

[0010] In some embodiments, at least one of the plurality of microneedles is coupled to a nucleic acid probe. In some embodiments, the nucleic acid probe comprises a homopolymeric sequence. In some embodiments, the homopolymeric sequence comprises thymine or uracil. In some embodiments, the nucleic acid probe comprises DNA. In some embodiments, the nucleic acid probe comprises thymine. In some embodiments, the nucleic acid probe comprises thymidine. In some embodiments, the nucleic acid probe is covalently linked to the microneedle. In some embodiments, the support substrate comprises a fiducial marker. In some embodiments, no more than three microneedles of the plurality of microneedles are less than 600 μm in length or more than 1050 μm in length.

[0011] Another aspect of the present disclosure provides a method of preparing a biological sample from a subject comprising: contacting skin of the subject with any one of the microneedle devices described herein, applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject; and allowing nucleic acids within the skin of the subject

to contact the microneedle device. In some embodiments, the method further comprises extracting nucleic acids, including, for example, RNA, mRNA. In some embodiments, the method further comprises converting mRNA to cDNA. In some embodiments, the subject is human. In some embodiments, the subject has psoriasis or symptoms of psoriasis. In some embodiments, the subject has a skin condition or symptoms of a skin condition.

[0012] Another aspect of the present disclosure provides a method of preparing a biological sample from a subject comprising: a)contacting skin of the subject with a microneedle device, wherein the microneedle device comprises a plurality of nucleic acid probes coupled to a microneedle; b) applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject; c) allowing the microneedle device to penetrate the skin of the subject for no more than 10 minutes to obtain a ribonucleic acid (RNA) sample hybridized to the nucleic acid probes, wherein the RNA sample comprises a population of RNA fragments comprising about 700 bases or more and a population of RNA fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of RNA fragments comprising about 700 bases or more to the population of RNA fragments comprising about 150 bases to about 200 bases is greater than 1; and d) removing the microneedle device from the skin of the subject, the microneedle device comprising the RNA sample hybridized to the nucleic acid probes after removal of the microneedle device from the skin of the subject. In some embodiments, the RNA sample is substantially free of contaminants. In some embodiments, the microneedle device is allowed to penetrate the skin of the subject for about 5 minutes. In some embodiments, the microneedle device penetrates the skin of the subject for less than 60 minutes, less than 45 minutes, less than 40 minutes, less than 30 minutes, less than 15 minutes, less than 10 minutes, less than 5 minutes, less than 3 minutes, less than 2 minutes, or less than 1 minute. In some embodiments, the microneedle device penetrates the skin of the subject for greater than 30 seconds, greater than 45 seconds, greater than 1 minutes, greater than 5 minutes, greater than 10 minutes, or greater than 30 minutes.

[0013] Another aspect of the present disclosure comprises method of treating a subject with an autoimmune disease of the skin comprising: a) collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA; b) determining an expression level of at least one gene based on the RNA; c)predicting that a subject with the autoimmune disease of the skin will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 80% based on the expression level of the at least one gene; and d)based on the predicting in (c), treating the subject with the

autoimmune therapeutic drug. In some cases, the PPV is greater than 90%. In some cases, the PPV is greater than 95% for a cohort that has greater than 100 patients. In some instances, the autoimmune therapeutic drug is a biologic or comprises an antibody. In some cases, the autoimmune therapeutic drug is an IL-17 mediated treatment, an IL-23 mediated treatment, or a TNFα mediated treatment. In some cases, the autoimmune therapeutic drug is at least one drug selected from the group consisting of: etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, or five genes. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, five genes, six genes, seven genes, eight genes, nine genes, or ten genes from Table 6, Table 12, or Table 13. In some instances, the at least one gene comprises at least two genes that do not share a common upstream regulator. In some cases, the autoimmune disorder is psoriasis. In some cases, the subject has a PASI greater than 8. In some cases, the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug. In some cases, the RNA comprises mRNA or microRNA, and is converted into cDNA, and then sequenced using next generation sequencing. In some cases, collecting a sample comprising RNA derived from skin from the subject comprises penetrating the skin of the subject with a microneedle device, wherein the microneedle device comprises microneedles conjugated to nucleic acid probes. In some embodiments, a trained algorithm is applied to data generated by the next generation sequence of the cDNA. In some embodiments, the algorithm was trained using samples from patients that were administered a single type of drug selected from the group consisting of: IL-17 mediated treatment, TNF-alpha mediated treatment and IL-23 mediated treatment. In some embodiments, the autoimmune therapeutic drug is an IL-17-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-17 mediated pathway. In some embodiments, the autoimmune therapeutic drug is an IL-23-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-23 mediated pathway. In some embodiments, the autoimmune therapeutic drug is a TNF-α-mediated treatment and the at least one gene comprises at least one gene that is not involved in a TNF-α-mediated pathway.

[0014] Another aspect of the present disclosure comprises a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising: collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA; converting the RNA into cDNA; determining expression of at least one

gene based on the cDNA; and predicting whether the subject with the skin lesion will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 80%. In some instances, the PPV is greater than 90%. In some instances, the PPV is greater than 95% for a cohort that has greater than 100 patients. In some embodiments, the autoimmune therapeutic drug is a biologic or comprises an antibody. In some embodiments, the autoimmune therapeutic drug is an IL-17 mediated treatment, an IL-23 mediated treatment, or a TNFα mediated treatment. In some embodiments, the autoimmune therapeutic drug is at least one drug selected from the group consisting of: etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab. In some embodiments, the autoimmune therapeutic drug is at least one drug selected from the group consisting of: certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, or five genes. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, five genes, six genes, seven genes, eight genes, nine genes, or ten genes from Table 6, Table 12, or Table 13. In some instances, the at least one gene comprises at least two genes that do not share a common upstream regulator. In some cases, the autoimmune disorder is psoriasis. In some cases, the subject has a PASI greater than 8. In some cases, the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug. In some cases, the RNA comprises mRNA or microRNA, and is converted into cDNA, and then sequenced using next generation sequencing. In some cases, collecting a sample comprising RNA derived from skin from the subject comprises penetrating the skin of the subject with a microneedle device, wherein the microneedle device comprises microneedles conjugated to nucleic acid probes. In some embodiments, a trained algorithm is applied to data generated by the next generation sequence of the cDNA. In some embodiments, the algorithm was trained using samples from patients that were administered a single type of drug selected from the group consisting of: IL-17 mediated treatment, TNF-alpha mediated treatment and IL-23 mediated treatment.

[0015] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug, comprising: penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; aligning the sequence reads with a signature of sequence reads associated with a positive

response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; and applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm has a greater than 80% positive predictive value for predicting response to the autoimmune disease therapeutic drug.

[0016] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising: penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; and aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; using the aligned sequence reads to determine an expression level of at least one RNA molecule; and applying a trained algorithm to the expression level of the at least one RNA molecule, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, an IL-23 mediated treatment, a TNFα mediated treatment, or any combination thereof. In some embodiments, the subject will be responsive to the to the IL-17 mediated treatment, the IL-23 mediated treatment, and the TNFα-mediated treatment. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, or five genes. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, five genes, six genes, seven genes, eight genes, nine genes, or ten genes from Table 6, Table 12, or Table 13. In some instances, the at least one gene comprises at least two genes that do not share a common upstream regulator. In some cases, the autoimmune disorder is psoriasis. In some cases, the subject has a PASI greater than 8. In some cases, the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug. In some cases, the RNA comprises mRNA or microRNA, and is converted into cDNA, and then sequenced using next generation sequencing. In some embodiments, the recommended treatment comprises one or more autoimmune therapeutic drugs for an autoimmune disease or condition. In some embodiments, the recommended treatment comprises etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, risankizumab, or any combination thereof.

[0017] In some embodiments, the method described herein further comprises performing high throughput sequencing on the RNA biomarkers to generate one or more sequence reads for the subject; aligning the one or more sequence reads for the subject with a known signature of

sequence reads, wherein the known signature of sequence reads is associated with a positive response to the recommended treatment, thereby obtaining aligned sequence reads; and classifying the subject as having a likelihood of positively responding to the recommended treatment by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm comprises a greater than 50% positive predictive value for predicting a positive response to the recommended treatment. In some embodiments, the trained algorithm has greater than 50% negative predictive value.

[0018] Another aspect of the present disclosure provides a method of determining whether a subject with an autoimmune disorder of the skin will be responsive to an autoimmune therapeutic drug comprising: extracting mRNA from skin of the subject; sequencing the mRNA from the skin of the subject; and predicting whether the subject with the autoimmune disorder will be responsive to etanercept, adalimumab, infliximab, certolizumab, secukinumab, ixekisumab, broadalumab, guselkumab, tildrakizumab, and risankizumab with a positive predictive value greater than 80%.

[0019] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an IL-23 mediated treatment comprising: contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle such that the microneedle device penetrates the skin of the subject; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; and aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; and applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-23 mediated treatment and the aligned sequence reads correspond to at least one gene from Table 13.

[0020] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an IL-17, IL-23, or TNF-alpha mediated treatment comprising: contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle such that the microneedle device penetrates the skin of the subject; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; and aligning the sequence reads with a signature of sequence reads associated with a positive response to an

autoimmune disease therapeutic drug to obtain aligned sequence reads; and applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, a TNF-alpha-mediated treatment, or IL-23 mediated treatment and the aligned sequence reads correspond to at least one gene from Tables 6, 12 or 13. In some embodiments, subject is treated with IL-17 mediated treatment, wherein the aligned sequence reads correspond to at least one gene, two genes, three genes, four genes, five genes, or six genes from the table 12. In some embodiments, subject is treated with TNF-alpha mediated treatment, wherein the aligned sequence reads correspond to at least one gene, two genes, three genes, four genes, five genes, or six genes from the table 6. In some embodiments, subject is treated with IL-23 mediated treatment, wherein the aligned sequence reads correspond to at least one gene, two genes, three genes, four genes, five genes, or six genes from the table 13.

[0021] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, PPIG, RAB31, C3, and EGR. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, and PPIG. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of PCDH7, PPIG, RAB31, C3, and EGR.

[0022] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of CNFN, CTSC, GBAP1, and CRABP2. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PPIG, RAB31, C3, and EGR. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PCDH7, PPIG, RAB31, and C3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of GBAP1, CRABP2, PCDH7, PPIG.

[0023] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of SERPINB3, SERPINB4, S100A7A, PI3, KRT6A, LCN2, DEFB4A, DEFB4B, SPRR1A, IL36G, MX1, IFI27, CD36, CD24, and IL4R. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of KRT6A, SPRR1A, CD36, IL4R, LCN2, and IFI27. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CD36, IL4R, S100A7A, SERPINB4, MX1, and SERPINB3. In some cases, the at least one gene is at least one gene, two genes, three

genes, four genes, five genes, or six genes from the group consisting of LCN2, IFI27, DEFB4A, IL36G, CD24, and PI3.

[0024] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of IL4R, LCN2, and IFI27. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PI3, IFI27, and SERPINB3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of IL4R, S100A7A, and MX1. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of CD36, LCN2, and SERPINB4.

[0025] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of MTCO1P12, MTATP6P1, CLSTN1, PDPN, LDLRAD2, and GSTM3. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of AL158847.1, DAD1, LDLRAD2, ZNF395, MGMT, and AL136982.4. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of NREP, PPIF, PRIM1, AL136982.5, MTATP6P1, and SMPD3. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of PDPN, TXNRD1, GSTM3, GPSM1, GLRX, and USP2.

[0026] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of MTCO1P12, CLSTN1, and GSTM3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of NREP, PPIF, and PRIM1. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of AL136982.5, MTATP6P1, and SMPD3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PDPN, TXNRD1, and GSTM3.

[0027] Also provided herein, are microneedle devices, methods, and systems for characterizing a subject's autoimmune disease and the likelihood that a subject may respond positive to a given therapeutic to treat said subject's autoimmune disease.

[0028] An aspect of the present disclosure comprises a microneedle device comprising: a microneedle region comprising (i) a plurality of microneedles protruding from a front surface of a microneedle base substrate, (ii) a back surface of said microneedle base substrate; and (iii) a minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate; and a support substrate adjacent to at least one side of said microneedle base substrate, said support substrate connected to or integral with said

microneedle base substrate and comprising a support substrate depth, wherein said support substrate depth is greater than said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate.

[0029] Another aspect of the present disclosure comprises a microneedle device comprising: a microneedle region comprising a plurality of microneedles protruding from a front surface of a microneedle base substrate, said microneedle base substrate also comprising a back surface, said back surface comprising a recess directly behind at least a portion of said microneedle region; and a support substrate adjacent to at least one side of said microneedle base substrate, said support substrate connected to or integral with said microneedle base substrate. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate is between about 1 μm to about 500 μm less than said depth of said support substrate. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate is between 150 μm to about 350 μm. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate and said support substrate depth comprise an about 1:5 ratio. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate and said support substrate depth comprise at least about a 0.1:5 ratio, at least about a 0.5:5 ratio, at least about 1:5 ratio, at least about 2:5 ratio, at least 3:5 ratio, at least 4:5 ratio, at least 1:1 ratio, at least 1:2 ratio, at least 1:3 ratio, at least 1:4 ratio, at least about a 1:10 ratio, at least about a 1:15 ratio, at least about a 1:20 ratio, at least about 1:25 ratio, or at least about 1:50 ratio. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate and said support substrate depth comprise at most about a 0.1:5 ratio, at most about a 0.5:5 ratio, at most about 1:5 ratio, at most about 2:5 ratio, at most 3:5 ratio, at most 4:5 ratio, at most 1:1 ratio, at most 1:2 ratio, at most 1:3 ratio, at most about 1:4 ratio, at most about a 1:10 ratio, at most about a 1:15 ratio, at most about a 1:20 ratio, at most about 1:25 ratio, or at most about 1:50 ratio. In some embodiments, said minimum distance between said front surface of said microneedle base substrate and said back surface of said microneedle base substrate and said support substrate depth comprise at least about a 2:1 ratio, at least about a 3:1 ratio, at least about 3:2 ratio, at least about 4:1 ratio, at least 5:1 ratio, at least 5:3 ratio, at least 6:1 ratio, at least 7:1 ratio, at least 10:1 ratio, at least 15:1 ratio, at least about a 20:1 ratio, at least about a 25:1 ratio, or at least about a 50:1 ratio. In some embodiments, said minimum distance between said front surface of said

microneedle base substrate and said back surface of said microneedle base substrate and said support substrate depth comprise at most about a 2:1 ratio, at most about a 3:1 ratio, at most about 3:2 ratio, at most about 4:1 ratio, at most 5:1 ratio, at most 5:3 ratio, at most about 6:1 ratio, at most 7:1 ratio, at most 10:1 ratio, at most 15:1 ratio, at most about a 20:1 ratio, at most about a 25:1 ratio, or at most about a 50:1 ratio. In some embodiments, said microneedle region comprises a perimeter and said support substrate is adjacent to at least half of said perimeter. In some embodiments, said back surface of said microneedle base substrate is not coplanar with a back surface of said support substrate. In some embodiments, said front surface of said microneedle base substrate is not coplanar with a surface of said support substrate. In some embodiments, said plurality of microneedles is plasma treated. In some embodiments, a plurality of probes is coupled to a microneedle of said plurality of microneedles. In some embodiments, said plurality of probes comprises a negative charge. In some embodiments, said plurality of microneedles comprises a polyolefine resin. In some embodiments, said polyolefine resin comprises one or both of Zeonor 1020Ror Zeonor 690R. In some embodiments, a microneedle of said plurality of microneedles is non-dissolvable. In some embodiments, a microneedle of said plurality of microneedles is pyramidal. In some embodiments, a microneedle of said plurality of microneedles is solid. In some embodiments, an angle between a base of said microneedle and said microneedle base substrate is between about 60° and about 90°. In some embodiments, said recess directly behind said at least a portion of said microneedle region comprises a width that is greater than a width of a mechanical applicator.

[0030] Another aspect of the present disclosure comprises a kit comprising (a) said device of some aspects described herein; and (b) a mechanical applicator that fits within said recess directly behind said at least a portion of said microneedle region. In some embodiments, at least one of said plurality of microneedles is coupled to a nucleic acid probe. In some embodiments, said nucleic acid probe comprises a homopolymeric sequence. In some embodiments, said homopolymeric sequence comprises thymine or uracil. In some embodiments said nucleic acid probe is covalently linked to said microneedle. In some embodiments, said support substrate comprises a fiducial marker. In some embodiments, no more than three microneedles of said plurality of microneedles are less than 600 μm in length or more than 1050 μm in length.

[0031] Another aspect of the present disclosure comprises a method of preparing a biological sample from a subject comprising: contacting skin of said subject with a microneedle device, wherein said microneedle device comprises a plurality of nucleic acid probes coupled to a microneedle; applying pressure to said microneedle device such that said microneedle device penetrates said skin of said subject; allowing said microneedle device to penetrate said skin of

said subject for no more than 10 minutes to obtain a ribonucleic acid (RNA) sample hybridized to said nucleic acid probes, wherein said RNA sample comprises a population of RNA fragments comprising about 700 bases or more and a population of RNA fragments comprising about 150 bases to about 200 bases, and wherein a ratio of said population of RNA fragments comprising about 700 bases or more to said population of RNA fragments comprising about 150 bases to about 200 bases is greater than 1; and removing said microneedle device from said skin of said subject, said microneedle device comprising said RNA sample hybridized to said nucleic acid probes after removal of said microneedle device from said skin of said subject. In some embodiments, said RNA sample is substantially free of contaminants. In some embodiments, the method comprises allowing said microneedle device to penetrate said skin of said subject for about 5 minutes.

[0032] Another aspect of the present disclosure comprises a method of predicting an individual patient's response to a class of biologic drugs, by the mechanism of action of that class of agent, to optimize treatment selection. By using a machine learning approach, a classifier is trained to predict the response of a patient to either the IL-17, IL-23, or TNF class of biologics using baseline transcriptomic biomarkers (e.g., certain genes) and with high positive predictive value, For example, by using a machine learning-derived classifier to predict response to the anti-IL-17 and anti-IL-23 biologics in patients with psoriasis with high level of positive predictive value.

[0033] Another aspect of the present disclosure comprises an algorithm for the prediction of response to biologics (for example, anti-IL-17, anti-IL-23) used for the treatment of patients with psoriasis, by comparing baseline transcriptomes with clinical response to biologic drugs after initial treatment, for example, at 12 weeks after.

[0034] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug, comprising: penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; quantifying the RNA molecules comprising sequence reads associated with a positive response to an autoimmune disease therapeutic drug to determine an expression level of at least one gene; and applying a trained algorithm to the expression level, wherein the trained algorithm has a greater than 80% positive predictive value for predicting response to the autoimmune disease therapeutic drug.

[0035] Another aspect of the present disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising: penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle; removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject; performing high throughput sequencing on the RNA molecules to generate sequence reads; and quantifying the RNA molecules comprising sequence reads associated with a positive response to an autoimmune disease therapeutic drug to determine an expression level of at least one gene; and applying a trained algorithm to the expression level of the at least one RNA molecule, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, an IL-23 mediated treatment, a TNFα mediated treatment, or any combination thereof. In some embodiments, the subject will be responsive to the to the IL-17 mediated treatment, the IL-23 mediated treatment, and the TNFα-mediated treatment. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, or five genes. In some embodiments, the at least one gene comprises at least two genes, three genes, four genes, five genes, six genes, seven genes, eight genes, nine genes, or ten genes from Table 6, Table 12, or Table 13. In some instances, the at least one gene comprises at least two genes that do not share a common upstream regulator. In some cases, the autoimmune disorder is psoriasis. In some cases, the subject has a PASI greater than 8. In some cases, the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug. In some cases, the RNA comprises mRNA or microRNA, and is converted into cDNA, and then sequenced using next generation sequencing. In some embodiments, the recommended treatment comprises one or more autoimmune therapeutic drugs for an autoimmune disease or condition. In some embodiments, the recommended treatment comprises etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, risankizumab, or any combination thereof.

[0036] In some embodiments, the method described herein further comprises performing high throughput sequencing on the RNA biomarkers to generate one or more sequence reads for the subject; quantifying the RNA molecules comprising one or more sequence reads for the subject with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the recommended treatment, thereby obtaining aligned sequence reads; and classifying the subject as having a likelihood of positively responding to the recommended treatment by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm comprises a greater than 50% positive predictive

value for predicting a positive response to the recommended treatment. In some embodiments, the trained algorithm has greater than 50% negative predictive value.

INCORPORATION BY REFERENCE

[0037] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0039] **FIG. 1** illustrates a cross section of a microneedle device as described herein.

[0040] **FIG. 2** illustrates a detail from the cross section of FIG. 1.

[0041] **FIG. 3** illustrates a front view of a microneedle device as described herein.

[0042] **FIG. 4** illustrates a microneedle of a microneedle device as described herein.

[0043] **FIGs. 5A-C** illustrate quality of different RNA samples collected with a microneedle device as described herein—measured in length of RNA fragments contained in the sample. **FIG. 5A** illustrates an undegraded sample. **FIGs. 5B and 5C** illustrate degraded samples.

[0044] **FIG. 6A** illustrates an example flow diagram for a method of treating one or more subjects with a mild or severe form of a skin condition, as described in some embodiments herein.

[0045] **FIG. 6B** shows an example flow diagram for a method of preparing a biological sample and identifying one or more therapeutic drugs for one or more subjects, as described in some embodiments herein.

[0046] **FIG. 6C** shows an example flow diagram for identifying the activation of one or more pathways that may be associated with a disease or condition in a subject.

[0047] **FIG. 7** shows study patient sample enrollment and analysis flow chart.

[0048] **FIG. 8A-C** shows Correlation between predicted response and patient week 12 PASI changes in psoriasis patients from independent validation data set. **FIG. 8A:** 43 patients treated with IL-23i; **FIG. 8B:** 31 patients treated with IL-17i; **FIG. 8C**: 11 patients treated with TNFαi. X-axis: predicted responder or non-responder; Y-axis: Week 12 PASI changes. Red dot: median PASI change value.

[0049] **FIG. 9** shows predicted response prevalence in patients enrolled in the study.

[0050] **FIG. 10** shows overview of RNA-Seq Analysis.

[0051] **FIG. 11** Lesional versus non-lesional samples compared using Mindera patches versus punch biopsies from 66 patients in the Mindera database.

[0052] **FIG. 12** shows heatmap generated from expression data for the 17 genes identified as correlating to response to anti-IL-17 biologic treatment.

DETAILED DESCRIPTION

Overview

[0053] Described herein are microneedle devices comprising features that result in precise, patient-friendly sample collection, including microneedle devices comprising features that may minimize pain during the sample collection process. The devices described herein can be specifically designed such that they can be fabricated by scalable processes. Features such as the varying thickness of the distinct areas of the devices described herein can result in uniform and precise microneedle devices. The specific features of the microneedle devices described herein may further result in sharper microneedles that are not described in the current state of the art. These sharp microneedles may result in an enhanced user or patient (e.g. the subject) experience wherein the user may experience much less pain as compared to microneedle devices of the current state of the art. Combining the precision of the microneedle devices with the enhanced user experience can result in more precise and accurate analysis of biological samples (e.g. the user's skin).

[0054] Generally, the microneedle devices provided herein may contain probes attached to the microneedle. In some cases, these probes are configured to bind to one or more biomarkers within a sample or tissue (e.g., skin) of a subject in such a manner as to permit extraction of the biomarker for further analysis. The extracted biomarkers can be analyzed, alone or in combination (e.g. generating a genetic signature or gene expression profile) to provide a diagnosis or prediction of a response to a drug or other therapeutic treatment with respect to the subject.

[0055] Further provided herein are methods and systems for determining a gene expression profile from a subject. In some cases, the gene expression profile is useful for determining an appropriate treatment regimen for an autoimmune disease. Thus, the methods of the disclosure are useful for personalized medicine, wherein treatment is tailored to a subject based on the particular characteristics of the disease in the subject.

[0056] In some embodiments, the present disclosure provides a microneedle device. In some embodiments, the microneedle device comprises a microneedle region and a support substrate.

The microneedle region may comprise a plurality of microneedles protruding from a front or first surface of a microneedle base substrate **110**, a back surface (or second base substrate surface) of the microneedle base substrate **160**; and a minimum distance between the front surface of the microneedle base substrate and the back surface of the microneedle base substrate **170**. In some embodiments, the minimum distance between the front surface of the microneedle base substrate and the back surface of the microneedle base substrate **170** configures the device such that during some methods of manufacturing the device, a molten medium (e.g. the polyolefin resins described herein) will fill a mold of the microneedles prior to filling the mold of other sections of the device. In some embodiments, the minimum distance between the front surface of the microneedle base substrate and the back surface of the microneedle base substrate **170** configures the device such that the device comprises uniform and/or sharp microneedles. In some embodiments, the uniform and/or sharp microneedles result in an enhanced user experience for a subject using the device. In some embodiments, the enhanced user experience comprises lessened pain experienced by the subject using the device.

[0057] This disclosure provides microneedle devices for detection and acquisition of biomarkers from a subject in situ. The microneedle-based device may contain one or more microneedles that can be pierced into biological barriers such as the skin or mucous membrane. Often, the microneedles are non-invasive, or minimally-invasive. When a plurality of microneedles is present, the device can also have a planar substrate which supports the microneedles. The substrate can be made of the same material as that of the microneedle. It can also be made of a different material.

Microneedle devices

[0058] Microneedle devices of the present disclosure may comprise a base substrate comprising a microneedle region, and a support substrate. **FIG. 1** illustrates a side view of an exemplary microneedle device **100**, comprising microneedles, **110**, on a microneedle base substrate, **120**. The microneedle base substrate comprises a front surface (used interchangeably herein with the term "first base substrate surface"), **140**, wherein the microneedles, **110**, protrude from the front surface (or first base substrate surface), and a back surface (used interchangeably herein with the term "second base substrate surface"), **160**. The device may comprise a support substrate, **130**, adjacent to at least one side of the interior microneedle base substrate, **120**, the support substrate connected to or integral with the microneedle base substrate. In some embodiments, an angle is formed between the back surface of the microneedle base substrate, **160** and the adjacent support substrate, **130**.

[0059] In some embodiments, the angle formed between the back surface of the microneedle base substrate, **160**, and the adjacent support substrate, **130**, is a right angle or obtuse angle, e.g., about 90 ° to about 179 °. The angle (Θ) formed between the back surface of the microneedle base substrate, **160**, and the adjacent support substrate, **130**, generally refers to an angle that spans a region of space interior to the device; while the adjacent support substrate generally comprises a complementary angle (α) spanning the substrate and that is often an acute angle (e.g., between 1 ° and 90 °). In some embodiments, the angle (Θ) formed between the back surface of the microneedle base substrate and the adjacent support substrate is about 110 °. In some embodiments, the angle (Θ) formed between the back surface of the microneedle base substrate and the adjacent support substrate is about 90 ° to about 100 °, about 90 ° to about 110 °, about 90 ° to about 120 °, about 90 ° to about 130 °, about 90 ° to about 140 °, about 90 ° to about 150 °, about 90 ° to about 160 °, about 90 ° to about 170 °, about 90 ° to about 179 °, about 100 ° to about 110 °, about 100 ° to about 120 °, about 100 ° to about 130 °, about 100 ° to about 140 °, about 100 ° to about 150 °, about 100 ° to about 160 °, about 100 ° to about 170 °, about 100 ° to about 179 °, about 110 ° to about 120 °, about 110 ° to about 130 °, about 110 ° to about 140 °, about 110 ° to about 150 °, about 110 ° to about 160 °, about 110 ° to about 170 °, about 110 ° to about 179 °, about 120 ° to about 130 °, about 120 ° to about 140 °, about 120 ° to about 150 °, about 120 ° to about 160 °, about 120 ° to about 170 °, about 120 ° to about 179 °, about 130 ° to about 140 °, about 130 ° to about 150 °, about 130 ° to about 160 °, about 130 ° to about 170 °, about 130 ° to about 179 °, about 140 ° to about 150 °, about 140 ° to about 160 °, about 140 ° to about 170 °, about 140 ° to about 179 °, about 150 ° to about 160 °, about 150 ° to about 170 °, about 150 ° to about 179 °, about 160 ° to about 170 °, about 160 ° to about 179 °, or about 170 ° to about 179 °. In some embodiments, the angle formed is between the back surface of the microneedle base substrate and the adjacent support substrate is about 90 °, about 100 °, about 110 °, about 120 °, about 130 °, about 140 °, about 150 °, about 160 °, about 170 °, or about 179 °. In some embodiments, the angle formed between the back surface of the microneedle base substrate and the adjacent support substrate is at least about 90 °, about 100 °, about 110 °, about 120 °, about 130 °, about 140 °, about 150 °, about 160 °, or about 170 °. In some embodiments, the angle formed between the back surface of the microneedle base substrate and the adjacent support substrate is at most about 100 °, about 110 °, about 120 °, about 130 °, about 140 °, about 150 °, about 160 °, about 170 °, or about 179 °.

[0060] The resulting shape of the support substrate, **130**, may also influence the speed and uniformity of the flow of polymer and/or resin into the section mold resembling the structure of the device during manufacturing of the device. In some embodiments, the resulting shape of the

support substrate, **130**, is circular. In some embodiments, the resulting shape of the support substrate, **130**, is linear.

[0061] In some cases, the arrangement of the microneedles is a circular pattern with a radius of the microneedle section being about 0.95 mm to about 4.15 mm. In some embodiments, the radius is about 4.0 mm. In some embodiments, the radius is about 0.95 mm to about 1.4 mm, about 0.95 mm to about 1.75 mm, about 0.95 mm to about 2.15 mm, about 0.95 mm to about 2.55 mm, about 0.95 mm to about 2.95 mm, about 0.95 mm to about 3.35 mm, about 0.95 mm to about 3.75 mm, about 0.95 mm to about 4.15 mm, about 1.4 mm to about 1.75 mm, about 1.4 mm to about 2.15 mm, about 1.4 mm to about 2.55 mm, about 1.4 mm to about 2.95 mm, about 1.4 mm to about 3.35 mm, about 1.4 mm to about 3.75 mm, about 1.4 mm to about 4.15 mm, about 1.75 mm to about 2.15 mm, about 1.75 mm to about 2.55 mm, about 1.75 mm to about 2.95 mm, about 1.75 mm to about 3.35 mm, about 1.75 mm to about 3.75 mm, about 1.75 mm to about 4.15 mm, about 2.15 mm to about 2.55 mm, about 2.15 mm to about 2.95 mm, about 2.15 mm to about 3.35 mm, about 2.15 mm to about 3.75 mm, about 2.15 mm to about 4.15 mm, about 2.55 mm to about 2.95 mm, about 2.55 mm to about 3.35 mm, about 2.55 mm to about 3.75 mm, about 2.55 mm to about 4.15 mm, about 2.95 mm to about 3.35 mm, about 2.95 mm to about 3.75 mm, about 2.95 mm to about 4.15 mm, about 3.35 mm to about 3.75 mm, about 3.35 mm to about 4.15 mm, or about 3.75 mm to about 4.15 mm. In some embodiments, the radius is about 0.95 mm, about 1.4 mm, about 1.75 mm, about 2.15 mm, about 2.55 mm, about 2.95 mm, about 3.35 mm, about 3.75 mm, or about 4.15 mm. In some embodiments, the radius is at least about 0.95 mm, about 1.4 mm, about 1.75 mm, about 2.15 mm, about 2.55 mm, about 2.95 mm, about 3.35 mm, or about 3.75 mm. In some embodiments, the radius is at most about 1.4 mm, about 1.75 mm, about 2.15 mm, about 2.55 mm, about 2.95 mm, about 3.35 mm, about 3.75 mm, or about 4.15 mm.

[0062] In some cases, the arrangement of the microneedles is a linear square pattern with total edge length of about 2.14 mm to about 9.34 mm. In some embodiments, the distance is about 4.0 mm. In some embodiments, the distance is about 2.14 mm to about 3.04 mm, about 2.14 mm to about 3.94 mm, about 2.14 mm to about 4.84 mm, about 2.14 mm to about 5.74 mm, about 2.14 mm to about 6.64 mm, about 2.14 mm to about 7.54 mm, about 2.14 mm to about 8.44 mm, about 2.14 mm to about 9.34 mm, about 3.04 mm to about 3.94 mm, about 3.04 mm to about 4.84 mm, about 3.04 mm to about 5.74 mm, about 3.04 mm to about 6.64 mm, about 3.04 mm to about 7.54 mm, about 3.04 mm to about 8.44 mm, about 3.04 mm to about 9.34 mm, about 3.94 mm to about 4.84 mm, about 3.94 mm to about 5.74 mm, about 3.94 mm to about 6.64 mm, about 3.94 mm to about 7.54 mm, about 3.94 mm to about 8.44 mm, about 3.94 mm to

about 9.34 mm, about 4.84 mm to about 5.74 mm, about 4.84 mm to about 6.64 mm, about 4.84 mm to about 7.54 mm, about 4.84 mm to about 8.44 mm, about 4.84 mm to about 9.34 mm, about 5.74 mm to about 6.64 mm, about 5.74 mm to about 7.54 mm, about 5.74 mm to about 8.44 mm, about 5.74 mm to about 9.34 mm, about 6.64 mm to about 7.54 mm, about 6.64 mm to about 8.44 mm, about 6.64 mm to about 9.34 mm, about 7.54 mm to about 8.44 mm, about 7.54 mm to about 9.34 mm, or about 8.44 mm to about 9.34 mm. In some embodiments, the distance is about 2.14 mm, about 3.04 mm, about 3.94 mm, about 4.84 mm, about 5.74 mm, about 6.64 mm, about 7.54 mm, about 8.44 mm, or about 9.34 mm. In some embodiments, the distance is at least about 2.14 mm, about 3.04 mm, about 3.94 mm, about 4.84 mm, about 5.74 mm, about 6.64 mm, about 7.54 mm, or about 8.44 mm. In some embodiments, the distance is at most about 3.04 mm, about 3.94 mm, about 4.84 mm, about 5.74 mm, about 6.64 mm, about 7.54 mm, about 8.44 mm, or about 9.34 mm.

[0063] In some embodiments, the surface from which the microneedles protrude from, and which is applied to the tissue of interest may be termed the bottom surface or front surface. In some embodiments, the surface that is opposite to the surface from which the microneedles protrude from, **160**, may be termed the back or top surface.

[0064] In some embodiments, the microneedle base substrate, **120**, is defined by the presence of microneedles. The microneedle base substrate, **120**, may be thinner and/or narrower than the support substrate, **130**, e.g. the distance between the front surface of the microneedle base substrate, **140**, and back surface of the microneedle base substrate, **160**, termed D_{BS} (**170** in **FIG. 1**) is less than the distance between the respective, adjacent surfaces of the support substrate, **130**, termed D_{SS} (**180** in **FIG. 1**). The difference in depth between the microneedle base substrate, **120**, and the support substrate, **130**, may create a recess in the device, the recess located adjacent to the microneedle base substrate, **120**. This structural feature is particularly advantageous because having a narrow microneedle base substrate, **120**, improves the flow and/or penetration of a polymer/resin used to fabricate the device into a mold resembling the structure of the device during manufacturing, specifically the flow and/or penetration of the polymer/resin into the section of the mold corresponding to the microneedles is increased due to this structural feature of a difference in depth between the microneedle base substrate, **120**, and the support substrate, **130**, resulting in more uniform and sharper microneedles. Sharper microneedles may cause less tissue damage when inserted, and therefore, may result in less pain to a subject. More uniform microneedles result in more standard and scalable manufacturing methods.

[0065] In some embodiments, the narrower microneedle base substrate may also concentrate pressure through the microneedles when the device is applied to skin or other tissues. In some cases, the minimum distance between the front surface of the microneedle base substrate, **140**, and the back surface of the microneedle base substrate, **150** is between about 1 μm to about 500 μm less than the distance from the front surface of the support substrate to the back surface of the support substrate.

[0066] In some embodiments, the difference in depth between the microneedle base substrate (D_{BS}) and the support substrate (D_{SS}) is about 1 μm to about 550 μm. In some embodiments, the difference in depth between the microneedle base substrate (D_{BS}) and the support substrate (D_{SS}) is about 250 μm. In some embodiments, the difference in depth between the microneedle base substrate (D_{BS}) and the support substrate (D_{SS}) is about 1 μm to about 50 μm, about 1 μm to about 100 μm, about 1 μm to about 150 μm, about 1 μm to about 200 μm, about 1 μm to about 250 μm, about 1 μm to about 300 μm, about 1 μm to about 350 μm, about 1 μm to about 400 μm, about 1 μm to about 450 μm, about 1 μm to about 500 μm, about 1 μm to about 550 μm, about 50 μm to about 100 μm, about 50 μm to about 150 μm, about 50 μm to about 200 μm, about 50 μm to about 250 μm, about 50 μm to about 300 μm, about 50 μm to about 350 μm, about 50 μm to about 400 μm, about 50 μm to about 450 μm, about 50 μm to about 500 μm, about 50 μm to about 550 μm, about 100 μm to about 150 μm, about 100 μm to about 200 μm, about 100 μm to about 250 μm, about 100 μm to about 300 μm, about 100 μm to about 350 μm, about 100 μm to about 400 μm, about 100 μm to about 450 μm, about 100 μm to about 500 μm, about 100 μm to about 550 μm, about 150 μm to about 200 μm, about 150 μm to about 250 μm, about 150 μm to about 300 μm, about 150 μm to about 350 μm, about 150 μm to about 400 μm, about 150 μm to about 450 μm, about 150 μm to about 500 μm, about 150 μm to about 550 μm, about 200 μm to about 250 μm, about 200 μm to about 300 μm, about 200 μm to about 350 μm, about 200 μm to about 400 μm, about 200 μm to about 450 μm, about 200 μm to about 500 μm, about 200 μm to about 550 μm, about 250 μm to about 300 μm, about 250 μm to about 350 μm, about 250 μm to about 400 μm, about 250 μm to about 450 μm, about 250 μm to about 500 μm, about 250 μm to about 550 μm, about 300 μm to about 350 μm, about 300 μm to about 400 μm, about 300 μm to about 450 μm, about 300 μm to about 500 μm, about 300 μm to about 550 μm, about 350 μm to about 400 μm, about 350 μm to about 450 μm, about 350 μm to about 500 μm, about 350 μm to about 550 μm, about 400 μm to about 450 μm, about 400 μm to about 500 μm, about 400 μm to about 550 μm, about 450 μm to about 500 μm, about 450 μm to about 550 μm, or about 500 μm to about 550 μm. In some embodiments, the difference in depth between the microneedle base substrate and the support substrate is about 1 μm, about 50 μm,

about 100 μm, about 150 μm, about 200 μm, about 250 μm, about 300 μm, about 350 μm, about 400 μm, about 450 μm, about 500 μm, or about 550 μm. In some embodiments, the difference in depth between the microneedle base substrate (D_{BS}) and the support substrate (D_{SS}) is at least about 1 μm, about 50 μm, about 100 μm, about 150 μm, about 200 μm, about 250 μm, about 300 μm, about 350 μm, about 400 μm, about 450 μm, or about 500 μm. In some embodiments, the difference in depth between the microneedle base substrate and the support substrate is at most about 50 μm, about 100 μm, about 150 μm, about 200 μm, about 250 μm, about 300 μm, about 350 μm, about 400 μm, about 450 μm, about 500 μm, or about 550 μm.

[0067] In some cases, the minimum distance between the front surface of the microneedle base substrate and the back surface of the microneedle base substrate (D_{BS}) is between 150 μm to about 350 μm. In some cases, the ratio of the distance between the front and back of the interior section and the front and back of the peripheral section is about a 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10 ratio.

[0068] In some cases, the microneedle section, or region, comprises a perimeter and the peripheral region, or support substrate, is adjacent to at least half of the perimeter. In some cases, the back surface of the microneedle base substrate is not coplanar with a back surface of the support substrate. In some cases, the front surface of the microneedle base substrate is not coplanar with a surface of the support substrate.

[0069] **FIG. 2** provides a detail view of the circled region in FIG. 1. In some embodiments, the interior section may also have a ridge, **200**, which surrounds the microneedle region. The ridge may protrude from the surface from which the microneedles protrude from. In some embodiments, the height of the ridge, H_R (**210** in FIG. 2), may be about 250 μm. In some embodiments, the height of the ridge, H_R may be about 50 μm, 100 μm, 150 μm, 200 μm, 250 μm, 254 μm, 300 μm, 350 μm, 400 μm, or 450 μm. In some cases, the ridge surrounding the interior section may increase or maintain surface tension of a tissue, such as skin, when the device is applied to the tissue. In some cases, the angle formed between the ridge and the bottom surface is about 5 ° to about 85 °. In some cases, the angle formed between the ridge and the bottom surface is about 45 °. In some cases, the angle formed between the ridge and the bottom surface is about 5 ° to about 10 °, about 5 ° to about 20 °, about 5 ° to about 30 °, about 5 ° to about 40 °, about 5 ° to about 50 °, about 5 ° to about 60 °, about 5 ° to about 70 °, about 5 ° to about 80 °, about 5 ° to about 85 °, about 10 ° to about 20 °, about 10 ° to about 30 °, about 10 ° to about 40 °, about 10 ° to about 50 °, about 10 ° to about 60 °, about 10 ° to about 70 °, about 10 ° to about 80 °, about 10 ° to about 85 °, about 20 ° to about 30 °, about 20 ° to about 40 °, about 20 ° to about 50 °, about 20 ° to about 60 °, about 20 ° to about 70 °, about 20 ° to about 80 °, about

20 ° to about 85 °, about 30 ° to about 40 °, about 30 ° to about 50 °, about 30 ° to about 60 °, about 30 ° to about 70 °, about 30 ° to about 80 °, about 30 ° to about 85 °, about 40 ° to about 50 °, about 40 ° to about 60 °, about 40 ° to about 70 °, about 40 ° to about 80 °, about 40 ° to about 85 °, about 50 ° to about 60 °, about 50 ° to about 70 °, about 50 ° to about 80 °, about 50 ° to about 85 °, about 60 ° to about 70 °, about 60 ° to about 80 °, about 60 ° to about 85 °, about 70 ° to about 80 °, about 70 ° to about 85 °, or about 80 ° to about 85 °. In some cases, the angle formed between the ridge and the bottom surface is about 5 °, about 10 °, about 20 °, about 30 °, about 40 °, about 50 °, about 60 °, about 70 °, about 80 °, or about 85 °. In some cases, the angle formed between the ridge and the bottom surface is at least about 5 °, about 10 °, about 20 °, about 30 °, about 40 °, about 50 °, about 60 °, about 70 °, or about 80 °. In some cases, the angle formed between the ridge and the bottom surface is at most about 10 °, about 20 °, about 30 °, about 40 °, about 50 °, about 60 °, about 70 °, about 80 °, or about 85 °.

[0070] In some embodiments, the microneedle section may also have a bottom surface which is lower than the bottom surface of the support substrate, e.g., the bottom surface of the microneedle section may protrude farther from the device compared to the bottom surface of the support substrate. In some cases, a bottom surface may overlap the base of the microneedles. In some cases, the bottom surface of the interior section may protrude by about 250 μm. In some cases, the bottom surface of the interior section may protrude by about 0 μm, 50 μm, 100 μm, 150 μm, 200 μm, 250 μm, 300 μm, 350 μm, 400 μm, or 450 μm. In some cases, the protruding bottom surface of the interior section may increase or maintain surface tension of a tissue such as skin when the device is applied to the tissue.

[0071] In some embodiments, the support substrate, **130**, may be formed of the same material as the microneedle section and the microneedles. The support substrate may be thicker than the interior section. In some cases, the peripheral section comprises a fiducial marker. FIG. 3 provides a front view of microneedle device **100**, with a fiducial marker, **310**.

[0072] In some embodiments, a device of the present disclosure may be manufactured using a mold. In some cases, a device may be manufactured by displacing a resin (e.g., polyolefin resin) into a mold for the device. The mold may comprise a plurality of cavities for forming a plurality of microneedles, cavity for forming an interior section comprising the plurality of microneedles; and one or more cavities for forming one or more peripheral sections adjacent to the interior section, wherein a width of the interior section is less than a width of the peripheral sections. In some cases, the polyolefin resin may generate the plurality of microneedles prior to generating the interior section. In some cases, the molded resin may be plasma treated to modify a surface.

[0073] In some embodiments, a device of the present disclosure may be manufactured using injection molding. In some cases, a device may be manufactured by injecting heated (e.g. molten) material (e.g. a polyolefin resin described herein) into a mold of the device. The mold may comprise a plurality of cavities for forming a plurality of microneedles, cavity for forming an interior section comprising the plurality of microneedles, and one or more cavities for forming one or more peripheral sections adjacent to the interior section, wherein a width (e.g. depth and/or thickness) of the interior section is less than a width of the peripheral sections. In some cases, the heated material may generate the plurality of microneedles prior to generating the interior or microneedle section.

[0074] In some cases, the entire device may be plasma treated, while in other cases a portion of the device, for example the microneedles, is plasma treated. In some cases, the method of plasma treating the device or portion thereof comprises the steps of providing the device or portion thereof, placing the device or portion thereof into the plasma vacuum chamber, closing the plasma vacuum chamber creating a sufficient vacuum seal, evacuating all of the gas present in the chamber, pumping a plasma treatment gas into the plasma vacuum chamber to a desirable pressure, enabling the generation of gas plasma for a desirable period of time at a desirable power, evacuating the chamber of the plasma treated gas and removing the device or portion thereof.

[0075] In some cases, the entire device may be excimer laser treated, while in other cases a portion of the device, for example the microneedles, is excimer laser treated. In some cases, the method of excimer laser treating the device or portion thereof comprises the steps of providing the device or portion thereof, placing the device or portion thereof into the excimer laser treating chamber, closing the excimer laser treating chamber, evacuating all of the gas present in the chamber, pumping a suitable excimer laser treatment gas into the excimer laser treating chamber to provide sufficient absorption of radiative laser energy by the device or portion thereof, enabling the radiative emission of an excimer laser for a desirable period of time at a desirable power, evacuating the excimer laser treatment gas and removing the device or portion thereof.

[0076] In some cases, one or more probes of the plurality of probes are attached to one or more microneedles of the plurality of microneedles. The probes may be covalently or non-covalently attached. In some cases, the probes are attached via a linker or a spacer. In some cases, the probes are attached to a microneedle as to achieve a maximum packing density on the surface of the microneedle.

Probes

[0077] In some embodiments, the microneedles comprise probes that can bind and extract biomarkers from a tissue. A plurality of probes can be attached to a microneedle of the disclosure. In some cases, the probes comprise polynucleotides (e.g., DNA, RNA, cDNA, cRNA, etc.). Often the polynucleotide probes are designed to bind or hybridize a specific polynucleotide biomarker. This disclosure also provides methods and devices for detecting peptide or protein biomarkers. In these embodiments, the probes attached to the microneedles can specifically recognize and bind to target peptides or proteins of interest. The probes can be any substance capable of binding to a specific peptide or protein biomarker. They can be, e.g., a protein (e.g., an antibody, antigen, or fragment thereof), carbohydrate, or a polynucleotide. The polynucleotide may possess sequence specificity for the biomarker, e.g., by having a complementary sequence.

[0078] In some embodiments, the probes to be used often depend on the biomarker or biomarkers to be detected. Thus, depending the nature and number of biomarkers to be detected, the number of probes immobilized to a microneedle can be 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. In some cases, the total number of probes immobilized to a microneedle can be from about 1 probe to about 1,000 probes, from about 1 probe to about 10,000 probes, from about 1 probe to about 100,000 probes, from about 1 probe to about 1,000,000 probes, from about 1 probe to about 10,000,000, from about 1 probe to about 100,000,000 probes, from about 1,000 probes to about 100,000,000 probes, from about 10,000 probes to about 100,000,000 probes, or from about 10,000,000 probes to about 100,000,000 probes. In some cases, the total number of probes in a microneedle is at least about 10, about 20, about 50, about 100, about 500, about 1000, about 10000, about 100000, about 1,000,000, about 10,000,000, or about 100,000,000 probes. In some cases, the total number of probes in a microneedle is less than about 10, about 100, about 1000, about 10000, about 100000, about 1,000,000, about 10,000,000, or about 100,000,000 probes.

[0079] In some embodiments, a probe for the biomarker can be immobilized to multiple microneedles in the device of the disclosure, particularly for detection of a biomarker of low concentration, as described further herein. In some cases, a single microneedle comprises multiple different probes capable of binding or detecting the same biomarker. In some cases, a single microneedle comprises at least 2 different probes, at least 3 different probes, at least 4 different probes, at least 6 different probes, at least 8 different probes, at least 10 different probes, at least 15 different probes, at least 20 different probes, or at least 50 different probes. In some cases, the same microneedle comprises more than 50 different types of probes for the same biomarker.

[0080] In some cases, the same microneedle comprises a plurality of different probes. The different probes can be specific for the same biomarker or for a different biomarker. A microneedle can comprise at least 2 different probes, at least 10 different probes, at least 100 different probes, at least 200 different probes at least 500 different probes, at least 1,000 different probes, at least 5,000 different probes, or at least 10,000 different probes.

[0081] In some cases, the individual probes of the plurality of probes are identical (e.g., identical copies of the same polynucleotide or antibody). In some embodiments, a microneedle can be associated with numerous copies of the same probe (e.g., greater than 2, 5, 10, 50, 100, 1000, 5000, 7500, 10000, or 50000 copies of the same probe). For example, a microneedle can comprise a plurality of copies of a polynucleotide probe designed to hybridize the same polymorphism or biomarker. In some cases, a microneedle may comprise a plurality of copies of an antibody probe designed to bind the same epitope.

[0082] In some cases, a probe may bind to a class of biomarkers, for example mRNAs. A probe which binds to mRNAs may comprise a polynucleotide with a homopolymeric sequence or a polynucleotide that comprises a homopolymeric sequence. In some cases, the probe comprises one or more thymine residues. For example, in some cases, the probe comprises at least 1, at least 5, at least 10, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, or at least 200 thymine residues (e.g., a thymidine residue). In some cases, the probe binds to a poly-A tail of an mRNA molecule. In some cases, the probe comprises a homopolymeric sequence. In these cases, the homopolymeric sequence is configured to bind to a complementary homopolymeric sequence. In some cases, a probe comprises a sequence of thymines which bind to the poly A tails of the mRNAs. A probe may comprise any fragment of a sequence of consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 250 consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 200 consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 150 consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 100 consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 50 consecutive thymines. In some embodiments, the sequence of consecutive thymines comprises about 50 and 250 consecutive thymines. In some cases, a probe may comprise thymines interspersed with other bases.

[0083] In some cases, a microneedle comprises polynucleotide probes. The probes may be designed to detect different biomarkers (e.g. nucleic acid molecules, proteins) associated with the same disease, disorder or condition. In some cases, a first probe may recognize a polymorphism (e.g., DNA polymorphism, RNA polymorphism) associated with a disease and a second probe

may recognize a different polymorphism associated with the same disease. For example, a first nucleic acid probe on a microneedle can be designed to detect a first polymorphism of an RNA biomarker associated with onchocerciasis, a skin condition. A second nucleic acid probe on a microneedle can be designed to detect a second polymorphism of an RNA biomarker associated with onchocerciasis. A polymorphism can be, for example, a single nucleotide polymorphism (SNP). Genetic and genomic variations can comprise a single SNP or a plurality of SNPs. SNPs can occur at a single locus, or at many loci. Individuals who carry a particular SNP on an allele at one locus can predictably carry additional SNPs at other loci. A correlation of SNPs can provide an association between alleles predisposing an individual to disease or condition. In some cases, the different polynucleotide probes are designed to detect different biomarkers associated with different conditions. For example, one probe may detect a biomarker of a disease (e.g. polynucleotides), while the other probe may detect a housekeeping gene or gene product (e.g. polypeptides). In some cases, the microneedles are attached to polynucleotides, polypeptides, or a mixture of polynucleotides and polypeptides. In some cases, the probes may comprise a negative charge. In some cases, a plurality of probes may comprise a residual negative charge. In some cases, the microneedles of the devices described herein are packed with probes at the maximum packing density for the microneedle. The residual negative charge generated from a maximum-packed microneedle can be strong enough to prevent the packed probes from non-specific binding to biomarkers that are not of interest.

[0084] In some cases, a microneedle can also be associated with a plurality of different protein or antibody probes. For example, a first antibody probe on a microneedle can be designed to detect a first epitope of an antigen associated with, for example, a skin cancer. A second antibody probe can be designed to detect a second epitope associated with the antigen. Or, in some cases, the second antibody probe can detect an epitope associated with a different skin condition and/or different antigen.

[0085] In some cases, the present disclosure provides a microneedle device comprising a set of microneedles, wherein each microneedle in the set comprises identical probes or set of probes. In some embodiments, an identical probe is attached to a plurality of microneedles of a device. An identical probe can be attached to, for example, about 1% of the microneedles, about 5% of the microneedles, about 10% of the microneedles, about 50% of the microneedles, about 90% of the microneedles, about 95% of the microneedles, or about 100% of the microneedles. In some embodiments, an identical probe is attached to no greater than 5% of the microneedles, no greater than 10% of the microneedles, no greater than 25% of the microneedles, no greater than 50% of

the microneedles, no greater than 95% of the microneedles, or no greater than 99% of the microneedles.

[0086] In some cases, a plurality of microneedles may comprise at least one microneedle attached to a first probe and at least one microneedle attached to a second probe that is different from the first probe. For example, as described herein, the first probe may be a polynucleotide or polypeptide (e.g., antibody, protein) that specifically binds a biomarker of a disease or disorder and the second probe may be a polynucleotide or polypeptide that specifically binds a different biomarker associated with the same disease or disorder. In some cases, the first probe may be a polynucleotide or polypeptide (e.g., antibody, protein) that specifically binds a biomarker of a disease or disorder and the second probe may be a polynucleotide or polypeptide that specifically binds a different biomarker associated with a different disease, disorder, or condition. In some cases, the different disease, condition, or disorder is associated with the same organ. For example, the first probe may be associated with a first disease, disorder, or condition associated with skin; and the second probe may be associated with a second disease, disorder, or condition associated with skin or eye. In some cases, the device may comprise an array of microneedles, wherein each microneedle comprises a probe that detects a biomarker associated with a different disease, disorder, or condition associated with the same organ. The array of microneedles may comprise greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 75, 100, 150, 200, 500, or 1000 microneedles associated with different diseases, disorders, or conditions. In some cases, the different diseases, disorders, or conditions are associated with different organs (e.g., greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 organs).

[0087] In some cases, the microneedle device comprises a plurality of arrays of microneedles; often, the plurality of arrays of microneedles are suitable for multiplexed reactions. In some cases, the plurality of arrays comprises two or more arrays of microneedles, wherein the arrays are designed to detect a different biomarker. In some cases, a first array of microneedles may be designed to detect a biomarker associated with disease, disorder, or condition, and the second array of microneedles is designed to detect a different biomarker associated with the same disease, disorder, or condition. In some cases, a first array of microneedles may be designed to detect a biomarker associated with disease, disorder, or condition, and a second array of microneedles may be designed to detect a different biomarker associated with a different disease, disorder, or condition. In some cases, a first array of microneedles may be designed to detect a plurality of biomarkers associated with a disease, disorder, or condition; and a second array of microneedles may be designed to detect a plurality of biomarkers associated with a different disease, disorder, or condition. In some cases, the second array of microneedles may be designed

to detect control biomarkers (e.g., housekeeping genes), either positive controls or negative controls.

[0088] In some embodiments, the probes are covalently attached to the microneedles. In some embodiments, the probes are attached to the microneedles at about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of a maximum packing density. A maximum packing density may be determined by the chemical structure of the microneedle surface. In some cases, a high density of probes may decrease non-specific binding. For example, a high density of polynucleotide probes will discourage non-specific polynucleotide binding due to the residual negative charge. In some cases, the microneedles may be treated to facilitate a high density of probe attachment. For example, the microneedles may be plasma treated. The plasma treatment may comprise treatment with an oxygen, nitrogen, or carbon dioxide plasma to modify the surface.

Microneedles

[0089] A microneedle can have a plurality of shapes, for example, a microneedle can be round, conical, triangular, square, rectangular, pentagonal, hexagonal, heptagonal, octagonal, pyramidal, or any other suitable shape. A microneedle can be a sharp microneedle, a blunt microneedle, or any combination thereof. For example, a device of the present disclosure comprising a plurality of sharp microneedles can be used to penetrate the skin of a subject thereby contacting the probe(s) on the microneedle with, for example, a biomarker. A sharp microneedle can be used to disrupt tissue of a biological sample, such as a layer of cells or the outer membrane of a cell. A blunt microneedle can be used to touch the surface of the skin of a subject thereby contacting the microneedle with, for example, a cell-surface biomarker on the skin. In some cases, the present disclosure provides microneedle devices that comprise microneedles with different shapes (e.g., sharp and blunt microneedles). In some cases, the microneedles may be solid rather than hollow.

[0090] In some cases, the present disclosure provides microneedle devices in which all needles have a same shape. In some cases, all microneedles on a device have the same shape and dimensions, within a 5%, 4%, 3%, 2%, 1.5%, 1%, 0.75%, 0.5%, or 0.25% error. In some cases, no more than three microneedles of a plurality of microneedles do not meet the height tolerance. For example, if a desired microneedle height is 950 μm, then a height tolerance may be no less than 600 μm in length and no more than 1050 μm in length.

[0091] In some embodiments, a microneedle may have a shape that allows non-specifically bound material to be wiped off needle upon microneedles' exit from skin. In some cases, the non-

specifically bound material may be wiped off via shearing forces generated as the microneedle is drawn through the skin.

[0092] In some embodiments, while the needle-shaped microneedles can be blunt-pointed objects, they are preferably sharp-pointed objects. In some embodiments, the microneedles have a circular cone structure with a diameter of the base generally in the range of 10 μm to 500 μm, preferably in the range of 20 μm to 200 μm. **FIG. 4** illustrates a surface of a device of the present disclosure with a plurality of microneedles wherein the diameter of the base is less than 10 mm in width.

[0093] In some embodiments, a microneedle of the disclosure can comprise a plurality of different diameters or base widths. The shape of the base of a microneedle can be, for example, round, rectangular, triangular, square, pentagonal, hexagonal, heptagonal, or other geometric shape. A microneedle of the disclosure can have a diameter or base width that is no greater than 500 μm , no greater than 400 μm, no greater than 300 μm, no greater than 200 μm, no greater than 100 μm, no greater than 50 μm, no greater than 40 μm, no greater than 30 μm, no greater than 20 μηι, no greater than 10 μηι, no greater than 1000 nm, no greater than 900 nm, no greater than 800 nm, no greater than 700 nm, no greater than 600 nm, no greater than 500 nm, no greater than 400 nm, no greater than 300 nm, no greater than 200 nm, or no greater than 100 nm.

[0094] In some cases, a microneedle with a circular cone structure may have an angle of the edge of the cone to the base of the cone from 70° to about 90°, from 71° to 89°, from 72° to 88°, from 73° to 86°, from 73° to 84°, from 73° to 80°, from 74° to 78°, from 74° to 76°, or from 75.5° to 76°. In some cases a microneedle with a circular cone structure may have an angle from the edge of the cone to the base of the cone of about 73°, 73.25°, 73.5°, 73.75°, 74°, 74.25°, 74.5°, 74.75°, 75°, 75.25°, 75.5°, 75.75°, 76°, 76.25°, 76.5°, 76.75°, or 77°. In some cases a microneedle with a circular cone structure may have an angle of the edge of the cone to the base of less than 73°, 73.25°, 73.5°, 73.75°, 74°, 74.25°, 74.5°, 74.75°, 75°, 75.25°, 75.5°, 75.75°, 76°, 76.25°, 76.5°, 76.75°, or 77°.

[0095] In some embodiments, the substrate and the microneedles of the arrays can be made of various biodegradable or non-biodegradable materials. Examples of the material for the microneedles or substrate include poly(methyl methacrylate), silicon, silicon dioxide, ceramic, metal (such as stainless steel, titanium, nickel, molybdenum, chromium, and cobalt), and synthetic or natural resin material. Some embodiments use a biodegradable polymer such as polylactic acid, polyglycolide, polylactic acid-co-polyglycolide, pullulan, capronolactone, polyurethane or polyanhydride. In some other embodiments, a non-degradable material is employed to fabricate the microneedle array, e.g., a polymer polycarbonate, a synthetic or natural

resin material such as polymethacrylic acid, ethylenevinylacetate, polytetrafluoroethylene, polysulfone, or polyoxymethylene. In some embodiments, the microneedles may be non-dissolvable. In some cases, the microneedle is fabricated with a thermoplastic polymer.

[0096] In some embodiments, the substrate and the microneedles of the arrays can be made of various thermoplastic polymers. Non-limiting examples of thermoplastic polymers include acrylic polymers, such as poly(methyl methacrylate) (PMMA), nylon, polyethylene, polypropylene, polystyrene, polyvinyl chloride, or Teflon. In some cases, a device of the present disclosure is fabricated with a thermoplastic polymer selected from the group consisting of polycarbonate, poly(methyl methacrylate), polyethylene and polypropylene.

[0097] Non-limiting examples of non-degradable polymers include, for example, silicone, hydrogels such as crosslinked poly(vinyl alcohol) and poly(hydroxy ethylmethacrylate), ethylene-vivyl acetate, acyl substituted cellulose acetates and alkyl derivatives thereof, partially and completely hydrolyzed alkylene-vinyl acetate copolymers, unplasticized polyvinyl chloride, crosslinked homo- and copolymers of polyvinyl acetate, crosslinked polyesters of acrylic acid and/or methacrylic acid, polyvinyl alkyl ethers, polyvinyl fluoride, polycarbonate, polyurethane, polyamide, polysulphones, styrene acrylonitrile copolymers, crosslinked poly(ethylene oxide), poly(alkylenes), poly(vinyl imidazole), poly(esters), poly(ethylene terephthalate), polyphosphazenes, and chlorosulphonated polyolefines, and combinations thereof. In some embodiments the polymer comprises ethylene vinyl acetate.

[0098] Further non-limiting examples of biodegradable polymers include polyesters such as 3-hydroxypropionate, 3-hydroxybutyrate, 3-hydroxyvalerate, 3-hydroxycaproate, 3-hydroxyheptanoate, 3-hydroxyoctanoate, 3-hydroxynonanoate, 3-hydroxydecanoate, 3-hydroxyundecanoate, 3-hydroxydodecanoate, 4-hydroxybutyrate, 5-hydroxyvalerate, polylactide or polylactic acid including poly(d-lactic acid), poly(1-lactic acid), poly(d,l-lactic acid), polyglycolic acid and polyglycolide, poly(lactic-co-glycolic acid), poly(lactide-co-glycolide), poly(ε-caprolactone) and polydioxanone. Polysaccharides including starch, glycogen, cellulose and chitin can also be used as biodegradable materials.

[0099] In some embodiments the substrate and the microneedles of the arrays can be made of a polyolefin resin. Examples of polyolefin resins include thermoplastic polyolefins: polyethylene (PE), polypropylene (PP), polymethylpentene (PMP), and polybutene-1 (PB-1). Further examples of polyolefin resins include polyolefin elastomers (POE): polyisobutylene (PIB), ethylene propylene rubber (EPR), and ethylene propylene diene monomer (M-class) rubber (EPDM rubber). In some cases, the polyolefin resin may be a Cyclo Olefin Polymer such

as Zeonor or Zeonex. In some cases, the Zeonor is Zeonor 1020R, Zeonar 690R or Zeonor 1060R.

[00100] In some embodiments, the microneedles employed in the present disclosure have a length (height) that is typically in the range of 20 μm to 1 mm, preferably in the range of 50 μm to 500 μm. The height of the needles may range from about 400 μm to about 1000 μm.

[00101] A microneedle device of the present disclosure may comprise any number of microneedles. In some embodiments, a device of the present disclosure comprises at least 1 microneedle, at least 100 microneedles, at least 200 microneedles, at least 300 microneedles, at least 400 microneedles, at least 500 microneedles, at least 1000 microneedles, at least 3000 microneedles, or at least 5000 microneedles.

[00102] In some embodiments, a device of the present disclosure comprises at most 10000 microneedles, at most 5000 microneedles, at most 2500 microneedles, at most 2000 microneedles, at most 1000 microneedles, at most 500 microneedles, at most 400 microneedles, at most 300 microneedles, at most 100 microneedles, at most 90 microneedles, at most 50 microneedles, at most 40 microneedles, at most 30 microneedles, at most 20 microneedles, at most 15 microneedles, at most 10 microneedles, at most 9 microneedles, at most 8 microneedles, at most 7 microneedles, at most 6 microneedles, at most 5 microneedles, at most 4 microneedles, at most 3 microneedles, at most 2 microneedles, or 1 microneedle.

[00103] In some embodiments, a device of the disclosure comprises from about 1 microneedle to about 100 microneedles, from about 1 microneedle to about 200 microneedles, from about 1 microneedle to about 1000 microneedles, from about 10 microneedles to about 200 microneedles, from about 50 microneedles to about 150 microneedles, or from about 1 microneedle to about 5000 microneedles.

[00104] In some embodiments, a device of the disclosure comprises 100 microneedles. In some embodiments, a device of the disclosure comprises from 90 to 110 microneedles. In some embodiments, a device of the disclosure comprises from 80 to 120 microneedles. In some embodiments, a device of the disclosure comprises 50 to 150 microneedles.

[00105] In some embodiments, for devices containing a plurality of microneedles, the microneedles can be present on the devices in rows. In some embodiments, the rows can be spaced at virtually equal intervals to the space of the needles aligned in the row. In some embodiments, the rows can be spaced at irregular intervals.

[00106] In some embodiments the density of the microneedles in the device may be at least about 10/cm^2, about 15/cm^2, about 20/cm^2, about 25/cm^2, about 30/cm^2, about 35/cm^2, about 40/cm^2, about 45/cm^2, about 50/cm^2, about 55/cm^2, about 60/cm^2, about 65/cm^2, about 70/cm^2,

about 75/cm^2, about 80/cm^2, about 85/cm^2, about 90/cm^2, about 95/cm^2, about 100/cm^2, about 110/cm^2, about 120/cm^2, about 130/cm^2, about 140/cm^2, about 150/cm^2, or about 200/cm^2. In some embodiments the density of the microneedles in the device may be less than about 10/cm^2, about 15/cm^2, about 20/cm^2, about 25/cm^2, about 30/cm^2, about 35/cm^2, about 40/cm^2, about 45/cm^2, about 50/cm^2, about 55/cm^2, about 60/cm^2, about 65/cm^2, about 70/cm^2, about 75/cm^2, about 80/cm^2, about 85/cm^2, about 90/cm^2, about 95/cm^2, about 100/cm^2, about 110/cm^2, about 120/cm^2, about 130/cm^2, about 140/cm^2, about 150/cm^2, or about 200/cm^2.

[00107] In some embodiments, a distance between the center of two microneedles on a device of the disclosure can be calculated to determine a density of the microneedles in the device. In some embodiments, the center-to-center distance between two microneedles can be less than 1000 μm, less than 900 μm, less than 800 μm, less than 700 μm, less than 600 μm, less than 500 μm, less than 400 μm, less than 300 μm, less than 200 μm, or less 100 μm. In some embodiments, the center-to-center distance between two microneedles can be no greater than 100 μm, no greater than 200 μm, no greater than 300 μm, no greater than 400 μm, no greater than 500 μm, no greater than 600 μm, no greater than 700 μm, no greater than 800 μm, no greater than 900 μm, or no greater than 1000 μm.

[00108] In some embodiments, microneedles of the disclosure can comprise a plurality of different diameters or base widths. The shape of the base of a microneedle can be, for example, round, rectangular, triangular, square, pentagonal, hexagonal, heptagonal, or other geometric shape. A microneedle of the disclosure can have a diameter or base width that is no greater than 500 μm, no greater than 400 μm, no greater than 300 μm, no greater than 200 μm, no greater than 100 μm, no greater than 50 μm, no greater than 40 μm, no greater than 30 μm, no greater than 20 μm, no greater than 10 μm, no greater than 1000 nm, no greater than 900 nm, no greater than 800 nm, no greater than 700 nm, no greater than 600 nm, no greater than 500 nm, no greater than 400 nm, no greater than 300 nm, no greater than 200 nm, or no greater than 100 nm.

Kits

[00109] In some embodiments, the present disclosure provides kits comprising a microneedle device as described herein. In some cases, a kit may comprise a microneedle device as described herein, wherein the microneedle device comprises a recess behind the microneedle region, and an applicator which fits within the recess. In some cases, the applicator is a mechanical applicator. In some cases, the microneedle device comprises a microneedle region comprising a plurality of microneedles protruding from a front surface of a microneedle base substrate, the microneedle base substrate also comprising a back surface, the back surface comprising a recess directly behind at least a portion of the microneedle region; and a support substrate adjacent to at least

one side of the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate.

Methods

[00110] In some embodiments, the present disclosure provides a method of obtaining a biological sample from a tissue, such as the skin of a subject. A subject can be of any age. For example, a subject can be an elderly adult, an adult, an adolescent, a pre-adolescent, a child, a toddler, or an infant. A subject can be a mammal, a bird, a fish, a reptile, or an amphibian. Non-limiting examples of a subject include humans, primates, dogs, cats, horses, pigs, and mice. A subject may be a patient. In some embodiments, the subject is a human.

[00111] In some cases, the method comprises contacting a device of the present disclosure with a tissue of a subject. In some cases, the subject may contact the device with their tissue, for example by applying the device to their skin. In some cases, the device may be applied to a subject's tissue by someone else. In some cases, the tissue may be skin, and the skin may be cleaned prior to application of the device.

[00112] In some cases, the method comprises contacting a device of the present disclosure with a tissue, such as skin, of a subject. In some cases, the tissue is an *in situ* tissue. In some cases, the tissue is a biopsy. In some cases, the tissue is skin tissue. Pressure may be applied to the device, thus causing the microneedles to penetrate the tissue. In some cases, pressure is applied by hand, for example thumb pressure. In other cases, pressure may be applied by an applicator. The pressure may be less than about 1 N/mm2, 5 N/mm2, 10 N/mm2, 50 N/mm2, 100 N/mm2, 200 N/mm2, 200 N/mm2, or within a range of about 1 N/mm2 and about 200 N/mm2. The applicator may be a manual applicator, or a mechanical or electronic applicator which provides a uniform pressure. In some cases, one end of the applicator has a width that is less than the width of the recess behind the microneedle section. The surface of the device distal to the microneedles may be referred to as the back surface. The pressure may be applied to the entire back surface, or may be concentrated on the back surface of the interior section of the device 120.

[00113] In some embodiments, the microneedle device may be retained in the tissue for up to 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 12 minutes, 14 minutes, 16 minutes, 18 minutes, 20 minutes, 22 minutes, 24 minutes, 26 minutes, 28 minutes, or 30 minutes. The residence time of the microneedles inside the tissue allows the probes to contact and bind to biomarkers, for example nucleic acid molecules, in the tissue. In some cases, the residence time of microneedles inside the tissue may be optimized for a particular tissue, biomarker or probe. For example, a biomarker such as an

RNA biomarker may start to degrade after a given period of time, and thus a residence time long enough to allow binding but short enough to minimize degradation would be preferred. In some cases, a residence time of about 1 minute to about 10 minutes may be preferred for RNA biomarkers. In some cases, a residence time of less than 5 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes may be preferred for RNA biomarkers. In some cases, a residence time of no more than 10 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 2 minutes to about 9 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 3 minutes to about 8 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 4 minutes to about 7 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes to about 6 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes to about 7 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes to about 8 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes to about 9 minutes may be preferred for RNA biomarkers. In some cases, a residence time of about 5 minutes to about 10 minutes may be preferred for RNA biomarkers.

[00114] In some cases, the optimal residence time of the microneedles inside the tissue is determined by the cycle threshold (Ct) for detection of a target biomarker. In some cases, for RNA biomarkers, the Ct is lowest at about 5 minutes. In some cases, for RNA biomarkers, the Ct of a sample collected after a residence time of 10 minutes is higher than the Ct of a sample collected after a residence time of 5 minutes. In some cases, an increase of Ct from a minimum Ct indicates degradation of the sample may be occurring.

[00115] In some cases, the optimal residence time generates a high-quality biomarker sample. In some cases, for RNA biomarkers, the quality of the RNA sample is determined by a ratio of larger RNA fragments (e.g. RNA fragments comprising about 700 bases or more) comprised in the RNA sample to smaller RNA fragments (e.g. fragments comprising about 150 bases to about 200 bases) comprised in the RNA sample. In some cases, a residence time of no more than 10 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 9 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA

fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 8 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 7 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 6 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 5 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of no more than 4 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In

some cases, a residence time of no more than 3 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1. In some cases, a residence time of about 5 minutes generates an RNA sample wherein the RNA sample comprises a population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and a population of small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of large RNA fragments, e.g. RNA fragments comprising about 700 bases or more, and the small RNA fragments, e.g. fragments comprising about 150 bases to about 200 bases, is greater than 1.

[00116] In some embodiments, after allowing a time for the probes to contact and bind the biomarkers, the device is removed from the tissue. Removal of the microneedles from the tissue may generate sheering pressures that remove non-specifically bound contaminants from the microneedles. In some cases, contaminants comprise any compound not intended for further genomic analysis.

[00117] In some embodiments, once removed from skin the device and extracted RNA biomarkers may be placed into a storage buffer, a transport buffer, or an analysis buffer. The device and extracted RNA biomarkers may be stored at -80 °C, -20 °C, -4 °C, 4 °C or room temperature. Alternatively, the device may be placed into a buffer to dissociate the extracted RNA biomarkers from the device, and the extracted RNA biomarkers may be stored at -80 °C, -20 °C, -4 °C, 4 °C or room temperature. The extracted RNA biomarkers (with or without the device) may be sent to a laboratory for further analysis.

[00118] In some embodiments, the present disclosure provides a method for preparing a sample for genomic or transcriptomic analysis. The method may comprise, contacting a device of the present disclosure with skin of a subject, extracting nucleic acid biomarkers from the skin onto the device, washing the nucleic acid biomarkers from the device to produce a sample solution. The nucleic acids of the sample solution may be analyzed by polymerase chain reaction. In some cases, the extracted nucleic acids of the sample solution may be amplified and analyzed by next generation sequencing (NGS). NGS of extracted mRNAs may allow determination of gene expression in the contacted skin of the subject.

[00119] In some embodiments, the present disclosure provides a method of determining whether a skin lesion on a subject suffering thereof will be responsive to an autoimmune

therapeutic drug. For example, the present disclosure provides a method of preparing a biological sample and identifying one or more autoimmune disease therapeutic drugs for a subject, the method comprising contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle and applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject. Extracted ribonucleic acid (RNA) molecules from the subject may be obtained by removing the microneedle device from the skin of the subject. Performing high throughput sequencing on the extracted RNA molecules can generate one or more sequence reads for the subject, which may be aligned with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the autoimmune disease therapeutic drug, thereby obtaining aligned sequence reads; and used to classify the subject as having a greater than 50%, 60%, 70%, 80% or 90% likelihood of positively responding to the autoimmune disease therapeutic drug by applying a trained algorithm to the aligned sequence reads. In some cases, the trained algorithm has a greater than 50%, 60%, 70%, 80% or 90% positive predictive value or greater than 50%, 60%, 70%, 80% or 90% negative predictive value. The autoimmune disease therapeutic drug may be an IL-17 mediated treatment, an IL-23 mediated treatment, an TNFα mediated treatment, or a combination thereof. The method may comprise the steps of extracting and sequencing mRNAs as described above, and comparing a gene signature of the lesion to known gene expression signatures using a trained algorithm, or analyzing the gene signature of the lesion using a trained algorithm. In some cases, the trained algorithm may have a greater than 50% positive predictive value or greater than 50% negative predictive value for predicting a greater than 50% likelihood of positive response to the autoimmune disease therapeutic drug. Comparing the gene expression signatures may allow classification of the contacted skin lesion as likely to be responsive to a treatment, wherein the treatment is either IL-17 mediated treatment, IL-23 mediated treatment, TNFα mediated treatment, or a combination thereof. Examples of autoimmune disease therapeutics include etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab.

[00120] In some embodiments, the present disclosure provides a method for determining a likelihood that a subject with an autoimmune disease will respond to a therapeutic drug for an autoimmune disease the method comprising obtaining a biological sample from the subject; extracting one or more nucleic acid molecules from the biological sample; performing high throughput sequencing on the nucleic acid molecules to generate one or more sequence reads for the subject; and performing a genomic analysis on the sequence reads. The genomic analysis may

comprise: determining an expression level of genes encoded by the sequence reads; comparing the expression level to a known positive-response genetic signature specific to a treatment for the autoimmune disease; and generating a treatment score from the comparison, wherein the treatment score is specific to a response to the treatment in the patient.

[00121] In some embodiments, the present disclosure provides a method of treating a skin lesion of a subject, the method comprising contacting the skin lesion with a device as described herein, extracting mRNAs from the skin lesion, using NGS to determine the gene expression signature, comparing the signature to known signatures or using a trained algorithm to select a treatment for the subject, and administering the selected treatment to the subject. In some cases, selecting a treatment comprises comparing an expression level of a known positive-response genetic signature specific to a treatment for the autoimmune disease.

[00122] This disclosure provides non-invasive and/or minimally invasive devices, methods, and systems that provide a subject specific dataset for disease diagnosis or guided clinical intervention. Further described herein are microneedle devices and methods and systems of using the same. For example, the methods of the invention described herein may comprise, in some embodiments, determining a gene expression profile, precise classification of a disease or condition, prospective guided therapy and/or clinical intervention for the diseases or conditions, retrospective analysis and modeling of past treatments, identifying an activated pathway for a disease or condition, or any combination thereof for one or more subjects. The microneedle devices described herein may provide precise, minimally and/or non-invasive sample collection that may be used in downstream analysis as described elsewhere herein, particularly in the aforementioned methods. Thus, the methods of the disclosure are useful for providing a means for practicing personalized medicine, wherein treatment is tailored to a subject based on characteristics of the disease in the subject (e.g. the gene expression profile of the subject suffering from the disease).

[00123] Generally, the microneedle devices may facilitate the collection of a subject's biological sample in situ for further analysis. In some cases, the microneedle device may comprise one or more microneedle features configured to puncture the skin or mucosal membrane of the subject. In some cases, the mucosal membrane may comprise oral mucosa, ophthalmic mucosal membranes, or any combination thereof. When a plurality of microneedles is present, the device may comprise a planar substrate which supports the microneedles. The substrate can be made of the same material as that of the microneedle. It can also be made of a different material. In some instances, the microneedles may further comprise probes adhered to the microneedle. These probes may be configured to bind to one or more biomarkers within a

sample or tissue (e.g., skin) of a subject in such a manner as to permit extraction of the biomarker for further analysis. The extracted biomarkers may be analyzed, alone or in combination (e.g. generating a genetic signature or gene expression profile) to provide a diagnosis or prediction of a response to a drug or other therapeutic treatment with respect to the subject. **Treating a Subject for a Mild, Moderate, or Severe Form of a Skin Condition**

[00124] In some cases, known treatments for known diseases may not be efficacious against a particular form of a disease as compared to an alternative form of the same disease. Differences between forms of the disease may arise as a result of varying genetic signatures from each particular form. In some embodiments, the methods described herein may provide targeted, specialized, personalized, or otherwise highly efficacious treatments or recommendation of treatments for subjects suffering from particular forms of known diseases.

[00125] In some embodiments, the present disclosure provides a method of determining whether a mild, moderate, or severe form of a skin condition of a subject suffering thereof will be responsive to a recommended treatment **600**, as seen in **FIG. 6**. In some cases, the method of determining whether a skin condition of a subject suffering thereof will be responsive to recommended treatment may comprise: (a) obtaining ribonucleic acid (RNA) biomarkers from a subject (as described elsewhere herein) **602; (**b) determining the likelihood that the skin condition will respond to a recommended treatment, wherein the recommended treatment comprises a 50% or less aggregate efficacy rate against known forms of the skin condition **604;** and (c) treating the subject with the recommended treatment **606**.

[00126] In some cases, the step of determining (i.e., step (b) of the above) may further comprise: (i) performing high throughput sequencing on the RNA biomarkers to generate one or more sequence reads for the subject; (ii) aligning the one or more sequence reads for the subject with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the recommended treatment, thereby obtaining aligned sequence reads; and (iii) classifying the subject as having a greater than 50% likelihood of positively responding to the recommended treatment by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm comprises a greater than 50% positive predictive value for predicting a greater than 50% likelihood of positive response to the recommended treatment. In some cases, the RNA biomarkers may comprise RNA molecules. In some instances, the RNA biomarkers are transcribed from one or more of the follow genes listed in **Table 6**, **Table 12, or Table 13**.

[00127] In some embodiments, the trained algorithm may further comprise a greater than 50% negative predictive value for predicting a greater than 50% likelihood of positive response

to the recommended treatment. In some cases, the positive predictive value may be greater than 50%, 60%, 70%, 80% or 90%. In some instances, the negative predictive value may be or greater than 50%, 60%, 70%, 80% or 90%.

[00128] In some embodiments, the recommended treatment may comprise one or more autoimmune therapeutic drugs for an autoimmune disease or condition. In some cases, the autoimmune disease or condition may be psoriasis. In some instances, the recommended treatment may comprise etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, risankizumab, or any combination thereof.

Recommending a Treatment by Positive Predictive Value

[00129] In some cases, one or more subject suffering from a similar disease or condition may respond to a treatment with varying efficacy. In some embodiments, the efficacy may comprise a responder, non-responder, or adverse responder. In particular, genetic variability between subjects suffering from similar disease or condition may further stratify the varying efficacy of treatment intended to broadly treat a general form of the disease or condition. Aspects of the disclosure provided herein, in some embodiments, comprise a method of preparing a biological sample and identifying one or more therapeutic drugs to effectively tailor treatment to the particular genetic variant of a subject's disease or condition **608,** as seen in **FIG. 6B**.

[00130] In some embodiments, this disclosure provides a method of treating a subject with an autoimmune disease of the skin (e.g., psoriasis) or recommending a treatment to such subject comprising collecting a sample comprising RNA derived from skin from the subject. Such collecting of a sample of RNA may involve use of a microneedle device or may involve lysis of a tissue biopsy or cell sample and extraction of nucleic acids (e.g., RNA) using an RNA extraction protocol. In some cases, the subject has not been administered the autoimmune therapeutic drug prior to collecting the sample. For example, in some embodiments, the subject has not been administered the autoimmune therapeutic drug greater than 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 15 days or 20 days prior to collecting the sample from the subject. In some cases, the method further comprises determining an expression level of at least one gene based on the RNA; predicting that a subject with the autoimmune disease of the skin will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 70%, a positive predictive value, a greater than 75% positive predictive value, a greater than 80% positive predictive value, a greater than 85% positive predictive value, a greater than 90% positive predictive value, or a greater than 95% positive predictive value based on the expression level of the at least one gene; and/or based on the predicting, treating the subject with the

autoimmune therapeutic drug. In some cases, the positive predictive value is determined using a cohort of a specific size. For example, the method may have a positive predictive value provided herein when evaluated in a cohort of greater than 5, 10, 25, 50, 100, 150, 200, 250, 500, or 1000 patients.

[00131] In some embodiments, this disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising collecting a sample comprising RNA derived from skin from the subject. Such collecting of a sample of RNA may involve use of a microneedle device or may involve lysis of a tissue biopsy or cell sample and extraction of nucleic acids (e.g., RNA) using an RNA extraction protocol. In some cases, the subject has not been administered the autoimmune therapeutic drug prior to collecting the sample. For example, in some embodiments, the subject has not been administered the autoimmune therapeutic drug greater than 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 15 days or 20 days prior to collecting the sample from the subject. In some cases, the method further comprises converting the RNA into cDNA and determining an expression level of at least one gene based on the cDNA. In some cases, the method further comprises predicting that a subject with the skin lesion will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 70%, a positive predictive value, a greater than 75% positive predictive value, a greater than 80% positive predictive value, a greater than 85% positive predictive value, a greater than 90% positive predictive value, or a greater than 95% positive predictive value based on the expression level of the at least one gene; and/or based on the predicting, treating the subject with the autoimmune therapeutic drug. In some cases, the positive predictive value is determined using a cohort of a specific size. For example, the method may have a positive predictive value provided herein when evaluated in a cohort of greater than 5, 10, 25, 50, 100, 150, 200, 250, 500, or 1000 patients.

[00132] In some embodiments, this disclosure provides a method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle. RNA molecules are obtained from the subject by removing the microneedle device from the skin of the subject. In some cases, high throughput sequencing on the RNA molecules is performed to generate sequence reads. The sequence reads are aligned with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads. A trained algorithm is applied to the aligned sequence reads, wherein the trained algorithm has a greater than 70%, 80%, 85%, 90% or 95% positive predictive value for predicting response

to the autoimmune disease therapeutic drug. In some cases, the aligned sequence reads is used to determine an expression level of at least one RNA molecule; and a trained algorithm is applied to the expression level of the at least one RNA molecule, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, an IL-23 mediated treatment, a TNFα mediated treatment, or any combination thereof. In some cases, high throughput sequencing is performed on the RNA biomarkers to generate one or more sequence reads for the subject. The one or more sequence reads is aligned with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the recommended treatment, thereby obtaining aligned sequence reads. The subject is classified as having a likelihood of positively responding to the recommended treatment by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm comprises a greater than 50%, 60%, 70%, 80%, 85%, 90% or 95% positive predictive value for predicting a positive response to the recommended treatment. In some case, the trained algorithm also has a greater than 50%, 60%, 70%, 80%, 85%, 90% or 95% negative predictive value. In some cases, the recommended treatment comprises one or more autoimmune therapeutic drugs for an autoimmune disease or condition, including, for example, etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab. In some cases, the autoimmune disease or condition is psoriasis, acne, atopic dermatitis (i.e., eczema), Raynaud's phenomenon, rosacea, skin cancer (e.g., basal cell carcinoma, squamous cell carcinoma and melanoma), or vitiligo.

[00133] In some embodiments, this disclosure provides a method of determining whether a subject with an autoimmune disorder of the skin will be responsive to an autoimmune therapeutic drug comprising extracting mRNA from skin of the subject, sequencing the mRNA from the skin of the subject; and predicting whether the subject with the autoimmune disorder will be responsive to etanercept, adalimumab, infliximab, certolizumab, secukinumab, ixekisumab, broadalumab, guselkumab, tildrakizumab, and risankizumab with a positive predictive value greater than than 50%, 60%, 70%, 80%, 85%, 90% or 95%.

[00134] In some cases, the at least one gene is at least one gene from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least two genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least three genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least four genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least five genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least six genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least ten

genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least fifteen genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least twenty genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least 25 genes from **Table 6, Table 12,** and/or **Table 13.** In some cases, the at least one gene is at least 50 genes from **Table 6, Table 12,** and/or **Table 13.**

[00135] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, PPIG, RAB31, C3, and EGR. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, and PPIG. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of PCDH7, PPIG, RAB31, C3, and EGR.

[00136] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of CNFN, CTSC, GBAP1, and CRABP2. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PPIG, RAB31, C3, and EGR. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PCDH7, PPIG, RAB31, and C3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of GBAP1, CRABP2, PCDH7, PPIG.

[00137] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of SERPINB3, SERPINB4, S100A7A, PI3, KRT6A, LCN2, DEFB4A, DEFB4B, SPRR1A, IL36G, MX1, IFI27, CD36, CD24, and IL4R. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of KRT6A, SPRR1A, CD36, IL4R, LCN2, and IFI27. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of CD36, IL4R, S100A7A, SERPINB4, MX1, and SERPINB3. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of LCN2, IFI27, DEFB4A, IL36G, CD24, and PI3.

[00138] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of IL4R, LCN2, and IFI27. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PI3, IFI27, and SERPINB3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of IL4R, S100A7A, and MX1. In some cases, the at least one gene is at

least one gene, two genes, or three genes from the group consisting of CD36, LCN2, and SERPINB4.

[00139] In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of MTCO1P12, MTATP6P1, CLSTN1, PDPN, LDLRAD2, and GSTM3. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of AL158847.1, DAD1, LDLRAD2, ZNF395, MGMT, and AL136982.4. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of NREP, PPIF, PRIM1, AL136982.5, MTATP6P1, and SMPD3. In some cases, the at least one gene is at least one gene, two genes, three genes, four genes, five genes, or six genes from the group consisting of PDPN, TXNRD1, GSTM3, GPSM1, GLRX, and USP2.

[00140] In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of MTCO1P12, CLSTN1, and GSTM3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of NREP, PPIF, and PRIM1. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of AL136982.5, MTATP6P1, and SMPD3. In some cases, the at least one gene is at least one gene, two genes, or three genes from the group consisting of PDPN, TXNRD1, and GSTM3.

[00141] The gene or genes used to detect a response to treatment may, in some cases, engage in a common pathway, such as an IL-17-mediated pathway, TNF-alpha pathway, or IL-23 pathway. In some cases, the at least one gene comprises at least 2, 3, 4, 5, 6, 7, 10, 15, or 20 genes that do not share a common upstream regulator or do not share a common pathway. In some cases, the genes have some relationship with the recommended treatment, e.g., the genes may be involved with a particular pathway (e.g., IL-17, Il-23, or TNF-alpha mediated pathway). In some cases, at least one gene does not have a known relationship with a recommended treatment. For example, the gene used to evaluate a response to treatment for a particular therapeutic may not be involved in the pathway targeted by the therapeutic. In some cases, the autoimmune therapeutic drug is an IL-17-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-17 mediated pathway. In some cases, the autoimmune therapeutic drug is an IL-23-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-23 mediated pathway. In some cases, the autoimmune therapeutic drug is a TNF-alpha-mediated treatment and the at least one gene comprises at least one gene that is not involved in a TNF-alpha-mediated pathway.

[00142] In some cases, a trained algorithm is applied to expression level of the at least one gene in order to predict the subject's likelihood of responding to the therapeutic. In some cases, the algorithm is trained using samples from patients that were administered a single type of drug. For example, the patients may have been administered an IL-17 mediated treatment, TNF-alpha mediated treatment or an IL-23 mediated treatment.

[00143] In some cases, the methods comprise use of a microneedle device. In some cases, the method of preparing a biological sample and identifying one or more therapeutic drugs may comprise : (a) contacting skin of a subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle, as described elsewhere herein **610**; (b) applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject **612**; (c) obtaining extracted RNA molecules from the subject by removing the microneedle device from the skin of the subject **614**; (d) performing high throughput sequencing on the extracted RNA molecules to generate one or more sequence reads for the subject **616**; (e) aligning the one or more sequence reads for the subject with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the one or more therapeutic drugs, thereby obtaining aligned sequence reads **618**; and classifying the subject as having a likelihood of positively responding to the one or more therapeutic drugs by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm has a greater than 70% positive predictive value, a greater than 75% positive predictive value, a greater than 80% positive predictive value, a greater than 85% positive predictive value, a greater than 90% positive predictive value, or a greater than 95% positive predictive value **620**. In some embodiments, the method of preparing a biological sample and identifying one or more therapeutic drugs may further comprise administering the one or more therapeutic drugs to the subject. In some instances, the autoimmune disease or condition is psoriasis.

[00144] In some cases, the subject has a PASI of at least 8, at least 9, at least 10, at least 15. In some cases, the subject has a PASI of at least 8 and at least a PASI 75, 80, 90, 95, or 100 after treatment with the recommended drug. In some cases, the subject has a PASI of at least 10 and a PASI 75, 80, 90, 95, or 100 after treatment. In some embodiments, the trained algorithm may further comprise a greater than 50% negative predictive value for predicting a greater than 50% likelihood of positive response to the one or more therapeutic drugs. In some cases, the positive predictive value may be greater than 50%, 60%, 70%, 80% or 90%. In some instances, the negative predictive value may be or greater than 50%, 60%, 70%, 80% or 90%. In some embodiments, the extracted RNA molecules may be transcribed from one or more of the

following genes listed in **Table 6**, **Table 12**, or **Table 13**. In some embodiments, the recommended treatment may comprise one or more autoimmune therapeutic drugs for an autoimmune disease or condition. In some cases, the autoimmune disease or condition may be psoriasis. In some instances, the recommended treatment may comprise etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, risankizumab, or any combination thereof.

Response to a Treatment

[00145] In some cases, the variability of gene expression between one or more subjects may be predictive of the response of a particular treatment for a disease or condition, particularly psoriasis. Aspects of the invention disclosed herein, in some embodiments, provide a method for determining the response to a treatment for a disease or condition in a subject afflicted with the disease or condition. In some embodiments, the method may further comprise recommending an additional treatment specific to the score. In some cases, the method may further comprise administering the treatment or the additional treatment to the subject. In some instances, the one or more nucleic acid molecules may comprise deoxyribonucleic acid (DNA) molecules. In some cases, the one or more nucleic acid molecules comprise RNA molecules. In some cases, the RNA molecules may comprise messenger RNA (mRNA) molecules.

[00146] In some embodiments, the disease or condition may be an autoimmune disease. In some cases, the autoimmune disease may be psoriasis.

Determining a Mild, Moderate or Severe Form of a Known Disease or Condition

[00147] In some cases, particular forms of a disease (e.g., severe, moderate, normal, etc.) may comprise different gene signatures. Certain known treatments for known diseases or conditions are not as efficacious against a particular form of a disease as compared to an alternate form of the same disease that may comprise. In some instances, the method may further comprise recommending a treatment specific to the score. In some cases, the method may further comprise administering the treatment to the subject. In some cases, the disease or condition may be an autoimmune disease. In some instances, the autoimmune disease may be psoriasis. In some cases, the one or more nucleic acid molecules may comprise DNA molecules. In some embodiments, the one or more nucleic acid molecules may comprise RNA molecules. In some cases, the RNA molecules may comprise mRNA molecules.

[00148] In some embodiments, the at least five genes from **Table 6, Table 12, or Table 13** may comprise genes associated with one or more pathways. In some cases, the at least five genes from **Table 6, Table 12, or Table 13** may comprise at least 2 genes associated with activation of the one or more pathways.

Identifying Activation of Biological Pathways Associated with a Disease or Condition

[00149] Aspects of the disclosure, in some embodiments, provide methods for identifying the activation of one or more pathways that may be associated with a disease or condition in a subject **642**, as seen in **FIG. 6C**. In some embodiments, the method for identifying the activation of one or more pathways that may be associated with a disease or condition in a subject may comprise the steps of: (a) contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle **644**; (b) applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject **646**; (c) obtaining extracted RNA molecules from the subject by removing the microneedle device form the skin of the subject **648**; (d) performing high throughput sequencing on the extracted RNA molecules to generate one or more sequence reads for the subject **650**; (e) aligning the one or more sequence reads for the subject with a known signature of sequence reads, wherein the known signature of sequence reads may be associated with activation of one or more pathways associated with a disease or condition **652**; and (f) classifying the subject as having a greater than 50% likelihood of developing the disease or condition associated with activation of the one or more pathways by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm has a greater than 50% positive predictive value or greater than 50% negative predictive value for predicting a greater than 50% likelihood of developing the disease associated with activation of the one or more pathways **654**. In some cases, the method may further comprise recommending a treatment specific to the activation of the one or more pathways. In some cases, the method may further comprise administering the treatment specific to the activation of the one or more pathways to the subject. In some instances, the microneedle may be a solid microneedle.

[00150] In some embodiments, the trained algorithm may further comprise a greater than 50% negative predictive value for predicting a greater than 50% likelihood of developing the disease or condition associated with activation of the one or more pathways. In some cases, the positive predictive value may be greater than 50%, 60%, 70%, 80% or 90%. In some instances, the negative predictive value may be or greater than 50%, 60%, 70%, 80% or 90%.

[00151] In some embodiments, the disease or condition may be an autoimmune disease or condition. In some cases, the autoimmune disease or condition may be psoriasis. In some embodiments, the extracted RNA molecules may be transcribed from one or more genes comprising at least one gene associated with activation of the one or more pathways. In some cases, the one or more genes may comprise at least two genes associated with activation of the

one or more pathways. In some cases, the one or more genes may comprise any one of the genes listed in **Table 6**, **Table 12 or Table 13.**

Subjects

[00152] As used herein, the term "subject" refers to any animal, including mammals and non-mammals), for example, humans, non-human primates (e.g., rhesus macaques, crab-eating macaques, stump-tailed macaques, pig-tailed macaques, squirrel monkeys, owl monkeys, baboons, chimpanzees, marmosets and spider monkeys, etc.), rodents (e.g., mice, rats, guinea pigs, etc.), dogs, cats, pigs, etc. In some cases, the subject is a human subject.

[00153] In some embodiments, the subject may be experiencing a skin condition or have symptoms of a skin condition. Examples of skin conditions that may be detected and/or treated by the methods and devices provided herein include: psoriasis, acne, atopic dermatitis (i.e., eczema), Raynaud's phenomenon, rosacea, skin cancer (e.g., basal cell carcinoma, squamous cell carcinoma and melanoma), and vitiligo. In some cases, the subject has, or has symptoms of, one or more skin conditions, for example, psoriasis and atopic dermatitis. In some cases, the subject may be experiencing different degrees of skin conditions, for example, mild, moderate, severe, and very severe. Examples of symptoms of psoriasis include red patches of skin covered with thick, silvery scales; small scaling spots; dry, cracked skin that may bleed or itch; itching, burning or soreness;

thickened, pitted or ridged nails; swollen and stiff joints.

Psoriasis Area and Severity Index (PASI) score

[00154] The Psoriasis Area and Severity Index (PASI) score is commonly used to measure discoloration, thickness, scaling, and coverage of these plaques. A caregiver (e.g., a medical doctor, nurse, nurse practitioner, physician's assistant, etc.) can use PASI score to measure the severity and extent of psoriasis and observe the effectiveness of psoriasis treatments. Using the tool also allows a caregiver to monitor the progression of the condition and evaluate the effectiveness of treatment.

[00155] The scoring involves rating the symptoms of psoriasis from none to very severe and estimating the percentage of the body that they affect. Researchers also use PASI scores to determine the effectiveness of psoriasis medications in clinical trials.

[00156] The range of absolute PASI scores is 0–72, with higher scores indicating a greater severity of psoriasis. A score of 0 indicates no psoriasis, while a score higher than 10 suggests severe psoriasis. The scoring system includes a section for intensity and another for body coverage. The intensity section of the score measures 1) discoloration (i.e., redness), 2) thickness,

and 3) scaling. The area section shows the extent to which psoriasis affects the following:1) head and neck, 2) upper limbs, 3) trunk, and 4) lower limbs.

[00157] PASI scores can calculate PASI scores as follows:

A scores	Add together the three intensity scores (discoloration, thickness, and scaling) for each of the four body regions to get four A scores. Each intensity score can be given based on the severity of the symptoms: 0: absent symptoms; 1: mild symptoms; 2: moderate symptoms; 3: severe symptoms; 4: very severe symptoms.
B scores	A B score is assigned for each of the four body regions: 0: absence of symptoms, 1: 1–9% coverage, 2: 10–29% coverage, 3: 30–49% coverage, 4: 50–69% coverage, 5: 70–89% coverage, and 6: 90–100% coverage.
C scores	Multiply the A score by the B score for each body region to get four C scores
D scores	Multiply each C score by the amount of body surface area that the region represents. This amount is 0.1 for the head and neck, 0.2 for the arms, 0.3 for the trunk, and 0.4 for the legs. This gives four D scores.
PASI score	Add together the four D scores.

[00158] Clinical researchers often use PASI percentage response rates to indicate treatment outcomes. For example, PASI 75 means that a person's PASI score has decreased 75% from baseline, which indicates a significant improvement in the condition. Conventional goal of treatment has been to achieve PASI 75. However, a PASI 90 or PASI 100 (i.e., a 90% or 100% reduction in PASI scores, respectively) is more desirable.

[00159] In some cases, a subject provided herein has a PASI of at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or at least 15. In some cases, a subject has moderate-to-severe psoriasis and a baseline PASI of greater than or equal to 10. In some cases, the methods provided herein provide a prediction for whether a subject will respond to a particular treatment. In some cases, following being treated by the recommended treatment, the subject responds to the treatment and has as a decrease in PASI score. In some cases, the subject's response to treatment may be a particular response at week 16 following treatment, such as week 16 PASI60; week 16 PASI75, week 16 PASI80, week 16 PASI80, week 16 PASI90, week 16 PASI95, week 16 PASI 99, or week 16 PASI 100. In some cases, the subject's response to treatment may be a particular response at week 12 following treatment, such as week 12 PASI60; week 12 PASI75, week 12 PASI80, week 12 PASI80, week 12 PASI90, week 12 PASI95, week 12 PASI 99, or

week 12 PASI 100. In some cases, the subject's response to a treatment is a response at week 8 following treatment, such as week 8 PASI60; week 8 PASI75, week 8 PASI80, week 8 PASI80, week 8 PASI90, week 8 PASI95, week 8 PASI 99, or week 8 PASI 100. In some cases, the subject's response to a treatment is a response at week 4 following treatment, such as week 4 PASI60; week 4 PASI75, week 4 PASI80, week 4 PASI80, week 4 PASI90, week 4 PASI95, week 4 PASI 99, or week 4 PASI 100. In some embodiments, the subject's response to treatment is evaluated at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 8 weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, or at least 24 weeks following treatment. In some embodiments, the subject's response to treatment is evaluated at most 2 weeks, at most 3 weeks, at most 4 weeks, at most 8 weeks, at most 12 weeks, at most 16 weeks, at most 20 weeks, at most 24 weeks following treatment, or at most 50 weeks following treatment. For example, the subject's response to treatment may, in some embodiments, be evaluated between 8 and 12 weeks following treatment, between 4 and 12 weeks following treatment, and/or between 8 and 16 weeks following treatment. In some embodiments, the patient's PASI response may be at least PASI60, at least PASI70, at least PASI80, at least PASI90, at least PASI95, at least PASI99, or PASI100 for any of the periods following treatment.

Sample Preparation

[00160] Nucleic acids can be extracted from a sample using the microneedle devices described herein, or extracted directly from a sample (e.g., biopsy sample) using an extraction method other than one that involves use of a microneedle device. The types of nucleic acids that can be extracted include any nucleic acid molecules that encode genetic information, including for example, messenger RNA (mRNA), microRNA, DNA (e.g., nuclear DNA or mitochondrial DNA), a mixture of mRNA and DNA, short RNA, isolated RNA, and isolated DNA. The biopsy samples that can be used for nucleic acids extraction can include any tissue removed from any part of the body, for example, from the surface of the body (e.g., skin, hair, scalp, surface skin, nails, skin debris, hair follicles); from inside the body (e.g., brain, heart, lungs, pancreas. kidney, liver, intestine, muscles, connective tissue, bone, cartilage, or blood). The biopsy sample can be obtained invasively, non-invasively, or minimally invasively. In some cases, the biopsy sample is obtained from one or more active lesions, dormant lesions, or normal tissues.

[00161] In some cases, once removed from skin, the device and extracted RNA biomarkers may be placed into a storage buffer, a transport buffer, or an analysis buffer. The device and extracted RNA biomarkers may be stored at -80 °C, -20 °C, -4 °C, 4 °C or room temperature. Alternatively, the device may be placed into a buffer to dissociate the extracted RNA biomarkers from the device, and the extracted RNA biomarkers may be stored at -80 °C, -20 °C, -4 °C, 4 °C

or room temperature. The extracted RNA biomarkers (with or without the device) may be sent to a laboratory for further analysis.

[00162] In some cases, DNA is sequenced using nanopore sequencing, in which a single molecule of DNA or RNA can be sequenced without the need for PCR amplification or chemical labeling of the sample. In some cases, the extracted nucleic acids of the sample s may be amplified and analyzed by next generation sequencing (NGS), high-throughput sequencing, massively parallel sequencing, or sequencing-by-synthesis. Different sequencing approaches can include, for example, pyrosequencing, sequencing by reversible terminator chemistry, sequencing-by-ligation mediated by ligase enzymes, or phosphor-linked fluorescent nucleotides or real-time sequencing. Methods for performing genomic analyses may also include microarray methods. In some cases, genomic analysis may be performed in combination with any of the other methods herein. For example, a sample may be obtained, tested for adequacy, and divided into aliquots. One or more aliquots may then be used for cytological analysis of the present invention, one or more may be used for gene expression profiling methods of the present invention, and one or more may be used for genomic analysis. It is further understood the present invention anticipates that one skilled in the art may wish to perform other analyses on the biological sample that are not explicitly provided herein.

[00163] In some embodiments, wherein the nucleic acid molecule is RNA, the method further comprises converting the captured RNA to DNA (e.g., cDNA) that is readily available for sequencing (e.g. sequencing by synthesis, or nanopore sequencing). In some cases, RNeasy Mini kits (Qiagen, Valencia, CA) are used for RNA extraction. RNAase-Free DNase digestion (Qiagen, Valencia, CA) can be used to remove genomic DNA from the RNA preparations. Taqman kits (Applied Biosystems, Foster City, CA) can be used for reverse transcription. For example, 200 ng total mRNA was added in 20 μl reverse-transcription reaction and the mixture was incubated 5 minutes at 25°C, 30 minutes at 48°C, then 5 minutes at 95°C. cDNAs were stored at -20°C for further use.

[00164] In some case, cDNA can be amplified and then sequenced by a commercial vendor (Psomagen, Inc., Rockville, MD) according to standard procedures. Library preparation can be accomplished using Illumina Nextera DNA Flex kits according to the manufacturer's instructions. Prepared indexed libraries can be loaded onto a NovaSeq6000 S4 with read length of 150PE for sequencing of 40 M reads per sample. During sequencing, the quality score (Q30) may be maintained at a certain level, e.g., over 75%. Upon completion of runs, FASTQ file quality may be checked with FASTQC and trimmed with the Trim_galore program. The trimmed FASTQ can be aligned and mapped to human reference genome (e.g., GRCh38) using

hisat2 program. In some embodiments, the number of reads is counted for each Ensemble gene ID using FeatureCounts program and Homo sapiens GRCH38.84.gtf. In some cases, RNA expression analysis is further processed using Bioconductor package edgeR. Genes may be filtered using filterByExpr before logCPM (counts per million reads) were calculated. Plots may be made with ggplot2 and heatmap.2 functions in R. Percent of genes detected may be determined using the ratio of genes detected (with >0 count) over the total number of Ensemble genes.

[00165] The general methods for determining gene expression product levels may include but are not limited to one or more of the following: additional cytological assays, assays for specific proteins or enzyme activities, assays for specific expression products including protein or RNA or specific RNA splice variants, in situ hybridization, whole or partial genome expression analysis, microarray hybridization assays, SAGE, enzyme linked immuno-absorbance assays, mass-spectrometry, immuno-histochemistry, or blotting. Gene expression product levels may be normalized to an internal standard such as total mRNA or the expression level of a particular gene including but not limited to glyceraldehyde 3 phosphate dehydrogenase, or tublin.

[00166] In some embodiments, microarray analysis begins with extracting and purifying nucleic acid from a biological sample, (e.g. a biopsy or fine needle aspirate) using methods known to the art. For expression analysis, it can be advantageous to extract and/or purify RNA from DNA. It can further be advantageous to extract and/or purify mRNA from other forms of RNA such as tRNA and rRNA.

[00167] In some embodiments, purified nucleic acid may further be labeled with a fluorescent, radionuclide, or chemical label such as biotin or digoxin for example by reverse transcription, PCR, ligation, chemical reaction or other techniques. The labeling can be direct or indirect which may further require a coupling stage. The coupling stage can occur before hybridization, for example, using aminoallyl-UTP and NHS amino-reactive dyes (like cyanine dyes) or after, for example, using biotin and labeled streptavidin. The modified nucleotides (e.g. at a 1 aaUTP:4 TTP ratio) are added enzymatically at a lower rate compared to normal nucleotides, typically resulting in 1 every 60 bases (measured with a spectrophotometer). The aaDNA may then be purified with, for example, a column or a diafiltration device. The aminoallyl group is an amine group on a long linker attached to the nucleobase, which reacts with a reactive label (e.g. a fluorescent dye).

[00168] In some embodiments, the labeled samples may then be mixed with a hybridization solution which may contain SDS, SSC, dextran sulfate, a blocking agent (such as COT1 DNA, salmon sperm DNA, calf thymum DNA, PolyA or PolyT), Denhardt's solution, formamine, or a

combination thereof. A hybridization probe is a fragment of DNA or RNA of variable length, which is used to detect in DNA or RNA samples the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe. The probe thereby hybridizes to single-stranded nucleic acid (DNA or RNA) whose base sequence allows probe-target base pairing due to complementarity between the probe and target. The labeled probe is first denatured (by heating or under alkaline conditions) into single DNA strands and then hybridized to the target DNA.

[00169] In some embodiments, to detect hybridization of the probe to its target sequence, the probe is tagged (or labeled) with a molecular marker; commonly used markers are ^{32}P or Digoxigenin, which is non-radioactive antibody-based marker. DNA sequences or RNA transcripts that have moderate to high sequence similarity to the probe are then detected by visualizing the hybridized probe via autoradiography or other imaging techniques. Detection of sequences with moderate or high similarity depends on how stringent the hybridization conditions were applied—high stringency, such as high hybridization temperature and low salt in hybridization buffers, permits only hybridization between nucleic acid sequences that are highly similar, whereas low stringency, such as lower temperature and high salt, allows hybridization when the sequences are less similar. Hybridization probes used in DNA microarrays refer to DNA covalently attached to an inert surface, such as coated glass slides or gene chips, and to which a mobile cDNA target is hybridized.

[00170] In some embodiments, the mix may then be denatured by heat or chemical means and added to a port in a microarray. The holes may then be sealed, and the microarray hybridized, for example, in a hybridization oven, where the microarray is mixed by rotation, or in a mixer. After an overnight hybridization, non-specific binding may be washed off (e.g. with SDS and SSC). The microarray may then be dried and scanned in a special machine where a laser excites the dye and a detector measures its emission. The image may be overlaid with a template grid and the intensities of the features (several pixels make a feature) may be quantified.

[00171] Various kits can be used for the amplification of nucleic acid and probe generation of the subject methods. Examples of kit that can be used in the present invention include but are not limited to Nugen WT-Ovation FFPE kit, cDNA amplification kit with Nugen Exon Module and Frag/Label module. The NuGEN WT-Ovation™ FFPE System V2 is a whole transcriptome amplification system that enables conducting global gene expression analysis on the vast archives of small and degraded RNA derived from FFPE samples. The system is comprised of reagents and a protocol required for amplification of as little as 50 ng of total FFPE RNA. The protocol can be used for qPCR, sample archiving, fragmentation, and labeling. The amplified cDNA can

be fragmented and labeled in less than two hours for GeneChip® 3′ expression array analysis using NuGEN's FL-Ovation™ cDNA Biotin Module V2. For analysis using Affymetrix GeneChip® Exon and Gene ST arrays, the amplified cDNA can be used with the WT-Ovation Exon Module, then fragmented and labeled using the FL-Ovation™ cDNA Biotin Module V2. For analysis on Agilent arrays, the amplified cDNA can be fragmented and labeled using NuGEN's FL-Ovation™ cDNA Fluorescent Module.

[00172] In some embodiments, Ambion WT-expression kit can be used. Ambion WT-expression kit allows amplification of total RNA directly without a separate ribosomal RNA (rRNA) depletion step. With the Ambion® WT Expression Kit, samples as small as 50 ng of total RNA can be analyzed on Affymetrix® GeneChip® Human, Mouse, and Rat Exon and Gene 1.0 ST Arrays. In addition to the lower input RNA requirement and high concordance between the Affymetrix® method and TaqMan® real-time PCR data, the Ambion® WT Expression Kit provides a significant increase in sensitivity. For example, a greater number of probe sets detected above background can be obtained at the exon level with the Ambion® WT Expression Kit as a result of an increased signal-to-noise ratio. Ambion WT-expression kit may be used in combination with additional Affymetrix labeling kit.

[00173] In some embodiments, AmpTec Trinucleotide Nano mRNA Amplification kit (6299-A15) can be used in the subject methods. The ExpressArt® TRinucleotide mRNA amplification Nano kit is suitable for a wide range, from 1 ng to 700 ng of input total RNA. According to the amount of input total RNA and the required yields of aRNA, it can be used for 1-round (input >300 ng total RNA) or 2-rounds (minimal input amount 1 ng total RNA), with aRNA yields in the range of >10 µg. AmpTec's proprietary TRinucleotide priming technology results in preferential amplification of mRNAs (independent of the universal eukaryotic 3′-poly(A)-sequence), combined with selection against rRNAs. This kit can be used in combination with cDNA conversion kit and Affymetrix labeling kit.

[00174] In some embodiments, the raw data may then be normalized, for example, by subtracting the background intensity and then dividing the intensities making either the total intensity of the features on each channel equal or the intensities of a reference gene and then the t-value for all the intensities may be calculated. More sophisticated methods include z-ratio, loess and lowess regression and RMA (robust multichip analysis) for Affymetrix chips.

[00175] In some cases, PolyA tailed mRNA is captured on a support via hybridization to a polyT DNA capture probe or primer coupled to the surface of a support. The polyT strand is next extended with a reverse transcriptase polymerase to make a double stranded molecule comprising a DNA:RNA duplex. Next, a transposome complex (e.g., Tn5 transposase bound with an adaptor

sequence and sequences complementary to surface amplification primers) is added to the support, which undergoes a transposition reaction with and tagments the duplex, ligating a DNA adaptor oligo to the 5′ end of the RNA strand. A strand displacing polymerase (e.g., Bst polymerase) can then be used to extend the 3′ end of the DNA strand, displacing the non-transferred strand of the transposome complex and copying the RNA strand to its 5′ DNA chimeric end. In some embodiments, the DNA adaptor oligo comprises a sequence complementary to an anchor primer on a substrate configured for sequencing. In some embodiments, the DNA adaptor oligo comprises a sequence that is complementary to sequencing primer for sequencing. The double-stranded molecule can then be amplified (e.g., cluster amplification) and sequenced with a sequencing primer. The primer partially comprises the adaptor sequence and the upstream adaptor sequence. Alternatively, the other end of the molecule (the polyT end) can be sequenced with a primer that anneals upstream of the polyT sequence and is extended with natural dATP nucleotides before commencing cycles of sequence by synthesis (SBS) chemistry.

[00176] In some cases, RNA is fragmented and treated with a phosphatase. A single stranded adaptor molecule is ligated to the 3′end of each RNA fragment comprising the complement of a surface bound primer. The fragments are then added to a support and captured via hybridization. The hybridized RNA molecules are converted to a DNA:RNA duplex with a reverse transcriptase polymerase. A transposome complex or composition comprising a transposase and an adaptor duplex (i.e., transposon) of an ME with P5 is used to tagment the duplex. Following extension of the DNA strand to the end with a strand displacing polymerase, the molecules can be amplified (e.g., cluster amplification) and sequenced.

Bioinformatic analysis of sequence reads

[00177] As used herein, positive predictive value (PPV) is the percentage of true positives among all subjects who test positive. It can be computed as follow: PPV = TP / (TP + FP) where TP and FP are the number of true positive and false positive results, respectively.

[00178] As used herein, negative predictive value (NPV) is the percentage of true negative among all subjects who test negative. It can be computed as follow: PPV = TN / (TN + FN) where TN and FN are the number of true negatives and false negatives results, respectively.

[00179] An algorithm (Mind.Px) is developed herein for the prediction of response to biologics (anti-IL-17, anti-IL-23) used for the treatment of patients with psoriasis, by comparing baseline transcriptomes with clinical response to biologic drugs at 12 weeks after initial treatment. The 62 patients (out of 75) who responded had Psoriasis Area and Severity Index changes of 0.75 or greater, whereas the non-responders had Psoriasis Area and Severity Index (PASI) changes of -0.2 to 0.75. Cross-validation using linear regression modeling showed a

balanced prediction accuracy of 0.71 using PASI75 as the cutoff for evaluating the classifier performance. A positive prediction value of 0.95 was achieved.

[00180] In some cases, a sequence read from a subject is compared with a sequence read from a positive or negative control. A positive control is a subject diagnosed with the disease or condition. A negative control is a healthy person who does not have any symptoms or history of symptoms of the disease or condition. Alternatively, a negative control can be the baseline for a subject prior to treatment.

Biologics

[00181] As used herein, the term "biologics" means medicinal products obtained from living organisms (e.g., humans, animals, or microorganisms), including recombinant biologics, which are produced by genetic engineering technique. For example, a biologic may contain proteins that control the action of other proteins and cellular processes, genes that control production of vital proteins, modified human hormones, or cells that produce substances that suppress or activate components of the immune system. In some cases, a biologic is an antibody (e.g., a monoclonal antibody). In some cases, the subject is treated with one or more of the following biologics: tumor necrosis factor inhibitors (e.g., adalimumab, etanercept, infliximab, golimumab and certolizumab pegol); IL-17 inhibitors (e.g., secukinumab, ixekizumab, and brodalumab); and the IL-23 inhibitors (e.g., tildrakizumab, guselkumab, ustekinumab, and risankizumab).

[00182] Whenever the term "at least," "greater than," or "greater than or equal to" precedes the first numerical value in a series of two or more numerical values, the term "at least," "greater than" or "greater than or equal to" applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

[00183] Whenever the term "no more than," "less than," or "less than or equal to" precedes the first numerical value in a series of two or more numerical values, the term "no more than," "less than," or "less than or equal to" applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

[00184] The term "bases," as used herein refers to nucleotides. In some cases, "bases" can refer to base pairs ("bp"), e.g. 1 base equals 1 base pair. As used herein, the terms, "bases," and "base pairs," are used interchangeably.

[00185] As used herein, the term "about" means within 10% above or below a given value. For example, "about 10", would include values from 9 to 11, unless otherwise indicated by the context in which the term is used.

[00186] The following examples are provided to further illustrate the advantages and features of the present invention, but are not intended to limit the scope of the invention. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLES

EXAMPLE 1: Manufacturing the Device

[00187] A device of the present disclosure is manufactured using injection molding. The device is manufactured by injecting heated (e.g. molten) material, e.g. a polyolefin resin disclosed herein, into a mold of the device, according to the features disclosed herein. The mold comprises a plurality of cavities for forming a plurality of microneedles, cavity for forming an interior section comprising the plurality of microneedles, and one or more cavities for forming one or more peripheral sections adjacent to the interior section. For this device, the width of the interior section is less than the width of the peripheral section(s). The width of the intersection is about a 1:5 ratio in size as compared to the width of the peripheral section(s).

[00188] As the heat material is injected through the mold, the smaller width of the interior section (as compared to the peripheral section(s)) facilitates the heated materials movement to the one or more cavities to generate the plurality of microneedles prior to generating the interior section and the peripheral section(s).

[00189] The result is a device as described herein comprising uniform, sharp microneedles. No more than 3% of the microneedles in the plurality of microneedles deviate (+/-). by more than about 50 μm from an average length of the microneedles of the plurality of microneedles.

EXAMPLE 2: Bonding Probes to the Device of Example 1

[00190] The microneedles of the device of Example 1 are plasma treated as described herein. The device or a portion thereof is placed into a plasma vacuum chamber, the plasma vacuum chamber is closed, creating a sufficient vacuum seal, all of the pre-existing gas present in the chamber is evacuated and a plasma treatment gas is pumped into the plasma vacuum chamber to a defined pressure, enabling the generation of gas plasma.

[00191] The gas plasma reacts with the heated material of the device (e.g. a polyolefin resin described herein) and renders the material more readily reactive to form covalent bonds with the probes described herein.

EXAMPLE 3: Kinetics of mRNA Extraction

[00192] The optimal time for mRNA extraction was determined by placing a microneedle device described herein on the skin of healthy subjects for varying periods of time, followed by removal of the patch from the skin and subsequent amplification, cDNA synthesis, and qPCR analysis. The devices were placed on subjects for 20 seconds, 1 minute, 5 minutes, and 10 minutes, and the cycle threshold (Ct) was measured for two biomarkers, *GAPDH* and *KRT10*, generated from the mRNA sample collected from the device at each residence time. All measurements were made in triplicate.

[00193] As can be seen in **Table 1**, optimal extraction occurs 5 minutes of residence time in the skin, with degradation starting to dominate by 10 minutes of skin insertion.

Table 1. Time course of mRNA stability (values in cycle threshold (Ct))

Gene	20 s insertion	1 m insertion	5 m insertion	10 m insertion
GAPDH	31.69	30.56	30.04	30.82
KRT10	28.49	27.45	25.98	27.07

EXAMPLE 4: Qualification of Extracted mRNA

[00194] To demonstrate the quality of intact mRNA, bioanalyzer QC and qPCR analysis were performed with the mRNA extracted from a microneedle device described herein.

[00195] For intact/high quality mRNA, successful cDNA synthesis and amplification showed a distinct amplification peak spanning from about 300 to about 10,000 bp, with a peak at about 1,500 bp (**FIG. 5A**). Quantification of the area under these curves returned a corrected area QC ratio (corrected area for large mRNA fragments (700-20,000 bp) / corrected area for small mRNA fragments (150-200 bp)) of greater than 1. In contrast, the amplification peak shift with degraded mRNA to smaller fragments of 100-1,000 in partially degraded samples (**FIG. 5B**). Further, in fully degraded samples, the peak around 1,500 bp was no longer visible (**FIG. 5C**). Importantly, in these partially and fully degraded samples, the corrected area QC ratio was less than 1. Additionally, qPCR analysis was performed on these samples. Using a typical gene (*B2M*), the Ct value from degraded RNA sample increased to 22.70 from a Ct of 19.25 for the control sample (intact RNA); this increase of 3.45 Ct indicated approximately 11-fold decrease in the quantity of mRNA in the degraded sample.

EXAMPLE 5: Prospective Prediction of Individual Patients Response to TNFα, IL-17 and IL-23 inhibitors

[00196] Response for patients with a psoriasis skin disease to particular drug classes (e.g., TNFα, IL-17i and IL-23i inhibitors) was determine prospectively prior to administering said drug classes via a predictive linear classifier modeling approach. To determine patient response a painless FDA-registered class I Dermal Biomarker Patch is was used to pull an individual patient's entire RNA transcriptome through exposure of the Dermal Biomarker Patch and the patient's skin for 5 minutes. The Dermal Biomarker Patch was then processed, and the RNA adhered on the Dermal Biomarker Patch was extracted and sequenced using next-generation sequencing. The RNA sequencing readout was then aligned and compared with a database of patients with a similar disease condition RNA gene profiles and their respective responsive or non-responsiveness to a particular drug class. The comparison between RNA sequencing readout and the database with a predictive linear classifier provided a score of the probability that a particular drug or drug class will provide an efficacious response to treating a disease condition.

[00197] Using the aforementioned methods, the predictive linear classifier's positive predictor value (PPV), sensitive, and balanced accuracy was assessed for TNFαi, (PASI75 @ W12), IL-17i (PASI75 @ W 12) and IL-23i (PASI150 @ W12) drug classes. The TNFαi inhibitor drug class was evaluated over a training set of 34 patients and a test set of 15. The resulting PPV, sensitivity and balanced accuracy for the linear classifier was 89%, 80%, and 80%, respectively. The IL-17i inhibitor drug class was evaluated over a training set of 75 patients and a test set of 6. The resulting PPV, sensitivity and balanced accuracy for the linear classifier was 100%, 100%, and 100%, respectively. The IL-23i inhibitor drug class was evaluated over a training set of 17 patients and a test set of 17. The resulting PPV, sensitivity and balanced accuracy for the linear classifier was 90%, 82%, and 83%, respectively. The average of the three inhibitor drug classes PPV, sensitivity, and was calculated to be 95% PPV, 87% sensitivity, and 88% specificity.

EXAMPLE 6. A Machine Learning-Based Test for Predicting Response to Psoriasis Biologics

[00198] This study was designed to develop and prospectively validate a machine learning based algorithm that could predict patient response to the most common biologic drug classes used in the management of psoriasis patients. This type of tool would allow clinicians to have greater confidence that a given patient will respond to a specific drug class, which could lead to improved health outcomes and reduced wasted healthcare spend.

[00199] Patients were enrolled into one of two observational studies (STAMP studies) where dermal biomarker patches (DBPs) were applied at baseline prior to drug exposure, followed by

clinical evaluations at 12 weeks after exposure. PASI measurements were made at baseline and 12 weeks to evaluate clinical response to a clinical phenotype. Responders were defined as those who reached PASI75 at 12 weeks. The transcriptomes obtained from the DBPs were sequenced and analyzed to derive and/or validate classifiers for each biologic class, which were then combined to yield predictive responses for all three biologic drug classes (IL-23i, IL-17i, and TNFαi).

[00200] A total of 242 psoriasis patients were enrolled in these studies, including 118 patients (49.6%) treated with IL-23i, 79 patients (33.2%) treated with IL-17i, 35 patients (14.7%) treated with TNFαi, and 6 patients (2.5%) treated with IL-12/23i. The IL-23i predictive classifier was developed from the earlier enrolled patients and independently validated with the latter enrolled patients. IL-17i and TNFαi predictive classifiers were developed using publicly available datasets and independently validated with patients from the STAMP studies. In the independent validation, positive predictive values for three classifiers (IL-23i, IL-17i, and TNFαi) were 93.1%, 92.3% and 85.7% respectively. Over the entire cohort, 99.5% of patients were predicted to respond to at least one drug class.

[00201] This study demonstrates the power of using baseline dermal biomarkers and machine learning methods as applied to the prediction of psoriasis biologic prior to drug exposure. Using this test, patients, physicians, and the health care system all can benefit in distinct ways. Precision medicine can be realized for individual patients as most will likely respond to their prescribed biologic the first time. Physicians can prescribe these drugs with increased confidence, and the healthcare system will realize lower net costs as well as greatly reduced wasted spend by significantly improving initial response rates to expensive biologic therapeutics.

[00202] Psoriasis is a T-cell mediated inflammatory skin disease characterized by discrete erythematous plaques and papules with micaceous scale. Worldwide, this is a common disease, with approximately 2.8% of the United States population, or 7.5 million people, diagnosed with psoriasis. Current treatment paradigms for psoriasis are distinguished by topical medications and/or phototherapy for mild to moderate patients, and systemic medications for patients who are classified as moderate to severe disease. The advent of biologic therapy as one of these systemic agents has revolutionized the management and treatment of psoriasis patients and is a direct result of the increased molecular understanding of the disease. Presently, there are eleven approved biologic agents approved for use in the United States for the treatment of psoriasis, with more under development. However, it is not clear which biologic would be effective for a given patient.

[00203] Disclosed herein is a novel biomarker capture platform that utilizes a dermal biomarker patch to capture the whole transcriptome including mRNA biomarkers from the epidermis and upper dermis. This platform showed excellent concordance with biopsy and provides a scalable method to access skin biomarkers in a minimally invasive manner. It is contemplated that the use of this platform in preliminary machine learning classifier builds for the prediction of response and non-response to IL-17 and IL-23 inhibitors. Also disclosed herein, is an extension of this preliminary study to the development and prospective validation of an actionable clinical test for predicting patient response to psoriasis biologics for all three drug classes.

METHODS

Dermal Biomarker Patch Platform

[00204] Dermal biomarker patches (DBPs) used in this study were fabricated and modified as previously described and used according to the manufacturer's specifications.

Human Subject Recruitment and Enrollment

[00205] Data was analyzed from past and ongoing observational, multicenter (20 centers), single-arm, open-label, 12-week studies, referred as STAMP studies. The protocols for these studies were approved by local institution ethics committees and conforms to the provisions of the Declaration of Helsinki and the International Council for Harmonisation (ICH) Guidelines on Good Clinical Practice (GCP). All patients who received treatment provided written informed consent. The primary objective of the study protocols was to examine if baseline or on-therapy transcriptomics can be used to help predict selection of medications and provide new therapeutic targets for drug development (**Table 2**). Visits included screening, baseline, week 1, week 4, week 8, and week 12. PASI, PGA, and BSA scoring was performed at every visit excluding the screening visit. Subjects were administered the Dermal Biomarker Patch at every visit excluding the screening visit. Subject medical history, physical exam, and demographics were collected at screening.

Table 2. Schedule of activities for STAMP studies.

Visit Number	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Study Week	**Screening[3]**	**Baseline[3]**	**Week 1**	**Week 4**	**Week 8**	**Week 12**
Informed Consent	X					
Inclusion/Exclusion Criteria	X					
Demographics	X					
Medical History[1]	X					
Physical Exam[2,4]	X					
PASI[4]	X	X		X		X
PGA[4]	X	X		X		X
BSA[4]	X	X		X		X
Mindera Dermal Biomarker Patch Application[4]	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X
IP accountability		X	X	X	X	X

Abbreviations: BMI = Body Mass Index; IP = Investigational Product; PASI = Psoriasis Area and Severity Index; PGA = Physician Global Assessment; BSA = Body Surface Area.

[1]Medical history includes prescription and over-the-counter medication history.

[2]Only applicable to subjects who have not been examined by a rheumatologist or dermatologist within 30 days prior to screening. Height, weight, and BMI are included.

[3]If screening and baseline occur on the same day, clinician must ensure subject has refrained from any topical steroid use 2 weeks prior to the application of the Mindera Dermal Biomarker Patch.

[4]For screening/baseline assessments, the physical exam, PASI, PGA, BSA, and/or Mindera Dermal Biomarker Patch application can be completed at either the screening visit or the baseline visit (PASI, PGA, and BSA should be completed at the same visit and before Mindera Dermal Biomarker Patch application).

Study population

[00206] These studies enrolled both male and female patients who were aged 18 years or older, diagnosed with psoriasis by either a rheumatologist or a dermatologist with at least one identifiable study lesion of 2 cm in diameter or greater, and were planned for treatment with IL-23 inhibitor (IL-23i), IL-17 inhibitor (IL-17i), or TNFα inhibitor (TNFαi) therapy once enrolled in the study. The exclusion criteria included use of topical steroids on the study lesion within 2 weeks prior to the baseline visit and concurrent use of Plaquenil. All study participants were also instructed to refrain from the use of all topical steroids throughout the study until the end of study treatment.

Dermal Biomarker Patch Application

[00207] To apply DBPs to the skin, a customized spring-loaded applicator was used. This applicator served to standardize the application pressure across subjects and users. The loaded applicator was placed against the skin and the trigger pressed, applying the patch to the skin. The patch was then held in place against the skin for 5 minutes by a ring of medical tape. After this time, the patch was removed from the subject, immediately placed into storage buffer (LiCl, Triton X-100, Tris-EDTA), and stored at 4 °C until processing.

Dermal Biomarker Patch Processing

[00208] Dermal transcriptomes were processed within 96 hours of collection from subjects. The applied DBPs were washed with chilled 1X PBS and then dried under a stream of nitrogen. mRNA extraction from the patch was performed by applying PCR grade water (50 µL, 95 °C) to the DBP. The patch was then heated 1 minute at 95 °C to elute the bound mRNA from the DBP. This eluted mRNA was then converted to cDNA using the Takara SMART-Seq® Single Cell kit according to the manufacturer's instructions. Amplified cDNA samples were then stored at 4 °C until analysis.

Next-Generation Sequencing Procedures

[00209] Amplified cDNA was sequenced by a commercial vendor (Psomagen, Inc., Rockville, MD) according to standard procedures. Library preparation was accomplished using Illumina Nextera DNA Flex kits according to the manufacturer's instructions. Prepared indexed libraries were then loaded onto a NovaSeq6000 S4 with read length of 150PE for sequencing of 40 M reads per sample. During sequencing, the quality score (Q30) was maintained over 75%. Upon completion of sequencing runs, FASTQ file quality was checked with FASTQC and trimmed with the Trim_galore program. The trimmed FASTQ files were aligned and mapped to human reference genome GRCh38 using the hisat2 program. The number of reads was counted for each Ensemble gene ID using the FeatureCounts program and Homo sapiens GRCH38.84.gtf. RNA expression analysis was further processed using the Bioconductor package edgeR. Genes were filtered using filterByExpr before logCPM (log counts per million reads) were calculated as a measure of gene expression level. For downstream classifier builds, logCPM values were used.

IL-23 Classifier Development

[00210] Five common classifiers were selected and applied for predicting responders under IL-23i treatment using the R package caret. The selected classifiers have been frequently used in the medical field for exploring predictive or prognostic biomarkers and included glmnet (Lasso

and Elastic-Net Regularized Generalized Linear Model), PAM (Nearest Shrunken Centroids), LM (Linear Regression Model), SVM (Support Vector Machine), and RF (Random Forest).

[00211] The five classifiers were compared for their predictive performance using the following experimental design: 1) the data set was split into ten stratified outer folds; 2) for each of the folds, the data were preprocessed for feature selection. The top 20, 50, or 200 differentially expressed genes (features) were selected using linear regression model; 3) The hyperparameters were tuned in the training set via a ten-fold cross-validation, and the process subsequently repeated five times; 4) Based on the selected hyperparameters, a model was derived from the training set and applied to the test set. Performance metrics on the test set were then calculated. This process was repeated five times for each classifier.

[00212] The earliest enrolled IL-23i treated patients in STAMP studies were used for IL-23i classifier training. Baseline PASI filter (none, 6+, 8+, and 10+) were applied to explore the impact of disease severity on classifier performance. Classifier training were performed using the machine learning approaches stated above and test performance was assessed using 10-fold cross validation. IL-23i classifier were locked once a desired performance (> 85% PPV and >85% sensitivity) were achieved. The IL-23i treatment patients enrolled after the classifier lock were used as the independent validation set.

IL-17 Classifier Development

[00213] Disclosed herein is a list of 17 genes which were predictive of psoriasis patients' response to IL-17i by analyzing a publicly available data set. In brief, moderate to severe psoriasis patients (baseline PASI $\geq$ 10) were treated with brodalumab and the patients were followed up for 12 weeks. PASI measurement were performed at baseline and week 12, and patients' treatment response was assessed using week 12 PASI75. Lesional and non-lesional skin biopsy samples were collected at baseline and week 12. RNA profiling was performed using an Affymetrix microarray platform. The lesional samples collected at baseline were used in the predictive biomarker analysis.

[00214] The 17 predictive genes were negatively correlating with patients' response to brodalumab. The 17 genes were mapped to 14 Ensemble gene IDs reported in RNASeq data from STAMP studies. The 14-gene classifier was validated in STAMP studies.

TNFai Classifier Development

[00215] Publicly available data sets in the NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and European Bioinformatics Institute (EMBL-EBI) big data database (https://www.ebi.ac.uk/) were used as a classifier training data sets. For initial data

selection, search terms of psoriasis patients with biologics treatment and transcriptome profiles were used to identify either array or sequencing data.

[00216] Supervised predictive biomarker selection was applied to individual training data to filter genes based on the following assessment: 1) correlation between gene expression and patient response; 2) median gene expression level; 3) gene expression dynamic range; 4) difference between average gene expression of responder and non-responders. Ratios of genes down-regulated and gene up-regulated in TNFαi responders were used to develop a prediction of TNFαi treatment responses.

Prospective classifier validation

[00217] IL-23i, IL-17i and TNFαi classifiers were independently validated using the patients enrolled in STAMP studies. Each classifier discretely predicted a patient as either a responder or non-responder for biologic class. Response was defined as achieving PASI75 at week 12. The cross-tabulation of observed and predicted classes with associated statistics was calculated with the confusionMatrix function of the R caret package.

(a) Characteristics of study subjects

[00218] A total of 242 psoriasis patients were enrolled in the STAMP studies (**FIG. 7**) at time of data lock, including 38 patients who were still in follow up. STAMP is an actively recruiting study designed to continue enrolling new patients to support psoriasis biomarker research. Varied demographics and clinical features of the study subjects were observed (**Table 3**). With regard to drug class, 49.6% patients were treated with IL-23i, 33.2% were treated with IL-17i, 14.7% were treated with TNFαi, and 2.5% were treated with IL-12/23i.

Table 3. STAMP study patient demographics and disease characteristics.

	Patient Population (N=242)
Sex	242
Male	112 (46.3%)
Female	130 (53.7%)
Race	241
White	193 (80.1%)
Asian	19 (7.9%)
Black or African American	16 (6.6%)
Others	13 (5.4%)

Age	241
	48.5 ± 13.7
Body Mass Index	238
	31.9 ± 7.0
PASI	238
	11.1 ± 9.1
Biologics Class	238
IL-17 Inhibitor	79 (33.2%)
IL-23 Inhibitor	118 (49.6%)
IL-12/23 Inhibitor	6 (2.5%)
TNFα Inhibitor	35 (14.7%)
Psoriasis Subtypes	239
Plaque Psoriasis	226 (94.6%)
Psoriatic Arthritis	9 (3.8%)
Others	4 (1.6%)
Prior Biologics History	214
Biologics Naive	134 (62.6%)
Treated with Biologics Within Past 12 Weeks	50 (23.4%)
Treated with Biologics Over 12 Weeks Ago	30 (14.0%)

Values are n (%) of patients or mean ± standard deviation

[00219] Of the 242 patients initially identified for this study, 185 patients completed the study, meaning that both baseline and week 12 PASI scores were collected, and 57 patients were either screen failures, lost to follow up, or still in follow up. Out of the 185 patients, 177 had baseline DBP samples collected, while 8 patients failed DBP sample collection. Of this subset, 167 samples passed sequencing data QC metrics and were included in the biomarker analysis, with 10 (5.4%, 10/177) samples failing either sample processing or sequencing data QC.

[00220] The patient response rate was 64.1% for the whole cohort, and ranged from 47.6% to 72.5% for different biologics (**Table 4**). High (baseline PASI ≥ 8) PASI patients had 26.4% higher response rate than low PASI patients across all drug classes.

Table 4. Psoriasis Patient Response Rates.

Biologics	No PASI Filter	PASI ≥ 8	PASI < 8
All Biologics	64.1% (118/184)	74.3% (84/113)	47.9% (34/71)
IL-23i	64.1% (68/106)	70.8% (46/65)	53.7% (22/41)
IL-17i	72.5% (37/51)	84.8% (28/33)	50.0% (9/18)
TNFαi	47.6% (10/21)	63.6% (7/11)	30.0% (3/10)

[00221] High (baseline PASI ≥ 8) PASI patients were used for predictive classifier development and validation. The IL-23i treated patient population was divided into two subsets, 17 IL-23i treated patients were used for training an IL-23i predictive classifier, and the remaining 43 patients were used for prospective validation. All high PASI IL-17i and TNFαi patients were used for the classifier validation.

(b) IL-23i Classifier Development and Performance in Training Set

[00222] A subset of 17 IL-23i treated high PASI (≥8) patients were used for IL-23i predictive classifier training, including 9 responders and 8 non-responders. The best performing model was built on glmnet using the top 50 features selected with linear regression model. Test performance was assessed with ten-fold cross validation and the positive predictive value (PPV), sensitivity and balanced accuracy were 89.7%, 96.3%, and 91.9%, respectively.

TNFαi Data Source and Predictive Biomarker Discovery

[00223] Four publicly available datasets (**Table 5**) were identified and used for the TNFαi response classifier development. A total of 73 patients were included in these datasets, out of which 58 patients had both transcriptome data and outcome assessment data for predictive biomarker discovery. Patient outcome was assessed with PASI75 at week 12 or 16, or histological response.

Table 5. Publicly available datasets for TNFαi classifier training.

ID	Dataset Accession #	Year Published	Treatment	Pt Response Criteria	Sample Size	Transcriptome Profiling Platform
#1	GSE85034	2017	Adalimumab	PASI75 at WK 16	14	Illumina HumanHT-12 V4.0 expression beadchip
#2	GSE11903	2009	Etanercept	Histological response (epidermal thickness) at WK 12	15	Affymetrix HG-U133A_2
#3	GSE117239	2019	Etanercept	PASI75 at WK 12	34	Affymetrix HG-U133_Plus_2
#4	E-MTAB-6556	2019	Etanercept	PASI75 at WK 12	10	RNASeq

[00224] Supervised predictive biomarkers selection was applied to the four training data sets. Nine genes were determined as predictive of TNFαi response in at least two datasets (**Table 6**). The output of the classifier was a TNFαi response prediction score; in this scoring system, the lower the prediction score, the higher chance the patient will respond to TNFαi treatment. The classifier performance showed PPV, sensitivity and balanced accuracy of 78.9%, 43.1%, and 63.7%, respectively with this training set (**Table 7**).

Table 6. List of the 9 genes identified as correlating to response to anti-α biologics

Gene Title	Gene Symbol
Cornefied Envelope Protein Cornefilin	CNFN
Cathepsin C	CTSC
Glucosylceramidase Beta Pseudogene 1	GBAP1
Cellular Retinoic Acid Binding Protein 2	CRABP2
Protocadherin 7	PCDH7
Peptidylprolyl Isomerase G	PPIG
Ras-related protein Rab-31	RAB31
Complement component 3	C3
Early growth response 1	EGR1

Table 7. Test performance of TNFαi classifier on the training set.

Sample Size	58
Number of Responder	34
Number of Non-Responder	24
PPV	78.9%
NPV	51.3%
Sensitivity	44.1%
Specificity	83.3%
Balanced Accuracy	63.7%

Mind.Px Classifier Validation

[00225] Patient demographics and disease characteristics for the 95 patients included in the prospective validation can be found in **Table 8**. Only patients with baseline PASI≥8 were included in the validation. For the three classifiers, positive predictive value ranged from 85.7% to 93.1% (**Table 9**). Correlation between observed W12 PASI changes and predicted drug response were assessed (**FIG. 8A-C**).

Table 8. Classifier Validation Set Patient Demographics and Disease Characteristics

	IL-23i Classifier (N=43)	**IL-17i Classifier (N=31)**	**TNFαi Classifier (N=11)**
Sex			
Male	23 (53.5%)	21 (32.3%)	4 (36.4%)
Female	20 (46.5%)	10 (67.7%)	7 (63.6%)
Race			
White	35 (81.4%)	23 (74.2%)	9 (81.8%)
Others	8 (18.6%)	8 (25.8%)	2 (18.2%)
Age			
	48.3 ± 16.9	48.9 ± 12.9	47.8 ± 13.4
Body Mass Index			
	31.1 ± 7.5	32.1 ± 6.3	32.6 ± 5.2
PASI			
	15.9 ± 8.5	17.5 ± 12.6	14.8 ± 5.4

Psoriasis Subtypes			
Plaque Psoriasis	41 (97.6%)	31 (96.8%)	11 (100%)
Others	1 (2.4%)	1 (3.2%)	0 (0%)

Table 9. Classifier validation test performance of three classifiers on patients with baseline PASI≥8.

Classifier	**Sample Size**	**Number of Responder**	**Number of Non-Responder**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**Balanced Accuracy**
IL-23i Classifier	43	37	6	93.1%	28.6%	73.0%	66.7%	69.8%
IL-17i Classifier	31	26	5	92.3%	22.2%	46.2%	80.0%	63.1%
TNFαi Classifier	11	7	4	85.7%	75.0%	85.7%	75.0%	80.4%

[00226] The same analysis was repeated for 66 moderate to severe disease patients (i.e., PASI≥10), and similar overall test performance was observed with PPV ranging from 90% to 100% in this smaller cohort (**Table 10**).

Table 10. Classifier validation test performance of three classifiers on patients with baseline PASI≥10.

Classifier	**Sample Size**	**Number of Responder**	**Number of Non-Responder**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**Balanced Accuracy**
IL-23i Classifier	35	30	5	95.7%	33.3%	73.3%	80.0%	76.7%
IL-17i Classifier	22	17	5	90.0%	33.3%	52.9%	80.0%	66.5%
TNFαi Classifier	9	7	2	100.0%	66.7%	85.7%	100.0%	92.9%

[00227] Patients with baseline PASI<8 were also analyzed to determine the classifier performance in milder patients. In this case, the balanced accuracy ranged from 44.4% to 52.8% for three classifiers, suggesting that the developed classifier was optimized for moderate to severe psoriasis patients.

Mind.Px Predicted Response Prevalence

[00228] The predicted response prevalence of patients by all three classifiers (IL-23i, IL-17i and TNFαi) was assessed using 195 patients who had baseline DBP samples and completed RNASeq sequencing data (**FIG. 9** and **Table 11**). Individually, the predicted response prevalence was 72.3%, 51.7% and 67.1% for IL-23i, IL-17i and TNFαi classifiers, respectively. Critically, 99.5% (194/195) patients were predicted as to responder to at least one of the three drug classes. All possible combinations of the three drug classes were represented with 17.4% (34/195) of patients predicted as a responder to all three drug classes, 56.9% (111/195) of patients predicted as a responder to two of the three drug classes, and 25.1% (49/195) of patients predicted as a responder to one of the three drug classes.

Table 11. Mind.Px predicted response prevalence in patients enrolled in the STAMP studies.

Class	Number of Patients	Patient Percentage
Non-Respond to All	1	0.5%
Respond to All	34	17.4%
Respond to IL17 & IL23	54	27.7%
Respond to IL17i & TNFαi	10	5.1%
Respond to IL23i & TNFαi	47	24.1%
Respond to IL23i Only	6	3.1%
Respond to IL17i Only	3	1.5%
Respond to TNFαi Only	40	20.5%

STAMP Study Demographics

[00229] There were 242 patients included in this analysis, with demographics that largely were consistent with previous studies with respect to gender, race, and age (**Table 3**). Similarly, the average patient in these studies was obese (BMI>30), with a mean age of 48.5 years. Most interestingly, in this study, the vast majority of patients (86%) were biologic naïve or had not been administered a biologic within the past 12 weeks. Given that many moderate to severe psoriasis patients have been exposed to biologics, this finding was particularly surprising, but analysis of the classifier response of biologic naïve versus biologic exposed patients showed no difference between the predictive value of the algorithms in either of these patient groups.

Classifier Development and Validation

[00230] The final IL-23i classifier was developed and validated using patients enrolled in the STAMP studies. A subset of the total IL-23i enrolled patients were used as the training set and the remaining patients in the cohort were used for classifier validation. Since the training and test set were from the same study with the sample and data processed in the same manner, the classifier developed with the training set can be applied to the test set without the need for additional normalization.

[00231] The strategy for the development of the IL-17i and TNFαi classifiers was different from the IL-23i classifier. The IL-17i and TNFαi patient sample sizes in STAMP studies were smaller than IL-23i patients and were not sufficient to divide into separate training and test sets, so publicly available datasets were used for the training of these two classifiers. It was noted that the training sets from the public data differed significantly than the test sets in some aspects, including sample collection method (punch biopsy vs. dermal patch), RNA preparation protocol, transcriptome profiling method (array vs. sequencing based). Due to these different natures of the training sets, the training sets primarily were used only for feature selection. Once the predictive genes were identified, a simplified algorithm that utilized gene expression values or the ratio of gene expression values, was applied as the predictive classifier. The cutoffs were preset prior to the validation using percentile data values calculated from the prediction scores of STAMP patients and this allowed for an assessment of classifier performance while minimizing the risk of overfitting.

[00232] Here, week 12 PASI75 was used as the patient outcome determination. In a clinical setting where a better response (e.g., PASI 90 or PASI 100) is desired, the classifier can potentially be used for the identification of this group of patients with certain clinical cutoff

adjustment. Further classifier development to conclusively identify "super-responders" or "super-non-responders" is ongoing and will be reported in due course.

[00233] All three classifiers were validated for baseline PASI ≥ 8 patients. However, the predictive value of these classifiers in patients with lower starting PASI scores was limited. This could be because the three classifiers were developed with high (≥ 10) PASI patients as the training set in order to match the types of patients that were enrolled in the pivotal clinical studies for each biologic. It is possible that a mild patient might biologically have different transcriptomic biomarkers. Alternatively, in patients with low starting PASI scores, the reliability of the response determination measure (PASI 75) is low given the reduced dynamic range of the measurement.

[00234] Other clinical variables have been previously used to stratify psoriasis patients or have been correlated with poorer outcomes. In particular, BMI and age have been reported as having clinical prognostic value in assessing biologic treatment response. The predictive significance of BMI and age as a possible orthogonal input variable in the classifier is tested. However, adding either variable as a covariate when exploring the predictive models, no improvement was observed in the predictive accuracy or positive predictive value.

[00235] The prevalence of the biomarker predicted response revealed key features of this test. Of those tested, only a single patient out of the 195 patients tested was predicted to not respond to any of the three biologic drug classes. These data concur with a widely accepted clinical fact that the treatment and management of psoriasis has dramatically changed since the introduction of biologics; almost all patients will respond to one of the three classes of biologics. However, a disconnect was observed between biologic prescribing behavior and the predicted biologic class from the test. While only 14.7% of the enrollees in the observational STAMP study were prescribed TNFαi biologics (49.6% of the patient cohort), 67% of the patient population was predicted to respond to this class.

[00236] Disclosed herein is an actionable machine learning-based precision medicine test that can predict psoriasis patient response to biologics (TNFαi, IL17i, or IL23i) with high positive predictive value, by combining dermal biomarker patch platform with machine learning methods. Interestingly, when the entire patient cohort was examined, almost all patients were predicted to respond to at least one biologic class, highlighting the tremendous efficacy of biologic drugs in treating psoriasis. Using baseline biomarkers combined with machine learning algorithm development, the proper biologic for a given patient can be prescribed the first time. This test could lead to improved patient outcomes while also translating into tremendous cost savings for healthcare systems. It is contemplated that this test can effectively minimize the trial-and-error

approach to the biologic treatment of psoriasis, and provide physicians, patients, and payers with a powerful tool to bring personalized medicine to the management of psoriasis patients.

EXAMPLE 7. Efficient Prediction of Response to Psoriasis Biologics Using a Machine Learning Classifier

[00237] In the United States, psoriasis affects upward of 3% of the population and treatment can cost from $87,585 to $366,645 annually with the most frequently used drugs. It generally takes 12 to 16 weeks for clinical response to treatment to be meaningful, and efficacy of presently available therapies ranges from 30% to 80%, due at least in part to the inability to predict response to a specific treatment regimen.

[00238] An algorithm (Mind.Px) is developed herein for the prediction of response to biologics (anti-IL-17, anti-IL-23) used for the treatment of patients with psoriasis, by comparing baseline transcriptomes with clinical response to biologic drugs at 12 weeks after initial treatment. The 62 patients (out of 75) who responded had Psoriasis Area and Severity Index changes of 0.75 or greater, whereas the non-responders had Psoriasis Area and Severity Index (PASI) changes of -0.2 to 0.75. Cross-validation using linear regression modeling showed a balanced prediction accuracy of 0.71 using PASI75 as the cutoff for evaluating the classifier performance. A positive prediction value of 0.95 was achieved. A total of 17 genes were finalized as correlating with patient response to anti-IL-17 biologics. Alternatively, a prospective classifier was built using patients receiving anti-IL-23 biologics using the same criteria for response (N=17). A total of 27 genes were finalized for use in this classifier and the positive predictive value (PPV) across both classifiers was 100%.

[00239] Classifiers for biologic prediction largely utilized orthogonal biomarkers and achieved high PPV. The use of Mind.Px results in better outcomes in patients with psoriasis and significantly reduced costs to the healthcare system.

Human Subject Recruitment and Enrollment

[00240] Subjects with active psoriasis and lesions >2 cm in diameter were enrolled. All procedures were approved by an independent Institutional Review Board (Integreview, Austin TX) and written consent was obtained from all subjects prior to the study. The study was not powered for statistical significance. For each subject, one Mindera dermal biomarker patch (DBP) was applied to the lesional skin. Samples were immediately placed into vials with storage buffer as per the manufacturer's instructions and placed at 4 °C until processing. Processing occurred as per the Mind.Px kit instructions.

Dermal Biomarker Patch Application

[00241] To apply patches to the skin, a customized spring-loaded applicator was used. This applicator served to standardize the application pressure across subjects and across users. The loaded applicator was placed against the skin and the trigger pressed, applying the patch to the skin. The patch was then held in place against the skin for 5 minutes by a ring of medical tape. After this time, the patch was removed from the subject, immediately placed into storage buffer (LiCl, Triton X-100, Tris-EDTA), and stored at 4 °C until processing.

Dermal Biomarker Patch Processing

[00242] Dermal transcriptomes were processed within 72 hours of collection from subjects. Samples were prepared by washing the applied DBP with chilled 1X PBS and then drying the patch under a stream of nitrogen. The dried DBP was then place on a heat block preset to 95 °C for 1 minute, at which time, 50 µL of PCR grade water previously heated to 95 °C was then applied to the microneedle array for 1 minute to elute the bound mRNA from the DBP. This eluted mRNA was then converted to cDNA using Takara SMART-Seq® Single Cell kit according to the manufacturer's instructions. Amplified cDNA samples were then stored at 4 °C until qPCR or NGS analysis.

Sequencing Procedures

[00243] Amplified cDNA was sequenced by a commercial vendor (Psomagen, Inc., Rockville, MD) according to standard procedures. Library preparation was accomplished using Illumina Nextera DNA Flex kits according to the manufacturer's instructions. Prepared indexed libraries were then loaded onto a NovaSeq6000 S4 with read length of 150PE for sequencing of 40 M reads per sample. During sequencing, the quality score (Q30) was maintained over 75%. Upon completion of runs, FASTQ file quality was checked with FASTQC and trimmed with the Trim_galore program. The trimmed FASTQ were aligned and mapped to human reference genome GRCh38 using hisat2 program. The number of reads was counted for each Ensemble gene ID using FeatureCounts program and Homo sapiens GRCH38.84.gtf. RNA expression analysis was further processed using Bioconductor package edgeR. Genes were filtered using filterByExpr before logCPM (counts per million reads) were calculated. Plots were made with ggplot2 and heatmap.2 functions in R. Percent of genes detected was determined using the ratio of genes detected (with >0 count) over the total number of Ensemble genes.

Bioinformatic Procedures

[00244] The Mind.Px algorithm has been implemented as an R-script on the DNAnexus® system (Mountain View, CA), a cloud-based data analysis and management platform for DNA

sequencing data. Cutoff points for anti-IL-17 and anti-IL-23 response prediction have been defined and implemented in the R-script.

[00245] Data were collected from three phase 3 randomized clinical trials: AMAGINE-1, AMAGINE-2, and AMAGINE-3. The endpoint was PASI 75 at week 12. Biopsy samples were collected from lesional areas. Transcriptome analyses were conducted for biopsy samples using the Affymetrix platform. A total of 75 patients were treated with brodalumab, including 62 responders (83%); and 12 non-responders (16%). A total of 17 patients enrolled in the Mindera-sponsored STAMP (Study Through the Application of the Mindera Patch) trial were also included in the analysis (STAMP is an ongoing clinical trial sponsored by Mindera Corp.) The enrollment included patients with psoriasis who were to be treated with either anti-IL-17 or anti-IL-23 biologics. RNA samples were collected from patients' skin prior to therapy and multiple times post-therapy for the identification of molecular biomarkers that could predict patient response to the therapy. Baseline samples were collected from all patients.

[00246] Post-sequencing, RNA-Seq FASTQ files were trimmed with the Trim_galore Program, which was followed by mapping to human reference genome GRCh38 using the HISAT2 Program. The number of reads was counted for each Ensemble gene ID using the FeatureCounts Program, and human GRCh38.84.gtf. RNA expression analysis was further processed using bioconductor package edgeR. Genes were filtered using filterByExpr before logCPM (counts per million reads) was calculated. Plots were made with ggplot2 and heatmap.2 functions compared with the standard R function. The percent of genes detected was determined using the ratio of genes detected (with > 0 count) divided by the total number of Ensemble genes. The overview of RNA-Seq analysis is summarized in **FIG. 12**.

Dermal Biomarker Patch Platform data

[00247] Disclosed herein is platform that allows for simple, rapid, and painless extraction of RNA from the skin using a dermal biomarker patch. Subsequent next-generation sequencing of the extracted RNA has allowed us to take a genetic and transcriptomic snapshot of the skin at the exact moment of the test. This rich patient-specific data set can then be analyzed by machine-learning algorithms to ask sophisticated questions of the data (e.g., predicting the appropriate biologic drug for a patient prior to therapeutic selection and treatment).

[00248] Lesional vs non-lesional samples were compared using Mindera patches versus punch biopsies from 66 patients in the Mindera database (**FIG. 13**). The entire transcriptome was analyzed by performing a paired t-test between lesional and non-lesional skin, and then plotting the heatmap for data that have the highest variance (e.g., best P value between the groups). Importantly, the biomarker data were equivalent between the Mindera patch samples and the

punch biopsy samples. The 30 highest variance genes were selected, and unsupervised clustering was performed for visualization, showing excellent discrimination between lesional and non-lesional skin in the same patient.

[00249] Also disclosed herein is an algorithm for the prediction of response to biologics (anti-IL-17, anti-IL-23) treatment in patients with psoriasis. The data set from Tomalin et al (2020) was used for the identification of genes whose RNA expression levels correlate with the responses to treatment with anti-IL-17 biologics in patients with psoriasis.

[00250] In this cohort, 62 patients (out of 75) who responded had PASI changes of ≥0.75, whereas the non-responders had PASI changes of -0.2 to 0.75. The data set was pre-filtered using the differentially expressed genes for psoriasis (lesional vs non-lesional) to minimize the chances of overfitting. Cross-validation using linear regression modeling showed a balanced prediction accuracy of 0.71 using PASI 75 as the cutoff for evaluating the classifier performance. Importantly, a positive prediction value of 0.95 was achieved. A total of 17 genes were finalized as correlating with patient response to brodalumab treatment using StepAIC (Akaike Information Criteria) linear regression modeling (**Table 12**). The Akaike information criterion is an estimator of out-of-sample prediction error and thereby relative quality of statistical models for a given set of data. Akaike information criterion estimates the relative amount of information lost by a given model: the less information a model loses, the higher the quality of that model.

Table 12. List of the 17 genes identified as correlating to response to anti-IL17 biologics

Affy ID	Gene Title	Gene Symbol
210413_x_at	serpin peptidase inhibitor, clade B (ovalbumin), member 3 /// serpin peptidase inhibitor, clade B (ovalbumin), member 4	SERPINB3 /// SERPINB4
232170 at	S100 calcium binding protein A7A	S100A7A
209719_x_at	serpin peptidase inhibitor, clade B (ovalbumin), member 3	SERPINB3
203691_at	peptidase inhibitor 3, skin-derived	PI3
209125_at	keratin 6A	KRT6A
212531_at	lipocalin 2	LCN2
207356_at	defensin, beta 4A /// defensin, beta 4B	DEFB4A // DEFB4B
214549_x_at	small proline-rich protein 1A	SPRR1A
220322 at	interleukin 36, gamma	IL36G
202086_at	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	MX1
202411_at	interferon, alpha-inducible protein 27	IFI27
206488_s_at	CD36 molecule (thrombospondin receptor)	CD36

209555_s_at	CD36 molecule (thrombospondin receptor)	CD36
208650_s_at	CD24 molecule	CD24
208651_x_at	CD24 molecule	CD24
266_s_at	CD24 molecule	CD24
203233_at	interleukin 4 receptor	IL4R

[00251] A heatmap was generated from expression data for these 17 genes (**FIG. 14**). Each column is a baseline sample collected from a patient. From left to right the patients were sorted by low to high PASI changes. The blue bar on the top shows the 12 non-responders (W12 PASI changes < 0.75); the yellow bar shows the 62 responders (W12 PASI changes > 0.75). As shown in the heatmap, a subset of responder patients (far right) had strong down-regulation (blue) of most of genes, suggesting the potential of using these 17 genes to predict patient response to anti-IL-17 treatment. The down-regulation of these 17 genes indicated the better responses to treatment, whereas the up-regulation indicated the worse responses.

[00252] An identical analysis was performed prospectively using patients receiving anti-IL-23 biologics. This cohort was derived from patients enrolling in the STAMP trial conducted by Mindera. In this cohort of 17 patients, 5 patients had achieved PASI-75 response by 12 weeks, while 12 patients did not achieve this level of response. Cross-validation using regression modeling showed a balanced prediction accuracy of 0.70 using PASI 75 as the cutoff for evaluating the classifier performance. Importantly, a positive prediction value of 1.00 was achieved. A total of 50 genes were finalized as correlating with patient response to anti-IL-23 biologic treatment (**Table 13**).

Table 13. List of the 50 genes identified as correlating to response to anti-IL23 biologics

Ensembl Gene ID	Gene Symbol
ENSG00000237973	MTCO1P12
ENSG00000248527	MTATP6P1
ENSG00000171603	CLSTN1
ENSG00000162493	PDPN
ENSG00000187942	LDLRAD2
ENSG00000241720	AL158847.1
ENSG00000134202	GSTM3
ENSG00000169509	CRCT1
ENSG00000235942	LCE6A
ENSG00000135916	ITM2C

ENSG00000170275	CRTAP
ENSG00000145040	UCN2
ENSG00000073282	TP63
ENSG00000124882	EREG
ENSG00000173221	GLRX
ENSG00000134986	NREP
ENSG00000197948	FCHSD1
ENSG00000151914	DST
ENSG00000078399	HOXA9
ENSG00000091136	LAMB1
ENSG00000101825	MXRA5
ENSG00000186918	ZNF395
ENSG00000019549	SNAI2
ENSG00000159899	NPR2
ENSG00000137098	SPAG8
ENSG00000160360	GPSM1
ENSG00000175274	TP53I11
ENSG00000165912	PACSIN3
ENSG00000077498	TYR
ENSG00000036672	USP2
ENSG00000108179	PPIF
ENSG00000271880	AGAP11
ENSG00000151303	AL136982.1
ENSG00000261011	AL136982.4
ENSG00000229969	AL136982.2
ENSG00000271573	AL136982.5
ENSG00000170430	MGMT
ENSG00000197757	HOXC6
ENSG00000198056	PRIM1
ENSG00000139233	LLPH
ENSG00000084110	HAL
ENSG00000198431	TXNRD1
ENSG00000257732	AC089983.1
ENSG00000255150	EID3
ENSG00000165799	RNASE7

ENSG00000129562	DAD1
ENSG00000184916	JAG2
ENSG00000140859	KIFC3
ENSG00000103056	SMPD3
ENSG00000161714	PLCD3

[00253] Disclosed herein is a platform that utilizes a simple, minimally invasive patch that takes a painless biomarker sample from a patient with psoriasis in a matter of minutes. The Mind.Px test has the ability to collect patient data at scale and, when combined with high-precision molecular testing, results in a powerful platform with excellent sensitivity and specificity. Use of this platform can potentially translate into huge cost savings for healthcare systems, particularly when applied to the prediction of response to expensive treatments—effectively eliminating the present trial-and-error approach to the treatment of psoriasis.

[00254] Biomarkers captured using Mind.Px include DNA, RNA, proteins, and small molecules. In particular, the role of RNA in chronic skin diseases, which is well characterized, has been exploited to provide the missing predictive link between a patient's genetic markers and responsiveness to different drug classes. By capturing RNA from a patient's psoriatic lesion, next-generation sequencing is used to evaluate more than 7000 biomarkers per test sample. The results of this biomarker analysis can be used by healthcare providers and payers to predict an individual patient's response to a class of biologic drugs, by the mechanism of action of that class of agent, to optimize treatment selection. The use of Mind.Px could result in better outcomes and significantly reduced costs to the healthcare system.

[00255] Analysis of the classifiers developed in this study demonstrated high positive predictive value in assessing if a patient will respond to biologic drug prior to initial exposure. Indeed, in both classifiers, >90% positive predictive value could be achieved, which could significantly impact medical practice and the treatment of psoriasis patients. Interestingly, comparison of the biomarker sets between algorithms showed some overlap (at least 2 genes overlap between the anti-IL-17 and anti-IL-23 classifier).

[00256] **Table 14**. List of the genes that can be used as classifies to determine the effectiveness of one or more biologics for treating psoriasis. In some embodiments, the methods disclosed herein directed to determining whether a subject suffering from psoriasis will positively respond to an IL-17, IL-23, or TNFα mediated treatment, further comprise determining the presence, absence, or expression level of at least 5 genes selected from **Table 14**. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least 6 genes selected from Table 14 to 17 genes selected from Table 14. In some

embodiments, the methods further comprise determining the presence, absence, or expression level of at least 6 genes selected from Table 14 to 7 genes selected from Table 14, 6 genes selected from Table 14 to 8 genes selected from Table 14, 6 genes selected from Table 14 to 9 genes selected from Table 14, 6 genes selected from Table 14 to 10 genes selected from Table 14, 6 genes selected from Table 14 to 11 genes selected from Table 14, 6 genes selected from Table 14 to 12 genes selected from Table 14, 6 genes selected from Table 14 to 13 genes selected from Table 14, 6 genes selected from Table 14 to 14 genes selected from Table 14, 6 genes selected from Table 14 to 15 genes selected from Table 14, 6 genes selected from Table 14 to 16 genes selected from Table 14, 6 genes selected from Table 14 to 17 genes selected from Table 14, 7 genes selected from Table 14 to 8 genes selected from Table 14, 7 genes selected from Table 14 to 9 genes selected from Table 14, 7 genes selected from Table 14 to 10 genes selected from Table 14, 7 genes selected from Table 14 to 11 genes selected from Table 14, 7 genes selected from Table 14 to 12 genes selected from Table 14, 7 genes selected from Table 14 to 13 genes selected from Table 14, 7 genes selected from Table 14 to 14 genes selected from Table 14, 7 genes selected from Table 14 to 15 genes selected from Table 14, 7 genes selected from Table 14 to 16 genes selected from Table 14, 7 genes selected from Table 14 to 17 genes selected from Table 14, 8 genes selected from Table 14 to 9 genes selected from Table 14, 8 genes selected from Table 14 to 10 genes selected from Table 14, 8 genes selected from Table 14 to 11 genes selected from Table 14, 8 genes selected from Table 14 to 12 genes selected from Table 14, 8 genes selected from Table 14 to 13 genes selected from Table 14, 8 genes selected from Table 14 to 14 genes selected from Table 14, 8 genes selected from Table 14 to 15 genes selected from Table 14, 8 genes selected from Table 14 to 16 genes selected from Table 14, 8 genes selected from Table 14 to 17 genes selected from Table 14, 9 genes selected from Table 14 to 10 genes selected from Table 14, 9 genes selected from Table 14 to 11 genes selected from Table 14, 9 genes selected from Table 14 to 12 genes selected from Table 14, 9 genes selected from Table 14 to 13 genes selected from Table 14, 9 genes selected from Table 14 to 14 genes selected from Table 14, 9 genes selected from Table 14 to 15 genes selected from Table 14, 9 genes selected from Table 14 to 16 genes selected from Table 14, 9 genes selected from Table 14 to 17 genes selected from Table 14, 10 genes selected from Table 14 to 11 genes selected from Table 14, 10 genes selected from Table 14 to 12 genes selected from Table 14, 10 genes selected from Table 14 to 13 genes selected from Table 14, 10 genes selected from Table 14 to 14 genes selected from Table 14, 10 genes selected from Table 14 to 15 genes selected from Table 14, 10 genes selected from Table 14 to 16 genes selected from Table 14, 10 genes selected from Table 14 to 17 genes selected from Table 14, 11 genes selected from Table 14 to 12 genes selected from Table 14, 11

genes selected from Table 14 to 13 genes selected from Table 14, 11 genes selected from Table 14 to 14 genes selected from Table 14, 11 genes selected from Table 14 to 15 genes selected from Table 14, 11 genes selected from Table 14 to 16 genes selected from Table 14, 11 genes selected from Table 14 to 17 genes selected from Table 14, 12 genes selected from Table 14 to 13 genes selected from Table 14, 12 genes selected from Table 14 to 14 genes selected from Table 14, 12 genes selected from Table 14 to 15 genes selected from Table 14, 12 genes selected from Table 14 to 16 genes selected from Table 14, 12 genes selected from Table 14 to 17 genes selected from Table 14, 13 genes selected from Table 14 to 14 genes selected from Table 14, 13 genes selected from Table 14 to 15 genes selected from Table 14, 13 genes selected from Table 14 to 16 genes selected from Table 14, 13 genes selected from Table 14 to 17 genes selected from Table 14, 14 genes selected from Table 14 to 15 genes selected from Table 14, 14 genes selected from Table 14 to 16 genes selected from Table 14, 14 genes selected from Table 14 to 17 genes selected from Table 14, 15 genes selected from Table 14 to 16 genes selected from Table 14, 15 genes selected from Table 14 to 17 genes selected from Table 14, or 16 genes selected from Table 14 to 17 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least 6 genes selected from Table 14, 7 genes selected from Table 14, 8 genes selected from Table 14, 9 genes selected from Table 14, 10 genes selected from Table 14, 11 genes selected from Table 14, 12 genes selected from Table 14, 13 genes selected from Table 14, 14 genes selected from Table 14, 15 genes selected from Table 14, 16 genes selected from Table 14, or 17 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least at least 6 genes selected from Table 14, 7 genes selected from Table 14, 8 genes selected from Table 14, 9 genes selected from Table 14, 10 genes selected from Table 14, 11 genes selected from Table 14, 12 genes selected from Table 14, 13 genes selected from Table 14, 14 genes selected from Table 14, 15 genes selected from Table 14, or 16 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least at most 7 genes selected from Table 14, 8 genes selected from Table 14, 9 genes selected from Table 14, 10 genes selected from Table 14, 11 genes selected from Table 14, 12 genes selected from Table 14, 13 genes selected from Table 14, 14 genes selected from Table 14, 15 genes selected from Table 14, 16 genes selected from Table 14, or 17 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least 20 genes selected from Table 14 to 50 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least 20 genes selected from Table 14 to 25 genes selected from

Table 14, 20 genes selected from Table 14 to 30 genes selected from Table 14, 20 genes selected from Table 14 to 35 genes selected from Table 14, 20 genes selected from Table 14 to 40 genes selected from Table 14, 20 genes selected from Table 14 to 45 genes selected from Table 14, 20 genes selected from Table 14 to 50 genes selected from Table 14, 25 genes selected from Table 14 to 30 genes selected from Table 14, 25 genes selected from Table 14 to 35 genes selected from Table 14, 25 genes selected from Table 14 to 40 genes selected from Table 14, 25 genes selected from Table 14 to 45 genes selected from Table 14, 25 genes selected from Table 14 to 50 genes selected from Table 14, 30 genes selected from Table 14 to 35 genes selected from Table 14, 30 genes selected from Table 14 to 40 genes selected from Table 14, 30 genes selected from Table 14 to 45 genes selected from Table 14, 30 genes selected from Table 14 to 50 genes selected from Table 14, 35 genes selected from Table 14 to 40 genes selected from Table 14, 35 genes selected from Table 14 to 45 genes selected from Table 14, 35 genes selected from Table 14 to 50 genes selected from Table 14, 40 genes selected from Table 14 to 45 genes selected from Table 14, 40 genes selected from Table 14 to 50 genes selected from Table 14, or 45 genes selected from Table 14 to 50 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least 20 genes selected from Table 14, 25 genes selected from Table 14, 30 genes selected from Table 14, 35 genes selected from Table 14, 40 genes selected from Table 14, 45 genes selected from Table 14, or 50 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least at least 20 genes selected from Table 14, 25 genes selected from Table 14, 30 genes selected from Table 14, 35 genes selected from Table 14, 40 genes selected from Table 14, or 45 genes selected from Table 14. In some embodiments, the methods further comprise determining the presence, absence, or expression level of at least at most 25 genes selected from Table 14, 30 genes selected from Table 14, 35 genes selected from Table 14, 40 genes selected from Table 14, 45 genes selected from Table 14, or 50 genes selected from Table 14.

AffyID	Gene Symbol	PublicID	EnsemblGeneID	Gene Regulation in Psoriasis
1405_i_at	CCL5	M21121	ENSG00000271503	Up-regulated in Psoriasis
1552312_a_at	MFAP3	NM_005927	ENSG00000037749	Up-regulated in Psoriasis
1552378_s_at	RDH10	NM_172037	ENSG00000121039	Up-regulated in Psoriasis

1552480_s_at	PTPRC	NM_080923	ENSG00000081237	Up-regulated in Psoriasis
1552497_a_at	SLAMF6	NM_052931	ENSG00000162739	Up-regulated in Psoriasis
1552536_at	VTI1A	NM_145206	ENSG00000151532	Up-regulated in Psoriasis
1552639_at	KLHDC7B	NM_138433	ENSG00000130487	Up-regulated in Psoriasis
1552671_a_at	SLC9A7	NM_032591	ENSG00000065923	Up-regulated in Psoriasis
1552980_at	HAS3	NM_138612	ENSG00000103044	Up-regulated in Psoriasis
1553055_a_at	SLFN5	NM_144975	ENSG00000166750	Up-regulated in Psoriasis
1553081_at	WFDC12	NM_080869	ENSG00000168703	Up-regulated in Psoriasis
1553102_a_at	CCDC69	BC016647	ENSG00000198624	Up-regulated in Psoriasis
1553423_a_at	SLFN13	NM_144682	ENSG00000154760	Up-regulated in Psoriasis
1553514_a_at	VNN3	NM_078625	ENSG00000093134	Up-regulated in Psoriasis
1553589_a_at	PDZK1IP1	NM_005764	ENSG00000162366	Up-regulated in Psoriasis
1553602_at	MUCL1	NM_058173	ENSG00000172551	Up-regulated in Psoriasis
1553695_a_at	NLRX1	NM_170722	ENSG00000160703	Up-regulated in Psoriasis
1553906_s_at	FGD2	NM_173558	ENSG00000146192	Up-regulated in Psoriasis
1554008_at	OSMR	BC010943	ENSG00000145623	Up-regulated in Psoriasis
1554016_a_at	USB1	BC006291	ENSG00000103005	Up-regulated in Psoriasis

1554079_at	GALNT18	BC037341	ENSG00000110328	Up-regulated in Psoriasis
1554119_at	USB1	BC010099	ENSG00000103005	Up-regulated in Psoriasis
1554240_a_at	ITGAL	BC008777	ENSG00000005844	Up-regulated in Psoriasis
1554406_a_at	CLEC7A	BC013385	ENSG00000172243	Up-regulated in Psoriasis
1554440_at	KIAA0513	BC030280	ENSG00000135709	Up-regulated in Psoriasis
1554452_a_at	HILPDA	BC001863	ENSG00000135245	Up-regulated in Psoriasis
1554462_a_at	DNAJB9	AF115512	ENSG00000128590	Up-regulated in Psoriasis
1554676_at	SRGN	BC022313	ENSG00000122862	Up-regulated in Psoriasis
1554707_at	SPATA6L	BC034293	ENSG00000106686	Up-regulated in Psoriasis
1554708_s_at	SPATA6L	BC034293	ENSG00000106686	Up-regulated in Psoriasis
1554724_at	SLC6A11	BC036083	ENSG00000132164	Up-regulated in Psoriasis
1554795_a_at	FBLIM1	BC019895	ENSG00000162458	Up-regulated in Psoriasis
1554807_a_at	SPIRE1	BC016825	ENSG00000134278	Up-regulated in Psoriasis
1554822_at	PHTF2	BC022419	ENSG00000006576	Up-regulated in Psoriasis
1554899_s_at	FCER1G	BC020763	ENSG00000158869	Up-regulated in Psoriasis
1554903_at	FRMD8	BC033851	ENSG00000126391	Up-regulated in Psoriasis
1554905_x_at	FRMD8	BC033851	ENSG00000126391	Up-regulated in Psoriasis

1554906_a_at	MPHOSPH6	BC029395	ENSG00000135698	Up-regulated in Psoriasis
1554914_at	PLA2G4D	BC034571	ENSG00000159337	Up-regulated in Psoriasis
1554920_at	SCEL	BC020726	ENSG00000136155	Down-regulated in Psoriasis
1554921_a_at	SCEL	BC020726	ENSG00000136155	Down-regulated in Psoriasis
1555019_at	CDHR1	BC038799	ENSG00000148600	Down-regulated in Psoriasis
1555120_at	CD96	BC020749	ENSG00000153283	Up-regulated in Psoriasis
1555167_s_at	NAMPT	BC020691	ENSG00000105835	Up-regulated in Psoriasis
1555213_a_at	CLEC7A	AF400602	ENSG00000172243	Up-regulated in Psoriasis
1555214_a_at	CLEC7A	AF400602	ENSG00000172243	Up-regulated in Psoriasis
1555292_at	STRIP2	BC019064	ENSG00000128578	Up-regulated in Psoriasis
1555326_a_at	ADAM9	AF495383	ENSG00000168615	Up-regulated in Psoriasis
1555407_s_at	FGD3	BC032232	ENSG00000127084	Up-regulated in Psoriasis
1555416_a_at	ALOX15B	AF468053	ENSG00000179593	Up-regulated in Psoriasis
1555464_at	IFIH1	BC046208	ENSG00000115267	Up-regulated in Psoriasis
1555480_a_at	FBLIM1	AF459643	ENSG00000162458	Up-regulated in Psoriasis
1555628_a_at	HAVCR2	BC020843	ENSG00000135077	Up-regulated in Psoriasis

1555638_a_at	SAMSN1	AF519621	ENSG00000155307	Up-regulated in Psoriasis
1555745_a_at	LYZ	U25677	ENSG00000090382	Up-regulated in Psoriasis
1555756_a_at	CLEC7A	AF400600	ENSG00000172243	Up-regulated in Psoriasis
1555759_a_at	CCL5	AF043341	ENSG00000271503	Up-regulated in Psoriasis
1555778_a_at	POSTN	AY140646	ENSG00000133110	Down-regulated in Psoriasis
1556122_at	RAB11B-AS1	CA431092	ENSG00000269386	Up-regulated in Psoriasis
1556123_a_at	RAB11B-AS1	CA431092	ENSG00000269386	Up-regulated in Psoriasis
1556263_s_at	PWRN1	AK058147	ENSG00000259905	Up-regulated in Psoriasis
1556346_at	COTL1	AJ227860	ENSG00000103187	Up-regulated in Psoriasis
1556606_at	NAV2	BU739339	ENSG00000166833	Up-regulated in Psoriasis
1557049_at	BTBD19	BQ183759	ENSG00000222009	Up-regulated in Psoriasis
1557078_at	SLFN5	AK054668	ENSG00000166750	Up-regulated in Psoriasis
1557116_at	APOL6	BM980001	ENSG00000221963	Up-regulated in Psoriasis
1557236_at	APOL6	BF512806	ENSG00000221963	Up-regulated in Psoriasis
1557275_a_at	TLCD2	BF726849	ENSG00000185561	Up-regulated in Psoriasis

1557285_at	AREG	AI891075	ENSG00000109321	Up-regulated in Psoriasis
1557309_at	DENND1B	BC016588	ENSG00000213047	Up-regulated in Psoriasis
1557389_at	SH3PXD2A-AS1	AK056784	ENSG00000280693	Up-regulated in Psoriasis
1557458_s_at	SHB	BU685917	ENSG00000107338	Up-regulated in Psoriasis
1557609_s_at	TBC1D12	AW188458	ENSG00000108239	Up-regulated in Psoriasis
1557636_a_at	C7orf57	BC031107	ENSG00000164746	Up-regulated in Psoriasis
1557890_at	STXBP5-AS1	BC035182	ENSG00000233452	Up-regulated in Psoriasis
1558105_a_at	SLC9A7	AK096921	ENSG00000065923	Up-regulated in Psoriasis
1558201_s_at	SLC4A1AP	AI889922	ENSG00000163798	Up-regulated in Psoriasis
1558217_at	SLFN13	AK074465	ENSG00000154760	Up-regulated in Psoriasis
1558290_a_at	PVT1	BG200951	ENSG00000249859	Up-regulated in Psoriasis
1558393_at	KRT7	BC042076	ENSG00000135480	Up-regulated in Psoriasis
1558407_at	PLEKHG2	AK095957	ENSG00000090924	Up-regulated in Psoriasis
1558549_s_at	VNN1	BG120535	ENSG00000112299	Up-regulated in Psoriasis
1558561_at	HM13	AK074686	ENSG00000101294	Up-regulated in Psoriasis
1558678_s_at	MALAT1	BE708432	ENSG00000251562	Up-regulated in Psoriasis

1558846_at	PNLIPRP3	AL833418	ENSG00000203837	Up-regulated in Psoriasis
1559018_at	PTPRE	AL832042	ENSG00000132334	Up-regulated in Psoriasis
1559263_s_at	ZC3H12D	BG397809	ENSG00000178199	Up-regulated in Psoriasis
1559268_at	ADAM23	AW022638	ENSG00000114948	Up-regulated in Psoriasis
1559501_at	CBR3-AS1	BC037580	ENSG00000236830	Up-regulated in Psoriasis
1559585_at	DDX60L	AK096369	ENSG00000181381	Up-regulated in Psoriasis
1559883_s_at	SAMHD1	AF147427	ENSG00000101347	Up-regulated in Psoriasis
1559917_a_at	CBR3-AS1	AB004851	ENSG00000236830	Up-regulated in Psoriasis
1561080_at	TXNRD1	BC042974	ENSG00000198431	Up-regulated in Psoriasis
1561336_at	DNASE1L3	CA449306	ENSG00000163687	Up-regulated in Psoriasis
1561948_at	COQ10B	AI793340	ENSG00000115520	Up-regulated in Psoriasis
1562364_at	GVINP1	AL833700	ENSG00000254838	Up-regulated in Psoriasis
1562460_at	CNDP2	BF512616	ENSG00000133313	Up-regulated in Psoriasis
1564031_a_at	RELL2	AK054889	ENSG00000164620	Up-regulated in Psoriasis
1564573_at	IFITM10	BC036199	ENSG00000244242	Up-regulated in Psoriasis
1565657_at	CMTM6	BC042995	ENSG00000091317	Up-regulated in Psoriasis
1566901_at	TGIF1	AL832409	ENSG00000177426	Up-regulated in Psoriasis

1569003_at	VMP1	AL541655	ENSG00000062716	Up-regulated in Psoriasis
1569149_at	PDLIM7	BC023629	ENSG00000196923	Up-regulated in Psoriasis
1569150_x_at	PDLIM7	BC023629	ENSG00000196923	Up-regulated in Psoriasis
1569555_at	GDA	BC012859	ENSG00000119125	Up-regulated in Psoriasis
1569617_at	OSBP2	BC018025	ENSG00000184792	Up-regulated in Psoriasis
200628_s_at	WARS	M61715	ENSG00000140105	Up-regulated in Psoriasis
200629_at	WARS	NM_004184	ENSG00000140105	Up-regulated in Psoriasis
200648_s_at	GLUL	NM_002065	ENSG00000135821	Up-regulated in Psoriasis
200670_at	XBP1	NM_005080	ENSG00000100219	Up-regulated in Psoriasis
200708_at	GOT2	NM_002080	ENSG00000125166	Up-regulated in Psoriasis
200766_at	CTSD	NM_001909	ENSG00000117984	Up-regulated in Psoriasis
200776_s_at	BZW1	AL518328	ENSG00000082153	Up-regulated in Psoriasis
200777_s_at	BZW1	NM_014670	ENSG00000082153	Up-regulated in Psoriasis
200790_at	ODC1	NM_002539	ENSG00000115758	Up-regulated in Psoriasis
200812_at	CCT7	NM_006429	ENSG00000135624	Up-regulated in Psoriasis
200831_s_at	SCD	AA678241	ENSG00000099194	Up-regulated in Psoriasis
200832_s_at	SCD	AB032261	ENSG00000099194	Up-regulated in Psoriasis

200886_s_at	PGAM1	NM_002629	ENSG00000171314	Up-regulated in Psoriasis
200887_s_at	STAT1	NM_007315	ENSG00000115415	Up-regulated in Psoriasis
200900_s_at	M6PR	AI583537	ENSG00000003056	Up-regulated in Psoriasis
200901_s_at	M6PR	NM_002355	ENSG00000003056	Up-regulated in Psoriasis
200923_at	LGALS3BP	NM_005567	ENSG00000108679	Up-regulated in Psoriasis
200935_at	CALR	NM_004343	ENSG00000179218	Up-regulated in Psoriasis
200989_at	HIF1A	NM_001530	ENSG00000100644	Up-regulated in Psoriasis
200998_s_at	CKAP4	AW029619	ENSG00000136026	Up-regulated in Psoriasis
200999_s_at	CKAP4	NM_006825	ENSG00000136026	Up-regulated in Psoriasis
201012_at	ANXA1	NM_000700	ENSG00000135046	Up-regulated in Psoriasis
201037_at	PFKP	NM_002627	ENSG00000067057	Up-regulated in Psoriasis
201118_at	PGD	NM_002631	ENSG00000142657	Up-regulated in Psoriasis
201195_s_at	SLC7A5	AB018009	ENSG00000103257	Up-regulated in Psoriasis
201242_s_at	ATP1B1	BC000006	ENSG00000143153	Up-regulated in Psoriasis
201243_s_at	ATP1B1	NM_001677	ENSG00000143153	Up-regulated in Psoriasis
201252_at	PSMC4	NM_006503	ENSG00000013275	Up-regulated in Psoriasis
201266_at	TXNRD1	NM_003330	ENSG00000198431	Up-regulated in Psoriasis

201348_at	GPX3	NM_002084	ENSG00000211445	Up-regulated in Psoriasis
201435_s_at	EIF4E	AW268640	ENSG00000151247	Up-regulated in Psoriasis
201436_at	EIF4E	AI742789	ENSG00000151247	Up-regulated in Psoriasis
201437_s_at	EIF4E	NM_001968	ENSG00000151247	Up-regulated in Psoriasis
201469_s_at	SHC1	AI809967	ENSG00000160691	Up-regulated in Psoriasis
201482_at	QSOX1	NM_002826	ENSG00000116260	Up-regulated in Psoriasis
201483_s_at	SUPT4H1	BC002802	ENSG00000213246	Up-regulated in Psoriasis
201484_at	SUPT4H1	NM_003168	ENSG00000213246	Up-regulated in Psoriasis
201487_at	CTSC	NM_001814	ENSG00000109861	Up-regulated in Psoriasis
201489_at	PPIF	BC005020	ENSG00000108179	Up-regulated in Psoriasis
201490_s_at	PPIF	NM_005729	ENSG00000108179	Up-regulated in Psoriasis
201510_at	ELF3	AF017307	ENSG00000163435	Up-regulated in Psoriasis
201531_at	ZFP36	NM_003407	ENSG00000128016	Up-regulated in Psoriasis
201564_s_at	FSCN1	NM_003088	ENSG00000075618	Up-regulated in Psoriasis
201594_s_at	PPP4R1	NM_005134	ENSG00000154845	Up-regulated in Psoriasis
201631_s_at	IER3	NM_003897	ENSG00000137331	Up-regulated in Psoriasis
201649_at	UBE2L6	NM_004223	ENSG00000156587	Up-regulated in Psoriasis

201695_s_at	PNP	NM_000270	ENSG00000198805	Up-regulated in Psoriasis
201739_at	SGK1	NM_005627	ENSG00000118515	Up-regulated in Psoriasis
201754_at	COX6C	NM_004374	ENSG00000164919	Up-regulated in Psoriasis
201858_s_at	SRGN	J03223	ENSG00000122862	Up-regulated in Psoriasis
201859_at	SRGN	NM_002727	ENSG00000122862	Up-regulated in Psoriasis
201860_s_at	PLAT	NM_000930	ENSG00000104368	Up-regulated in Psoriasis
201884_at	CEACAM5	NM_004363	ENSG00000105388	Up-regulated in Psoriasis
201890_at	RRM2	BE966236	ENSG00000171848	Up-regulated in Psoriasis
201945_at	FURIN	NM_002569	ENSG00000140564	Up-regulated in Psoriasis
201999_s_at	DYNLT1	NM_006519	ENSG00000146425	Up-regulated in Psoriasis
202081_at	IER2	NM_004907	ENSG00000160888	Up-regulated in Psoriasis
202082_s_at	SEC14L1	AV748469	ENSG00000129657	Up-regulated in Psoriasis
202083_s_at	SEC14L1	AI017770	ENSG00000129657	Up-regulated in Psoriasis
202084_s_at	SEC14L1	NM_003003	ENSG00000129657	Up-regulated in Psoriasis
202086_at	MX1	NM_002462	ENSG00000157601	Up-regulated in Psoriasis
202100_at	RALB	BG169673	ENSG00000144118	Up-regulated in Psoriasis
202101_s_at	RALB	NM_002881	ENSG00000144118	Up-regulated in Psoriasis

202112_at	VWF	NM_000552	ENSG00000110799	Up-regulated in Psoriasis
202266_at	TDP2	NM_016614	ENSG00000111802	Up-regulated in Psoriasis
202269_x_at	GBP1	BC002666	ENSG00000117228	Up-regulated in Psoriasis
202270_at	GBP1	NM_002053	ENSG00000117228	Up-regulated in Psoriasis
202284_s_at	CDKN1A	NM_000389	ENSG00000124762	Up-regulated in Psoriasis
202345_s_at	FABP5	NM_001444	ENSG00000164687	Up-regulated in Psoriasis
202376_at	SERPINA3	NM_001085	ENSG00000196136	Up-regulated in Psoriasis
202381_at	ADAM9	NM_003816	ENSG00000168615	Up-regulated in Psoriasis
202391_at	BASP1	NM_006317	ENSG00000176788	Up-regulated in Psoriasis
202411_at	IFI27	NM_005532	ENSG00000165949	Up-regulated in Psoriasis
202430_s_at	PLSCR1	NM_021105	ENSG00000188313	Up-regulated in Psoriasis
202446_s_at	PLSCR1	AI825926	ENSG00000188313	Up-regulated in Psoriasis
202459_s_at	LPIN2	U55968	ENSG00000101577	Up-regulated in Psoriasis
202460_s_at	LPIN2	NM_014646	ENSG00000101577	Up-regulated in Psoriasis
202497_x_at	SLC2A3	AI631159	ENSG00000059804	Up-regulated in Psoriasis
202498_s_at	SLC2A3	BE550486	ENSG00000059804	Up-regulated in Psoriasis
202499_s_at	SLC2A3	NM_006931	ENSG00000059804	Up-regulated in Psoriasis

202510_s_at	TNFAIP2	NM_006291	ENSG00000185215	Up-regulated in Psoriasis
202524_s_at	SPOCK2	NM_014767	ENSG00000107742	Up-regulated in Psoriasis
202525_at	PRSS8	NM_002773	ENSG00000052344	Up-regulated in Psoriasis
202531_at	IRF1	NM_002198	ENSG00000125347	Up-regulated in Psoriasis
202557_at	HSPA13	AI718418	ENSG00000155304	Up-regulated in Psoriasis
202558_s_at	HSPA13	NM_006948	ENSG00000155304	Up-regulated in Psoriasis
202575_at	CRABP2	NM_001878	ENSG00000143320	Up-regulated in Psoriasis
202594_at	LEPROTL1	NM_015344	ENSG00000104660	Up-regulated in Psoriasis
202595_s_at	LEPROTL1	AF161461	ENSG00000104660	Up-regulated in Psoriasis
202625_at	LYN	AI356412	ENSG00000254087	Up-regulated in Psoriasis
202626_s_at	LYN	NM_002350	ENSG00000254087	Up-regulated in Psoriasis
202637_s_at	ICAM1	AI608725	ENSG00000090339	Up-regulated in Psoriasis
202638_s_at	ICAM1	NM_000201	ENSG00000090339	Up-regulated in Psoriasis
202655_at	MANF	NM_006010	ENSG00000145050	Up-regulated in Psoriasis
202687_s_at	TNFSF10	U57059	ENSG00000121858	Up-regulated in Psoriasis
202688_at	TNFSF10	NM_003810	ENSG00000121858	Up-regulated in Psoriasis
202728_s_at	LTBP1	AI986120	ENSG00000049323	Up-regulated in Psoriasis

202729_s_at	LTBP1	NM_000627	ENSG00000049323	Up-regulated in Psoriasis
202787_s_at	MAPKAPK3	U43784	ENSG00000114738	Up-regulated in Psoriasis
202788_at	MAPKAPK3	NM_004635	ENSG00000114738	Up-regulated in Psoriasis
202804_at	ABCC1	AI539710	ENSG00000103222	Up-regulated in Psoriasis
202805_s_at	ABCC1	NM_004996	ENSG00000103222	Up-regulated in Psoriasis
202831_at	GPX2	NM_002083	ENSG00000176153	Up-regulated in Psoriasis
202842_s_at	DNAJB9	AL080081	ENSG00000128590	Up-regulated in Psoriasis
202843_at	DNAJB9	NM_012328	ENSG00000128590	Up-regulated in Psoriasis
202859_x_at	CXCL8	NM_000584	ENSG00000169429	Up-regulated in Psoriasis
202869_at	OAS1	NM_016816	ENSG00000089127	Up-regulated in Psoriasis
202887_s_at	DDIT4	NM_019058	ENSG00000168209	Up-regulated in Psoriasis
202888_s_at	ANPEP	NM_001150	ENSG00000166825	Up-regulated in Psoriasis
202901_x_at	CTSS	BC002642	ENSG00000163131	Up-regulated in Psoriasis
202902_s_at	CTSS	NM_004079	ENSG00000163131	Up-regulated in Psoriasis
202917_s_at	S100A8	NM_002964	ENSG00000143546	Up-regulated in Psoriasis
202934_at	HK2	AI761561	ENSG00000159399	Up-regulated in Psoriasis
202952_s_at	ADAM12	NM_003474	ENSG00000148848	Up-regulated in Psoriasis

202957_at	HCLS1	NM_005335	ENSG00000180353	Up-regulated in Psoriasis
202988_s_at	RGS1	NM_002922	ENSG00000090104	Up-regulated in Psoriasis
203002_at	AMOTL2	NM_016201	ENSG00000114019	Up-regulated in Psoriasis
203010_at	STAT5A	NM_003152	ENSG00000126561	Up-regulated in Psoriasis
203021_at	SLPI	NM_003064	ENSG00000124107	Up-regulated in Psoriasis
203132_at	RB1	NM_000321	ENSG00000139687	Up-regulated in Psoriasis
203153_at	IFIT1	NM_001548	ENSG00000185745	Up-regulated in Psoriasis
203180_at	ALDH1A3	NM_000693	ENSG00000184254	Up-regulated in Psoriasis
203201_at	PMM2	NM_000303	ENSG00000140650	Up-regulated in Psoriasis
203233_at	IL4R	NM_000418	ENSG00000077238	Up-regulated in Psoriasis
203234_at	UPP1	NM_003364	ENSG00000183696	Up-regulated in Psoriasis
203252_at	CDK2AP2	NM_005851	ENSG00000167797	Up-regulated in Psoriasis
203256_at	CDH3	NM_001793	ENSG00000062038	Up-regulated in Psoriasis
203261_at	DCTN6	NM_006571	ENSG00000104671	Up-regulated in Psoriasis
203313_s_at	TGIF1	NM_003244	ENSG00000177426	Up-regulated in Psoriasis
203315_at	NCK2	BC000103	ENSG00000071051	Up-regulated in Psoriasis
203363_s_at	ATG13	AU153525	ENSG00000175224	Up-regulated in Psoriasis

203364_s_at	ATG13	NM_014741	ENSG00000175224	Up-regulated in Psoriasis
203367_at	DUSP14	NM_007026	ENSG00000276023	Up-regulated in Psoriasis
203369_x_at	PDLIM7	AI825846	ENSG00000196923	Up-regulated in Psoriasis
203370_s_at	PDLIM7	NM_005451	ENSG00000196923	Up-regulated in Psoriasis
203416_at	CD53	NM_000560	ENSG00000143119	Up-regulated in Psoriasis
203423_at	RBP1	NM_002899	ENSG00000114115	Up-regulated in Psoriasis
203470_s_at	PLEK	AI433595	ENSG00000115956	Up-regulated in Psoriasis
203471_s_at	PLEK	NM_002664	ENSG00000115956	Up-regulated in Psoriasis
203499_at	EPHA2	NM_004431	ENSG00000142627	Up-regulated in Psoriasis
203507_at	CD68	NM_001251	ENSG00000129226	Up-regulated in Psoriasis
203535_at	S100A9	NM_002965	ENSG00000163220	Up-regulated in Psoriasis
203550_s_at	FAM189B	NM_006589	ENSG00000160767	Up-regulated in Psoriasis
203560_at	GGH	NM_003878	ENSG00000137563	Up-regulated in Psoriasis
203595_s_at	IFIT5	N47725	ENSG00000152778	Up-regulated in Psoriasis
203596_s_at	IFIT5	NM_012420	ENSG00000152778	Up-regulated in Psoriasis
203663_s_at	COX5A	NM_004255	ENSG00000178741	Up-regulated in Psoriasis
203665_at	HMOX1	NM_002133	ENSG00000100292	Up-regulated in Psoriasis

203691_at	PI3	NM_002638	ENSG00000124102	Up-regulated in Psoriasis
203710_at	ITPR1	NM_002222	ENSG00000150995	Up-regulated in Psoriasis
203740_at	MPHOSPH6	NM_005792	ENSG00000135698	Up-regulated in Psoriasis
203757_s_at	CEACAM6	BC005008	ENSG00000086548	Up-regulated in Psoriasis
203779_s_at	MPZL2	NM_005797	ENSG00000149573	Up-regulated in Psoriasis
203780_at	MPZL2	AF275945	ENSG00000149573	Up-regulated in Psoriasis
203797_at	VSNL1	AF039555	ENSG00000163032	Up-regulated in Psoriasis
203798_s_at	VSNL1	NM_003385	ENSG00000163032	Up-regulated in Psoriasis
203821_at	HBEGF	NM_001945	ENSG00000113070	Up-regulated in Psoriasis
203858_s_at	COX10	NM_001303	ENSG00000006695	Up-regulated in Psoriasis
203894_at	TUBG2	NM_016437	ENSG00000037042	Up-regulated in Psoriasis
203897_at	LYRM1	BE963444	ENSG00000102897	Up-regulated in Psoriasis
203915_at	CXCL9	NM_002416	ENSG00000138755	Up-regulated in Psoriasis
203922_s_at	CYBB	AI308863	ENSG00000165168	Up-regulated in Psoriasis
203923_s_at	CYBB	NM_000397	ENSG00000165168	Up-regulated in Psoriasis
203978_at	NUBP1	NM_002484	ENSG00000103274	Up-regulated in Psoriasis
204014_at	DUSP4	NM_001394	ENSG00000120875	Up-regulated in Psoriasis

204015_s_at	DUSP4	BC002671	ENSG00000120875	Up-regulated in Psoriasis
204057_at	IRF8	AI073984	ENSG00000140968	Up-regulated in Psoriasis
204058_at	ME1	AL049699	ENSG00000065833	Up-regulated in Psoriasis
204059_s_at	ME1	NM_002395	ENSG00000065833	Up-regulated in Psoriasis
204094_s_at	TSC22D2	NM_014779	ENSG00000196428	Up-regulated in Psoriasis
204095_s_at	ELL	AL521391	ENSG00000105656	Up-regulated in Psoriasis
204096_s_at	ELL	AL136771	ENSG00000105656	Up-regulated in Psoriasis
204116_at	IL2RG	NM_000206	ENSG00000147168	Up-regulated in Psoriasis
204137_at	GPR137B	NM_003272	ENSG00000077585	Up-regulated in Psoriasis
204153_s_at	MFNG	NM_002405	ENSG00000100060	Up-regulated in Psoriasis
204166_at	SBNO2	NM_014963	ENSG00000064932	Up-regulated in Psoriasis
204169_at	IMPDH1	NM_000883	ENSG00000106348	Up-regulated in Psoriasis
204174_at	ALOX5AP	NM_001629	ENSG00000132965	Up-regulated in Psoriasis
204205_at	APOBEC3G	NM_021822	ENSG00000239713	Up-regulated in Psoriasis
204232_at	FCER1G	NM_004106	ENSG00000158869	Up-regulated in Psoriasis
204351_at	S100P	NM_005980	ENSG00000163993	Up-regulated in Psoriasis
204357_s_at	LIMK1	NM_002314	ENSG00000106683	Up-regulated in Psoriasis

204385_at	KYNU	NM_003937	ENSG00000115919	Up-regulated in Psoriasis
204415_at	IFI6	NM_022873	ENSG00000126709	Up-regulated in Psoriasis
204420_at	FOSL1	BG251266	ENSG00000175592	Up-regulated in Psoriasis
204439_at	IFI44L	NM_006820	ENSG00000137959	Up-regulated in Psoriasis
204440_at	CD83	NM_004233	ENSG00000112149	Up-regulated in Psoriasis
204465_s_at	INA	NM_004692	ENSG00000148798	Up-regulated in Psoriasis
204470_at	CXCL1	NM_001511	ENSG00000163739	Up-regulated in Psoriasis
204502_at	SAMHD1	NM_015474	ENSG00000101347	Up-regulated in Psoriasis
204529_s_at	TOX	AI961231	ENSG00000198846	Up-regulated in Psoriasis
204530_s_at	TOX	NM_014729	ENSG00000198846	Up-regulated in Psoriasis
204546_at	KIAA0513	NM_014732	ENSG00000135709	Up-regulated in Psoriasis
204580_at	MMP12	NM_002426	ENSG00000262406	Up-regulated in Psoriasis

[00257] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the

invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

1. A microneedle device comprising:

a. a microneedle region comprising (i) a microneedle base substrate comprising a first base substrate surface and a second base substrate surface, wherein the first base substrate surface and the second base substrate surface are positioned on opposite sides of the microneedle base substrate; and (ii) a plurality of microneedles protruding from the first base substrate surface; and

b. a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate and comprising a support substrate depth, wherein the support substrate depth is greater than the minimum distance between the first base substrate surface and the second base substrate surface.

2. A microneedle device comprising:

a. a microneedle region comprising a microneedle base substrate comprising a first base substrate surface with a plurality of microneedles protruding from the first base substrate surface, the microneedle base substrate also comprising a second base substrate surface on a side opposite from the first base substrate surface, the second base substrate surface comprising a recess aligned with at least a portion of the plurality of microneedles; and

b. a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate.

3. The device of claim 1, wherein the minimum distance between the first base substrate surface and the second base substrate surface of the microneedle base substrate is between about 1 μm to about 500 μm less than the depth of the support substrate.
4. The device of claim 1 or 2, wherein the minimum distance between the first base substrate surface and the second base substrate surface is between 150 μm to about 350 μm.
5. The device of claim 1, wherein a ratio between (a) the minimum distance between the first base substrate surface and the second base substrate surface, and (b) the support substrate depth is at least 1:5.
6. The device of claim 1 or 2, wherein the microneedle region comprises a perimeter and the support substrate are adjacent to at least half of the perimeter.
7. The device of claim 1 or 2, wherein the support substrate comprises a first support substrate surface proximal to the plurality of microneedles and a second support substrate surface distal to the plurality of microneedles and positioned opposite to the first support substrate surface, and wherein the second base substrate surface is not coplanar with the second support substrate surface distal to the plurality of microneedles.

8. The device of claim 1 or 2, wherein the first base substrate surface is not coplanar with a surface of the support substrate.
9. The device of claim 1 or 2, wherein the plurality of microneedles is plasma treated.
10. The device of claim 1 or 2, wherein a plurality of probes is coupled to a microneedle of the plurality of microneedles.
11. The device of claim 9, wherein the plurality of probes comprises a negative charge.
12. The device of claim 1 or 2, wherein the plurality of microneedles comprises a polyolefine resin.
13. The device of claim 12, wherein the polyolefine resin comprises one or both of Zeonor 1020R or Zeonor 690R.
14. The device of claim 1 or 2, wherein a microneedle of the plurality of microneedles is non-dissolvable.
15. The device of claim 1 or 2, wherein a microneedle of the plurality of microneedles is pyramidal.
16. The device of claim 1 or 2, wherein a microneedle of the plurality of microneedles is solid.
17. The device of claim 15, wherein an angle between a base of the microneedle and the microneedle base substrate is between about 60° and about 90°.
18. The device of claim 2, wherein the recess aligned with at least a portion of the plurality of microneedles comprises a width that is greater than a width of a mechanical applicator.
19. A kit comprising (a) the device of claim 2; and (b) a mechanical applicator that fits within the recess aligned with at least a portion of the plurality of microneedles.
20. The device of claim 1 or 2, wherein at least one of the plurality of microneedles is coupled to a nucleic acid probe.
21. The device of claim 20, wherein the nucleic acid probe comprises a homopolymeric sequence.
22. The device of claim 21, wherein the homopolymeric sequence comprises thymine or uracil.
23. The device of claim 20, wherein the nucleic acid probe comprises DNA.
24. The device of claim 20, wherein the nucleic acid probe comprises thymine.
25. The device of claim 20, wherein the nucleic acid probe comprises thymidine.
26. The device of claim 20, where the nucleic acid probe is covalently linked to the microneedle.
27. The device of claim 1 or 2, wherein the support substrate comprises a fiducial marker.
28. The device of claim 1 or 2, wherein no more than three microneedles of the plurality of microneedles are less than 600 μm in length or more than 1050 μm in length.
29. A method of preparing a biological sample from a subject comprising:

a. contacting skin of the subject with the microneedle device of any one of claim 1-29;

b. applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject; and

c. allowing nucleic acids within the skin of the subject to contact the microneedle device of any one of claims 1-28.

30. The method of claim 29, further comprising extracting the nucleic acids.

31. The method of claim 29 or 30, wherein the nucleic acids comprise RNA.

32. The method of claim 27 or 28, wherein the nucleic acids comprise mRNA.

33. The method of claim 30, further comprising converting the mRNA to cDNA.

34. The method of any one of claims 27-31, wherein the subject is human.

35. The method of any one of claim 27-32, wherein the subject has psoriasis or symptoms of psoriasis.

36. The method of any one of claims 27-33, wherein the subject has a skin condition or symptoms of a skin condition.

37. A method of preparing a biological sample from a subject comprising:

a. contacting skin of the subject with a microneedle device, wherein the microneedle device comprises a plurality of nucleic acid probes coupled to a microneedle;

b. applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject;

c. allowing the microneedle device to penetrate the skin of the subject for no more than 10 minutes to obtain a ribonucleic acid (RNA) sample hybridized to the nucleic acid probes, wherein the RNA sample comprises a population of RNA fragments comprising about 700 bases or more and a population of RNA fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of RNA fragments comprising about 700 bases or more to the population of RNA fragments comprising about 150 bases to about 200 bases is greater than 1; and

d. removing the microneedle device from the skin of the subject, the microneedle device comprising the RNA sample hybridized to the nucleic acid probes after removal of the microneedle device from the skin of the subject.

38. The method of claim 37, wherein the RNA sample is substantially free of contaminants.

39. The method of claim 37, comprising allowing the microneedle device to penetrate the skin of the subject for about 5 minutes.

40. A method of treating a subject with an autoimmune disease of the skin comprising:

a. collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA;
b. determining an expression level of at least one gene based on the RNA;
c. predicting that a subject with the autoimmune disease of the skin will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 80% based on the expression level of the at least one gene; and
d. based on the predicting in (c), treating the subject with the autoimmune therapeutic drug.

41. The method in claim 40, wherein the PPV is greater than 90%.
42. The method in claim 40, wherein the PPV is greater than 95% for a cohort that has greater than 100 patients.
43. The method in claim 40, wherein the autoimmune therapeutic drug is a biologic.
44. The method in claim 40, wherein the autoimmune therapeutic drug comprises an antibody.
45. The method in claim 40, wherein the autoimmune therapeutic drug is an IL-17 mediated treatment, an IL-23 mediated treatment, or a TNFα mediated treatment.
46. The method in claim 40, wherein the autoimmune therapeutic drug is at least one drug selected from the group consisting of: etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab.
47. The method in claim 40, wherein the at least one gene comprises at least five genes.
48. The method in claim 40, wherein the at least one gene comprises at least one gene from **Table 6**.
49. The method in claim 40, wherein the at least one gene comprises at least one gene from **Table 12.**
50. The method in claim 40, wherein the at least one gene comprises at least one gene from **Table 13.**
51. The method in claim 40, wherein the autoimmune disorder is psoriasis.
52. The method in claim 40, wherein the subject has a PASI greater than 8.
53. The method in claim 40, wherein the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug.
54. The method in claim 40, wherein the RNA comprises mRNA.
55. The method in claim 40, wherein the RNA comprises microRNA.

56. The method in claim 40, wherein the collecting a sample comprising RNA derived from skin from the subject comprises penetrating the skin of the subject with a microneedle device, wherein the microneedle device comprises microneedles conjugated to nucleic acid probes.
57. The method in claim 40, further comprising converting the RNA into cDNA.
58. The method in claim 40, further comprising performing next generation sequencing of the cDNA.
59. The method in claim 40, further comprising applying a trained algorithm to data generated by the next generation sequence of the cDNA.
60. The method in claim 40, wherein the at least one gene comprises at least two genes that do not share a common upstream regulator.
61. The method in claim 40, wherein the algorithm was trained using samples from patients that were administered a single type of drug selected from the group consisting of: IL-17 mediated treatment, TNF-alpha mediated treatment and IL-23 mediated treatment.
62. The method in claim 40, wherein the autoimmune therapeutic drug is an IL-17-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-17 mediated pathway.
63. The method in claim 40, wherein the autoimmune therapeutic drug is an IL-23-mediated treatment and the at least one gene comprises at least one gene that is not involved in an IL-23 mediated pathway.
64. The method in claim 40, wherein the autoimmune therapeutic drug is a TNF-α-mediated treatment and the at least one gene comprises at least one gene that is not involved in a TNF-α-mediated pathway.
65. A method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising:
    a. collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA;
    b. converting the RNA into cDNA;
    c. determining expression of at least one gene based on the cDNA; and
    d. predicting whether the subject with the skin lesion will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 80%.
66. The method in claim 65, wherein the PPV is greater than 90%.
67. The method in claim 65, wherein the PPV is greater than 95% for a cohort that has greater than 100 patients.

68. The method in claim 65, wherein the autoimmune therapeutic drug is a biologic.
69. The method in claim 65, wherein the autoimmune therapeutic drug comprises an antibody.
70. The method in claim 65, wherein the autoimmune therapeutic drug is a IL-17 mediated treatment, an IL-23 mediated treatment, or a TNFα mediated treatment.
71. The method in claim 65, wherein the autoimmune therapeutic drug is at least one drug selected from the group consisting of: etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab.
72. The method in claim 65, wherein the autoimmune therapeutic drug is at least one drug selected from the group consisting of: certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, and risankizumab.
73. The method in claim 65, wherein the at least one gene comprises at least five genes.
74. The method in claim 65, wherein the at least one gene comprises at least one gene from Table 6.
75. The method in claim 65, wherein the at least one gene comprises at least one gene from Table 12.
76. The method in claim 65, wherein the at least one gene comprises at least one gene from Table 13.
77. The method in claim 65, wherein the autoimmune disorder is psoriasis.
78. The method in claim 65, wherein the subject has a PASI greater than 8.
79. The method in claim 65, wherein the subject has a PASI of at least 75 following the treating of the subject with the autoimmune therapeutic drug
80. The method in claim 65, wherein the RNA comprises mRNA.
81. The method in claim 65, wherein the RNA comprises microRNA.
82. The method in claim 65, wherein the extracting RNA from the skin of the subject comprises penetrating the skin of the subject with a microneedle device, wherein the microneedle device comprises microneedles conjugated to nucleic acid probes.
83. The method in claim 65, further comprising performing next generation sequencing of the cDNA.
84. The method in claim 65, further comprising applying a trained algorithm to data generated by the next generation sequencing of the cDNA.
85. A method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising:

a. penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle;
b. removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject;
c. performing high throughput sequencing on the RNA molecules to generate sequence reads;
d. aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; and
e. applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm has a greater than 80% positive predictive value for predicting response to the autoimmune disease therapeutic drug.

86. A method of determining whether a skin lesion of a subject will be responsive to an autoimmune therapeutic drug comprising:
a. penetrating skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a microneedle;
b. removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject;
c. performing high throughput sequencing on the RNA molecules to generate sequence reads;
d. aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads;
e. using the aligned sequence reads to determine an expression level of at least one RNA molecule; and
f. applying a trained algorithm to the expression level of the at least one RNA molecule, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, an IL-23 mediated treatment, a TNFα mediated treatment, or any combination thereof.

87. The method of claim 86, wherein the subject will be responsive to the to the IL-17 mediated treatment, the IL-23 mediated treatment, and the TNFα-mediated treatment.

88. The method of claim 86, further comprising converting RNA to cDNA.

89. The method of claim 86, wherein the expression level of the at least one RNA molecule corresponds to expression of at least one gene from **Table 6**.

90. The method of claim 86, wherein the expression level of the at least one RNA molecule corresponds to expression of at least one gene from **Table 12**.

91. The method of claim 86, wherein the expression level of the at least one RNA molecule corresponds to expression of at least one gene from **Table 13**.

92. The method of claim 86, wherein (b) further comprises:

i) performing high throughput sequencing on the RNA biomarkers to generate one or more sequence reads for the subject;

ii) aligning the one or more sequence reads for the subject with a known signature of sequence reads, wherein the known signature of sequence reads is associated with a positive response to the recommended treatment, thereby obtaining aligned sequence reads; and

iii) classifying the subject as having a likelihood of positively responding to the recommended treatment by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm comprises a greater than 50% positive predictive value for predicting a positive response to the recommended treatment.

93. The method of claim 92, wherein the trained algorithm has greater than 50% negative predictive value.

94. The method of claim 92, wherein the recommended treatment comprises one or more autoimmune therapeutic drugs for an autoimmune disease or condition.

95. The method of claim 94, wherein the autoimmune disease or condition is psoriasis.

96. The method of claim 92, wherein the RNA biomarkers are transcribed from at least one gene from **Table 6**.

97. The method of claim 92, wherein the RNA biomarkers are transcribed from at least one gene from **Table 12**.

98. The method of claim 92, wherein the RNA biomarkers are transcribed from at least one gene from **Table 13**.

99. The method of claim 92, wherein the recommended treatment comprises etanercept, infliximab, adalimumab, certolizumab, ustekinumab, secukinumab, ixekizumab, brodalumab, guselkumab, tildrakizumab, risankizumab, or any combination thereof.

100. The method of claim 92, wherein the one or more therapeutic drugs comprise autoimmune therapeutic drugs for an autoimmune disease or condition.

101. The method of claim 100, wherein the autoimmune disease or condition is psoriasis.

102. A method of determining whether a subject with an autoimmune disorder of the skin will be responsive to an autoimmune therapeutic drug comprising:

a. extracting mRNA from skin of the subject;
b. sequencing the mRNA from the skin of the subject; and
c. predicting whether the subject with the autoimmune disorder will be responsive to etanercept, adalimumab, infliximab, certolizumab, secukinumab, ixekisumab, broadalumab, guselkumab, tildrakizumab, and risankizumab with a positive predictive value greater than 80%.

103. A method of determining whether a skin lesion of a subject will be responsive to an IL-23 mediated treatment comprising:
a. contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle such that the microneedle device penetrates the skin of the subject;
b. removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject;
c. performing high throughput sequencing on the RNA molecules to generate sequence reads; and
d. aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; and
e. applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-23 mediated treatment and the aligned sequence reads correspond to at least one gene from **Table 13**.

104. A method of determining whether a skin lesion of a subject will be responsive to an IL-17, IL-23, or TNF-alpha mediated treatment comprising:
a. contacting skin of the subject with a microneedle device, wherein the microneedle device comprises one or more nucleic acid probes coupled to a solid microneedle such that the microneedle device penetrates the skin of the subject;
b. removing the microneedle device from the skin of the subject, thereby obtaining RNA molecules from the subject;
c. performing high throughput sequencing on the RNA molecules to generate sequence reads; and
d. aligning the sequence reads with a signature of sequence reads associated with a positive response to an autoimmune disease therapeutic drug to obtain aligned sequence reads; and

e. applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm predicts whether the subject with the skin lesion will be responsive to an IL-17 mediated treatment, a TNF-alpha-mediated treatment, or IL-23 mediated treatment and the aligned sequence reads correspond to at least one gene from **Table 6, 12 or 13.**

105. The method of claim 104, further comprising treating the subject with TNF-alpha mediated treatment wherein the aligned sequence reads correspond to at least one gene from the **Table 6**.

106. The method of claim 104, further comprising treating the subject with IL-17 mediated treatment, wherein the aligned sequence reads correspond to at least one gene from the **Table 12**.

107. The method of claim 104, further comprising treating the subject with IL-23 mediated treatment, wherein the aligned sequence reads correspond to at least one gene from the **Table 13**.

108. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, and PPIG.

109. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of CNFN, CTSC, GBAP1, CRABP2, PCDH7, and PPIG.

110. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of PCDH7, PPIG, RAB31, C3, and EGR.

111. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes or four genes selected from the group consisting of CNFN, CTSC, GBAP1, and CRABP2.

112. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes or four genes selected from the group consisting of PPIG, RAB31, C3, and EGR.

113. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes or four genes selected from the group consisting of PCDH7, PPIG, RAB31, and C3.

114. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes or four genes selected from the group consisting of GBAP1, CRABP2, PCDH7, and PPIG.

115. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of KRT6A, SPRR1A, CD36, IL4R, LCN2, and IFI27.

116. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of CD36, IL4R, S100A7A, SERPINB4, MX1, and SERPINB3.

117. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of LCN2, IFI27, DEFB4A, IL36G, CD24, and PI3.

118. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of IL4R, LCN2, and IFI27.

119. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of PI3, IFI27, and SERPINB3.

120. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of IL4R, S100A7A, and MX1.

121. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of CD36, LCN2, and SERPINB4.

122. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of MTCO1P12, MTATP6P1, CLSTN1, PDPN, LDLRAD2, and GSTM3.

123. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of AL158847.1, DAD1, LDLRAD2, ZNF395, MGMT, and AL136982.4.

124. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of NREP, PPIF, PRIM1, AL136982.5, MTATP6P1, and SMPD3.

125. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes, four genes, five genes, or six genes selected from the group consisting of PDPN, TXNRD1, GSTM3, GPSM1, GLRX, and USP2.

126. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of MTCO1P12, CLSTN1, and GSTM3.

127. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of NREP, PPIF, and PRIM1.

128. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of AL136982.5, MTATP6P1, and SMPD3

129. The method of any one of claims 40, 65, 86, 92, and 104, wherein the at least one gene is three genes selected from the group consisting of PDPN, TXNRD1, and GSTM3.

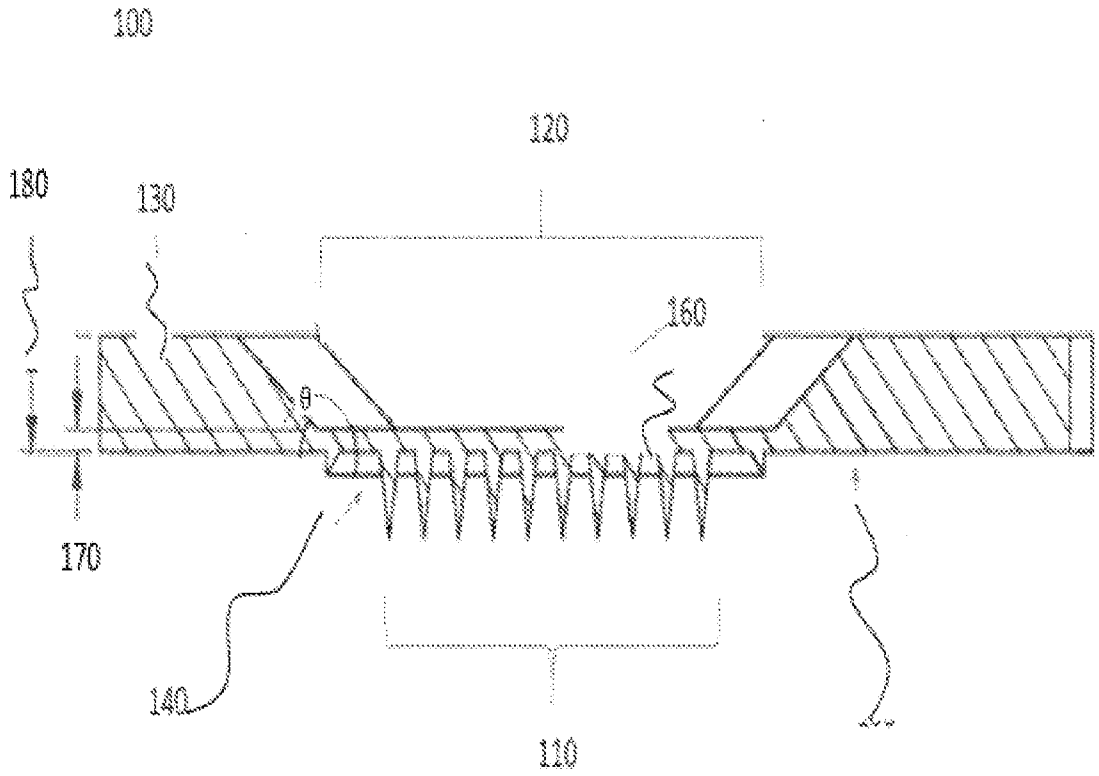


FIG. 1

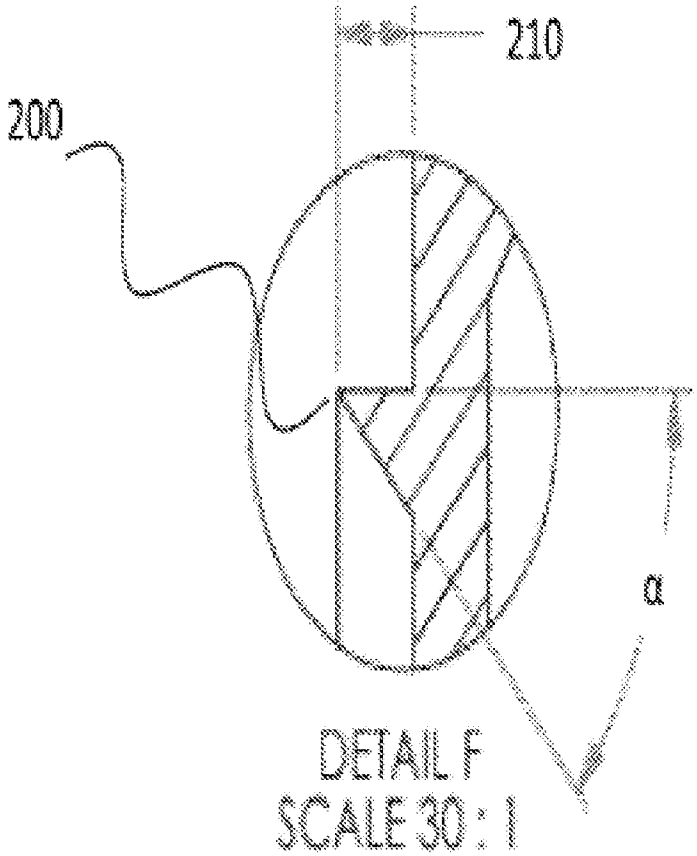


FIG. 2

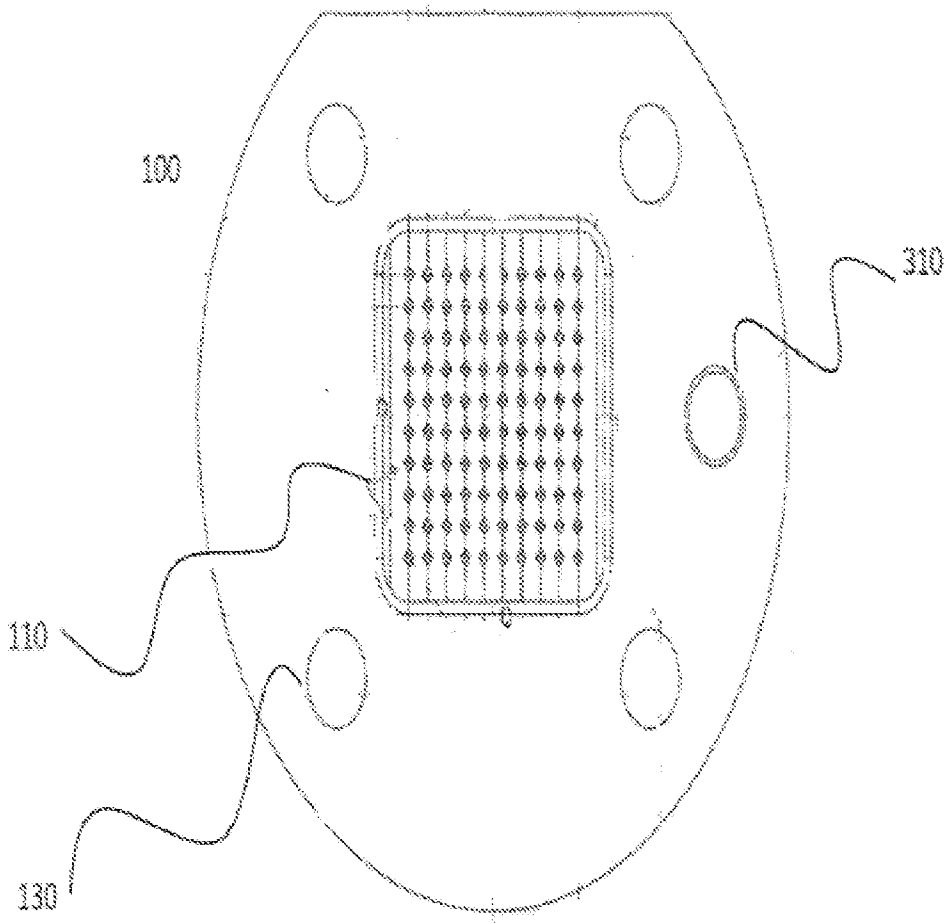


FIG. 3

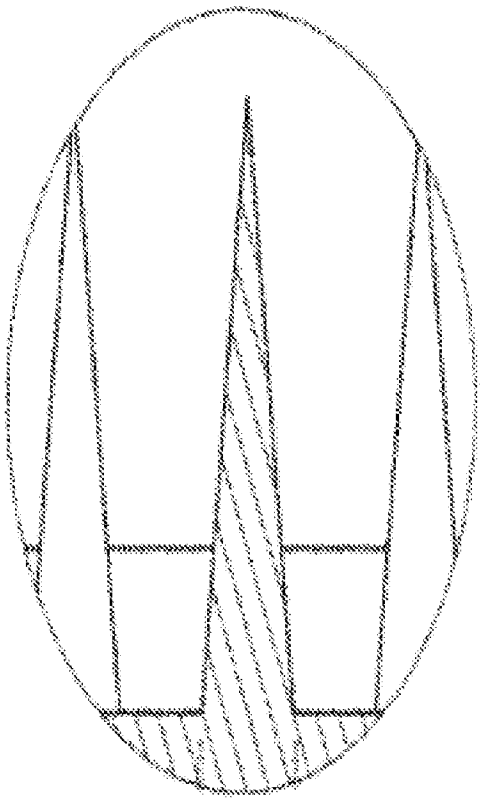


FIG. 4

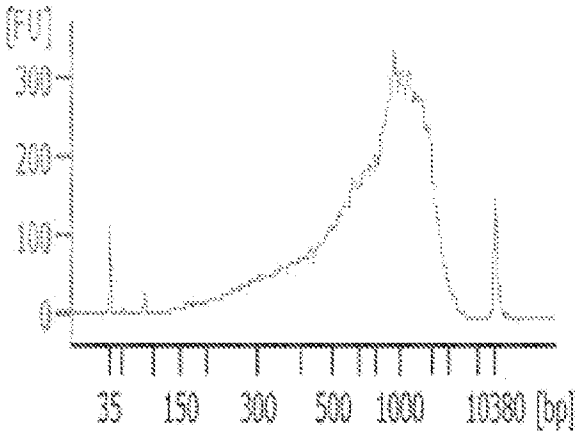


Figure 5A

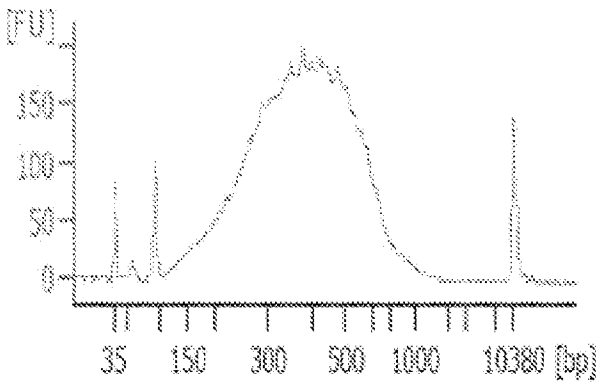


Figure 5B

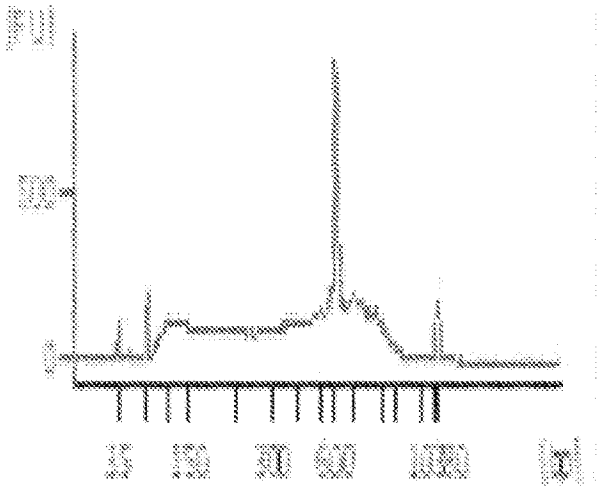


Figure 5C

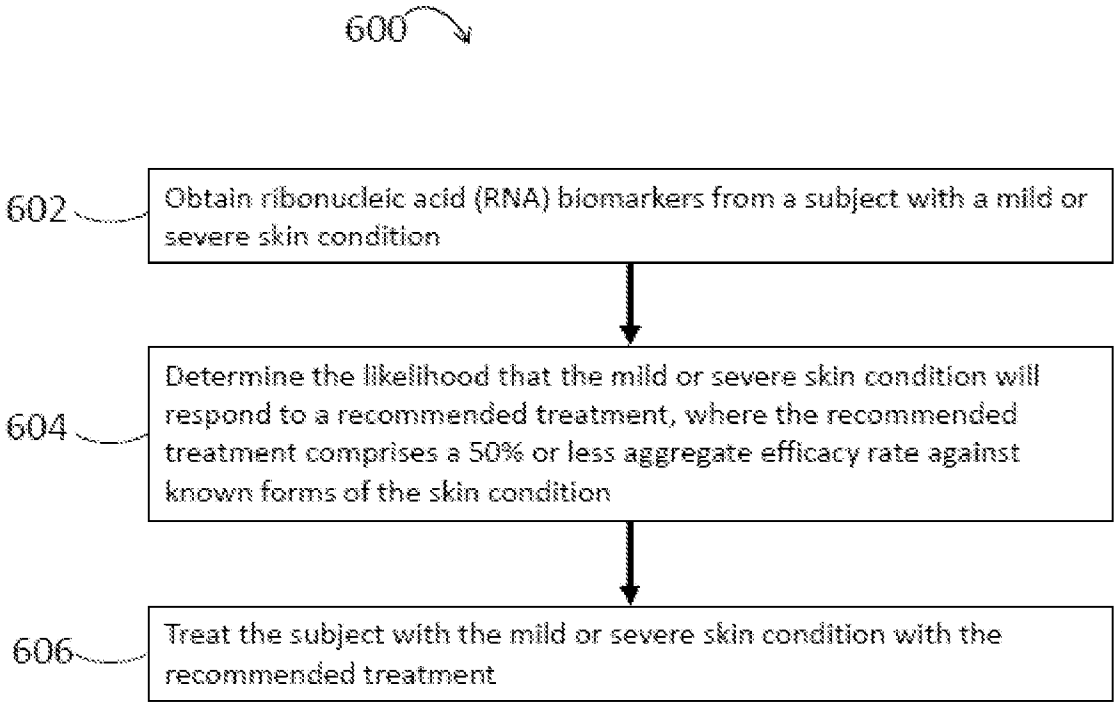

600
602
Obtain ribonucleic acid (RNA) biomarkers from a subject with a mild or severe skin condition
604
Determine the likelihood that the mild or severe skin condition will respond to a recommended treatment, where the recommended treatment comprises a 50% or less aggregate efficacy rate against known forms of the skin condition
606
Treat the subject with the mild or severe skin condition with the recommended treatment

FIG. 6A

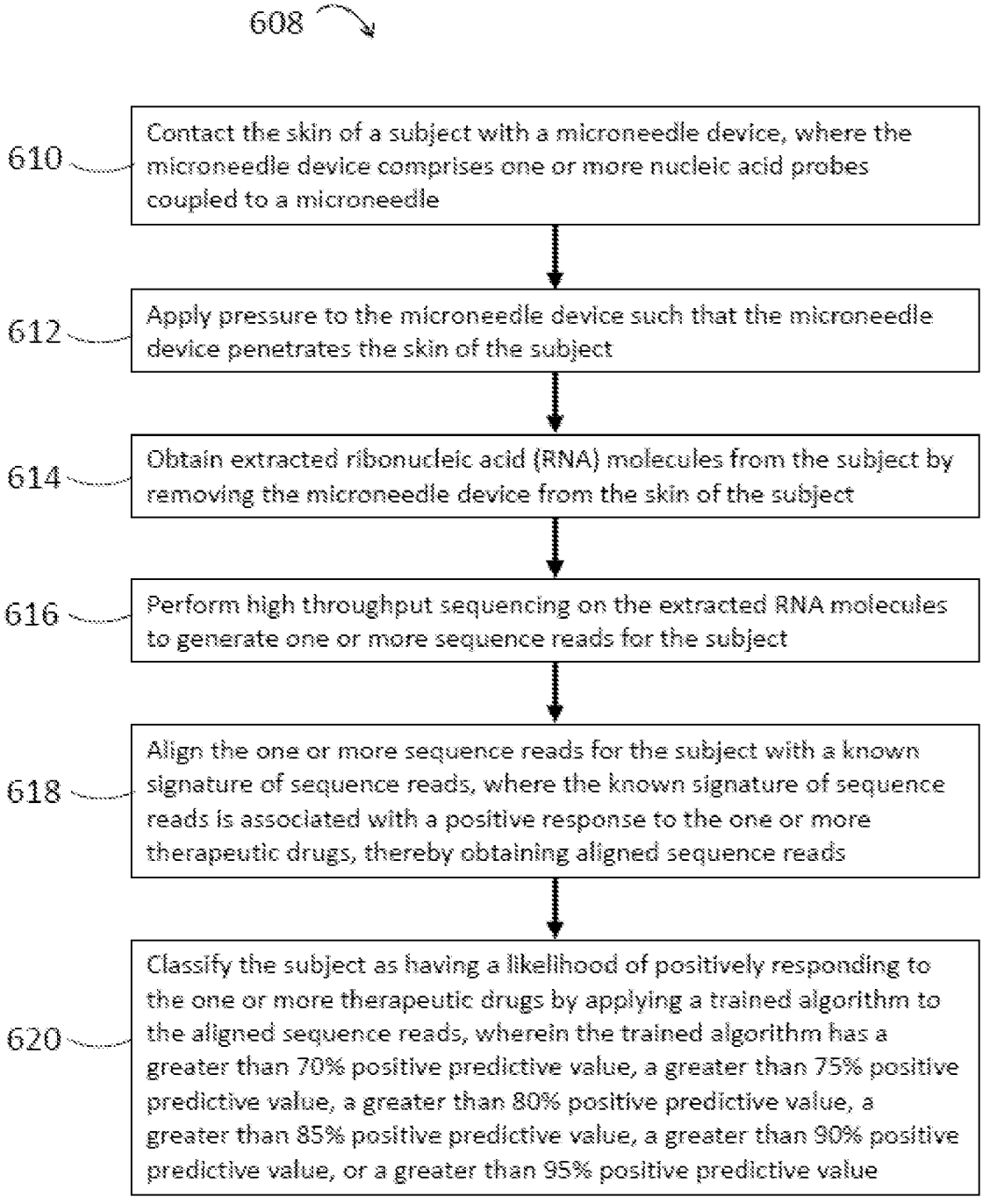

608
610
Contact the skin of a subject with a microneedle device, where the microneedle device comprises one or more nucleic acid probes coupled to a microneedle
612
Apply pressure to the microneedle device such that the microneedle device penetrates the skin of the subject
614
Obtain extracted ribonucleic acid (RNA) molecules from the subject by removing the microneedle device from the skin of the subject
616
Perform high throughput sequencing on the extracted RNA molecules to generate one or more sequence reads for the subject
618
Align the one or more sequence reads for the subject with a known signature of sequence reads, where the known signature of sequence reads is associated with a positive response to the one or more therapeutic drugs, thereby obtaining aligned sequence reads
620
Classify the subject as having a likelihood of positively responding to the one or more therapeutic drugs by applying a trained algorithm to the aligned sequence reads, wherein the trained algorithm has a greater than 70% positive predictive value, a greater than 75% positive predictive value, a greater than 80% positive predictive value, a greater than 85% positive predictive value, a greater than 90% positive predictive value, or a greater than 95% positive predictive value

FIG. 6B

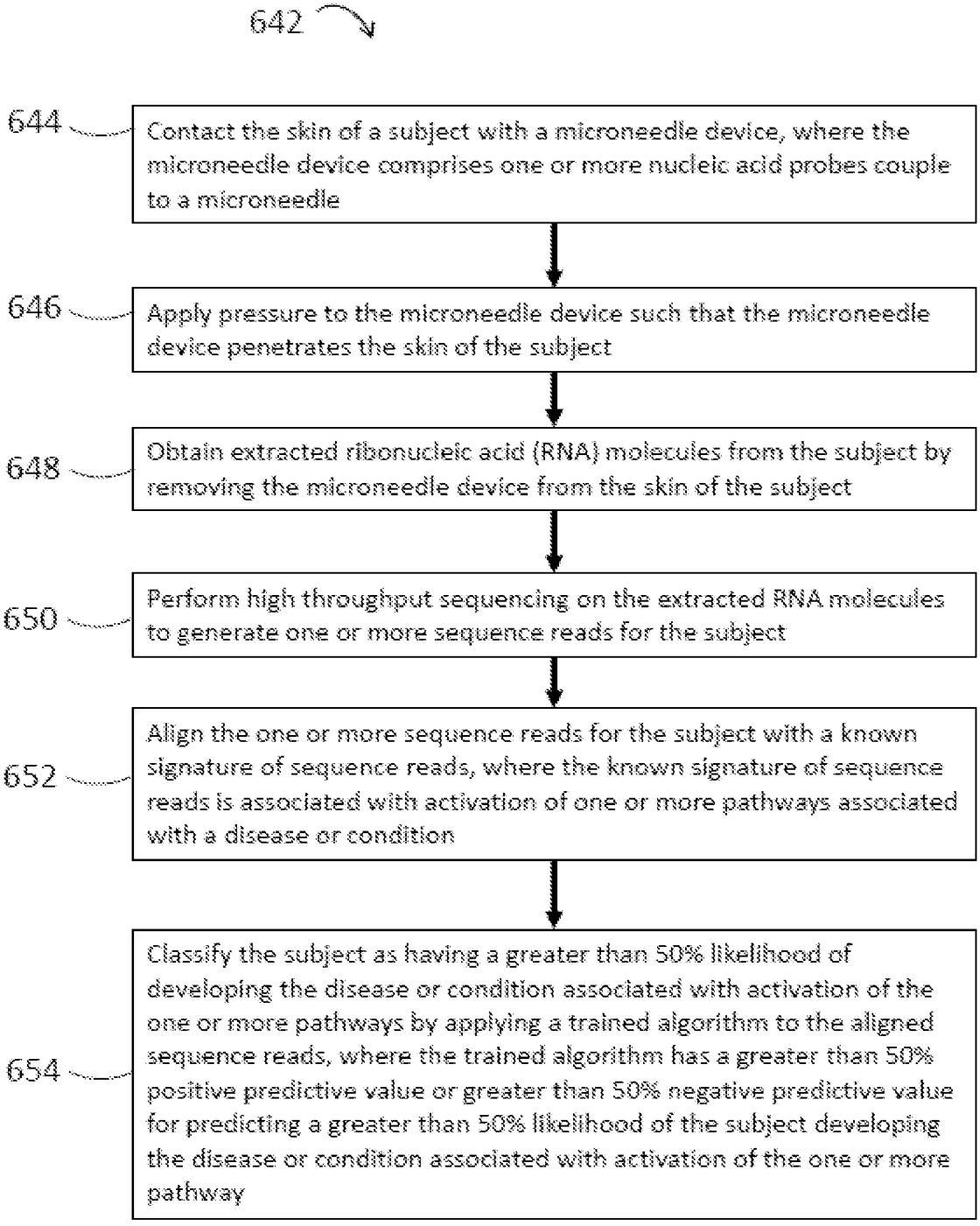

642
644
Contact the skin of a subject with a microneedle device, where the microneedle device comprises one or more nucleic acid probes couple to a microneedle
646
Apply pressure to the microneedle device such that the microneedle device penetrates the skin of the subject
648
Obtain extracted ribonucleic acid (RNA) molecules from the subject by removing the microneedle device from the skin of the subject
650
Perform high throughput sequencing on the extracted RNA molecules to generate one or more sequence reads for the subject
652
Align the one or more sequence reads for the subject with a known signature of sequence reads, where the known signature of sequence reads is associated with activation of one or more pathways associated with a disease or condition
654
Classify the subject as having a greater than 50% likelihood of developing the disease or condition associated with activation of the one or more pathways by applying a trained algorithm to the aligned sequence reads, where the trained algorithm has a greater than 50% positive predictive value or greater than 50% negative predictive value for predicting a greater than 50% likelihood of the subject developing the disease or condition associated with activation of the one or more pathway

FIG. 6C

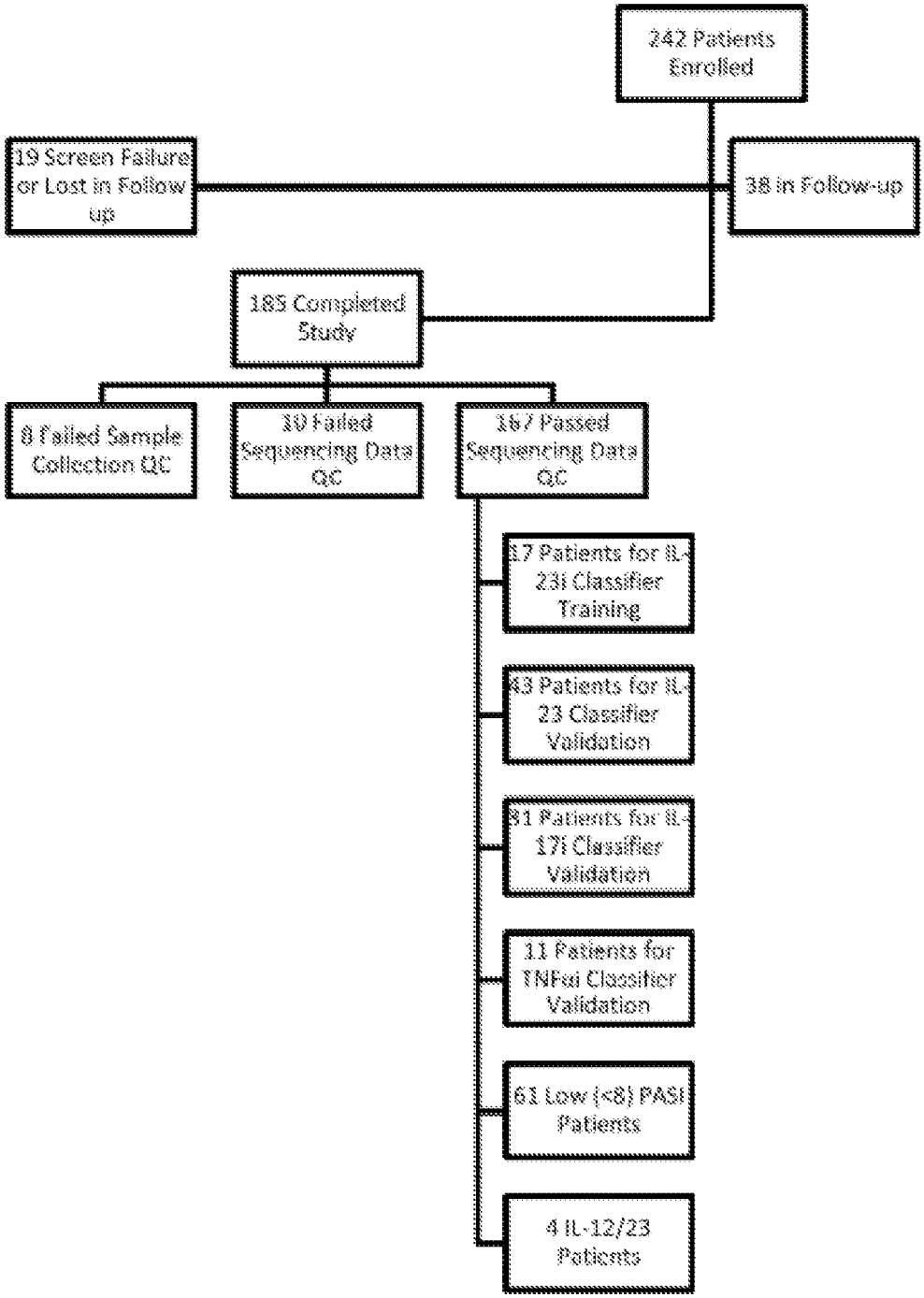

242 Patients Enrolled
19 Screen Failure or Lost in Follow up
38 in Follow-up
185 Completed Study
8 Failed Sample Collection QC
10 Failed Sequencing Data QC
167 Passed Sequencing Data QC
17 Patients for IL-23i Classifier Training
43 Patients for IL-23 Classifier Validation
81 Patients for IL-17i Classifier Validation
11 Patients for TNFsi Classifier Validation
61 Low (<8) PASI Patients
4 IL-12/23 Patients

FIG. 7

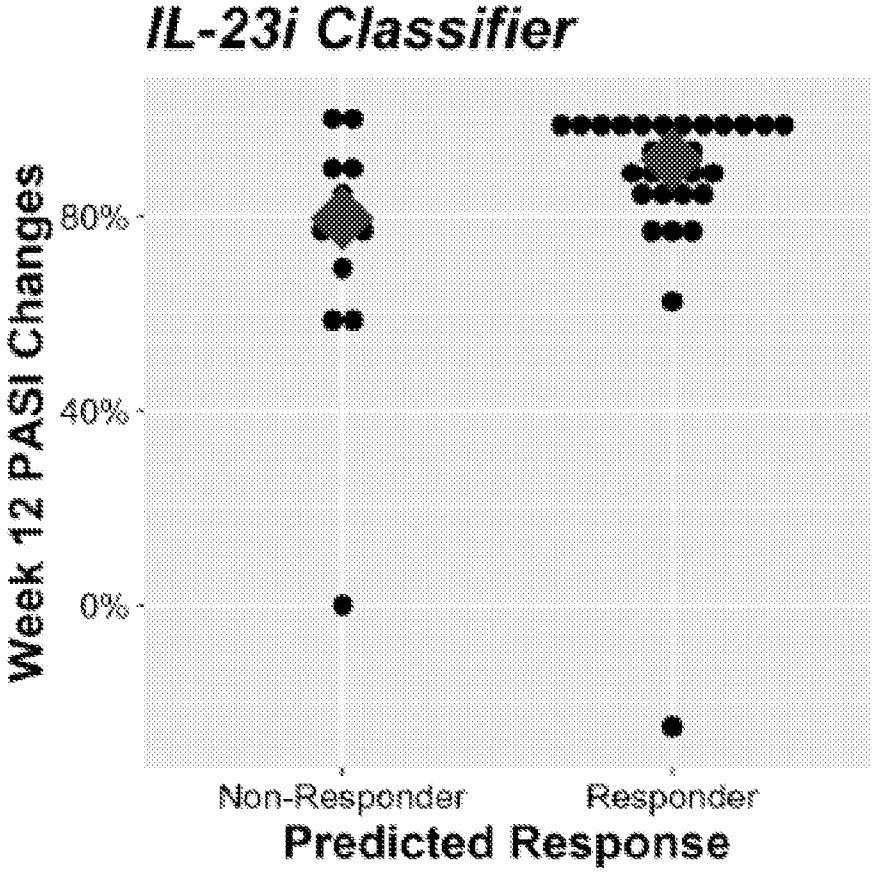

IL-23i Classifier
Week 12 PASI Changes
80%
40%
0%
Non-Responder
Responder
Predicted Response

FIG. 8A

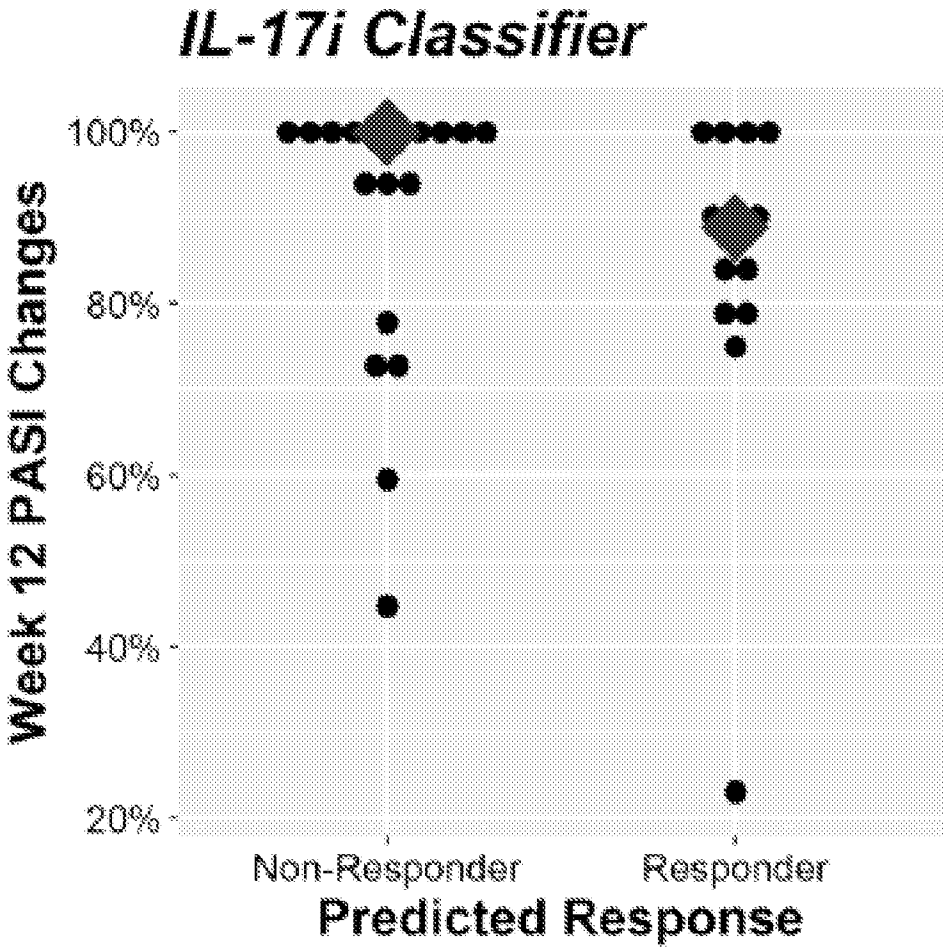

IL-17i Classifier
Week 12 PASI Changes
100%
80%
60%
40%
20%
Non-Responder
Responder
Predicted Response

FIG. 8B

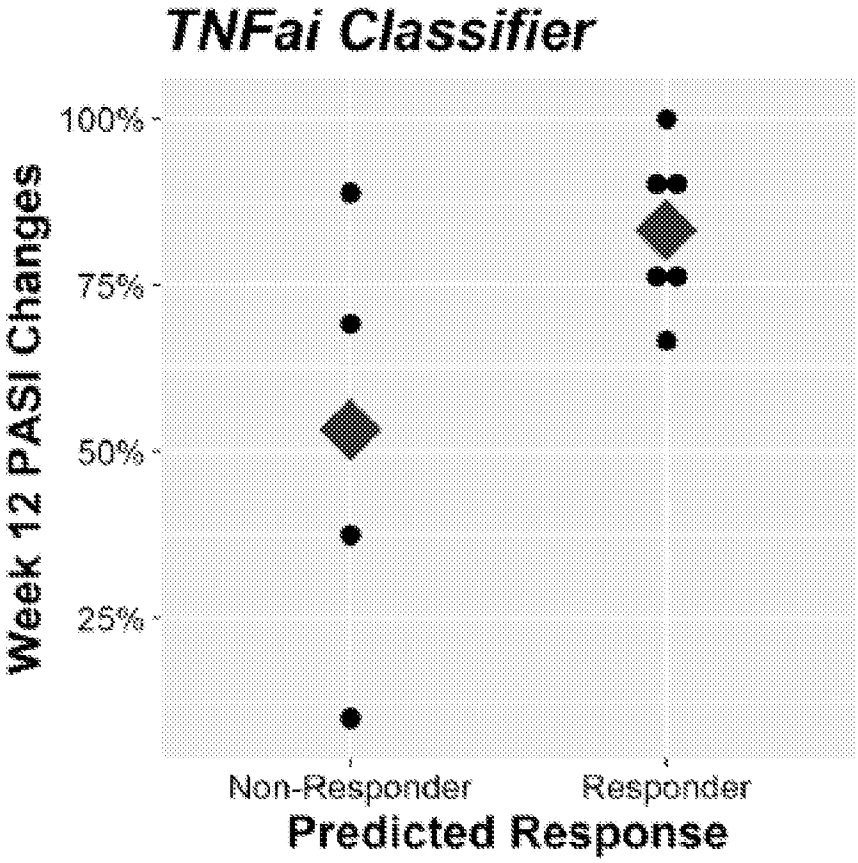

TNFai Classifier
100%
75%
50%
25%
Week 12 PASI Changes
Non-Responder
Responder
Predicted Response

FIG. 8C

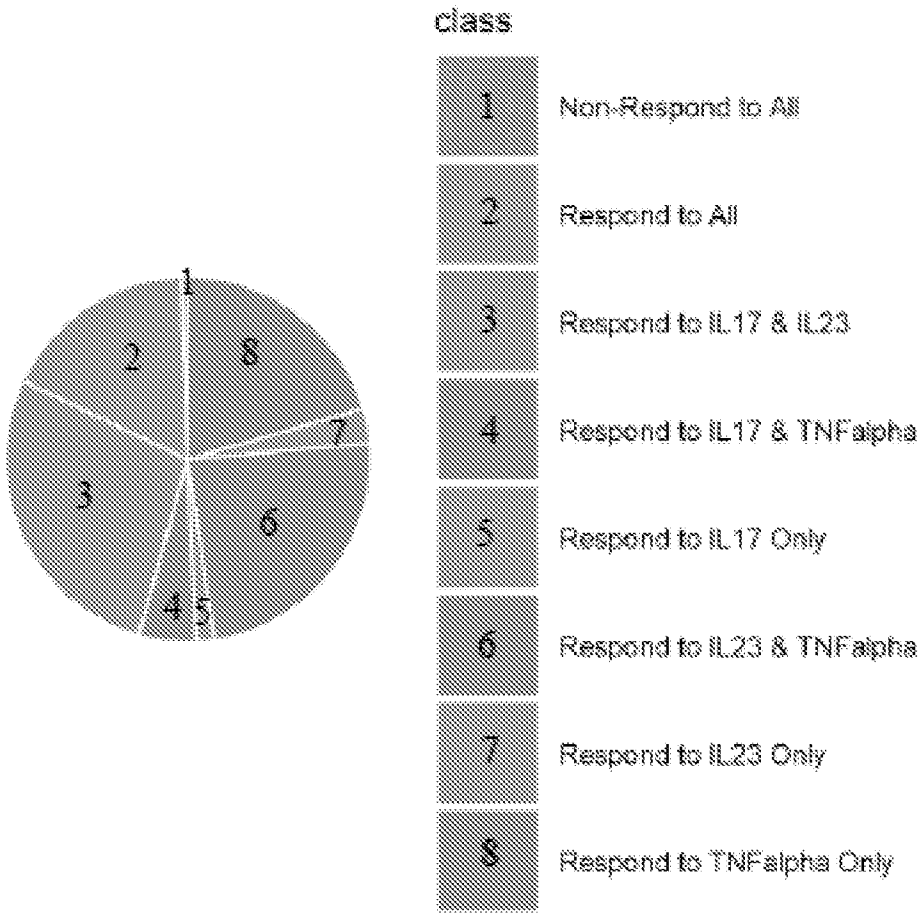

class
1
2
3
4
5
6
7
8
Non-Respond to All
Respond to All
Respond to IL17 & IL23
Respond to IL17 & TNFalpha
Respond to IL17 Only
Respond to IL23 & TNFalpha
Respond to IL23 Only
Respond to TNFalpha Only

FIG. 9

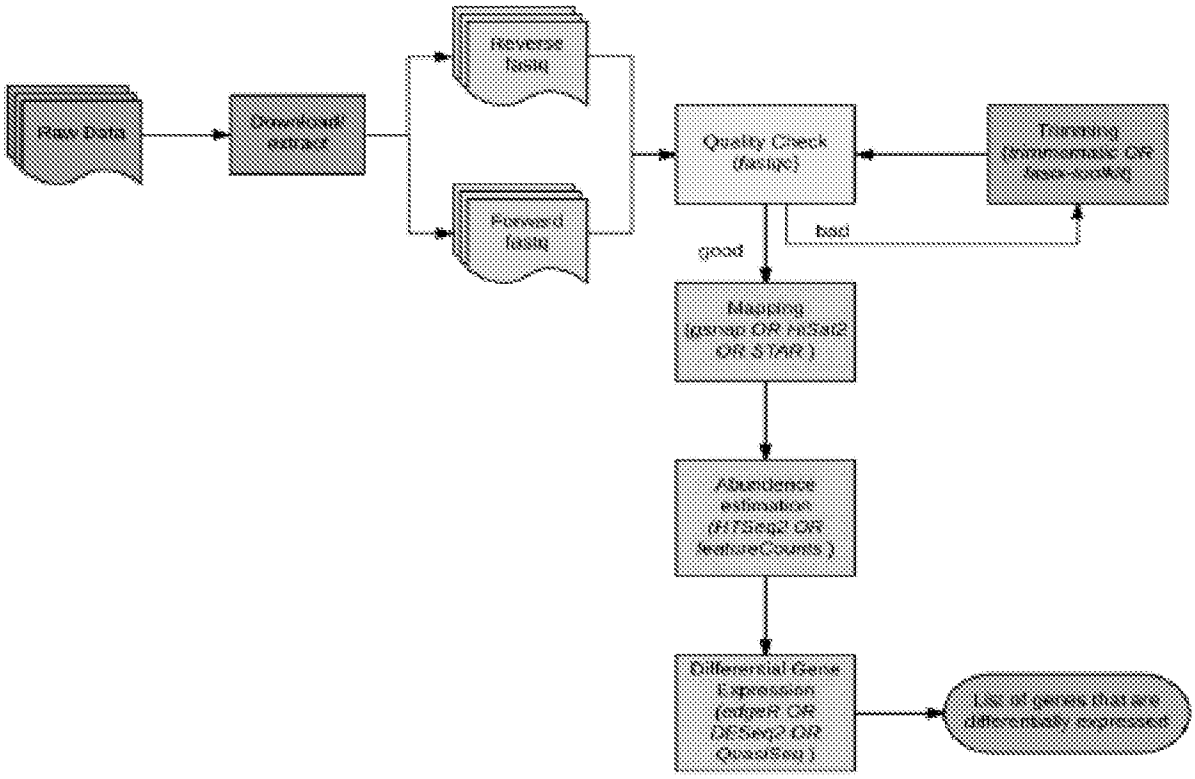

Reverse fastq
Quality Check (fastqc)
good
bad
Mapping
Abundance estimation
Differential Gene Expression

FIG. 10

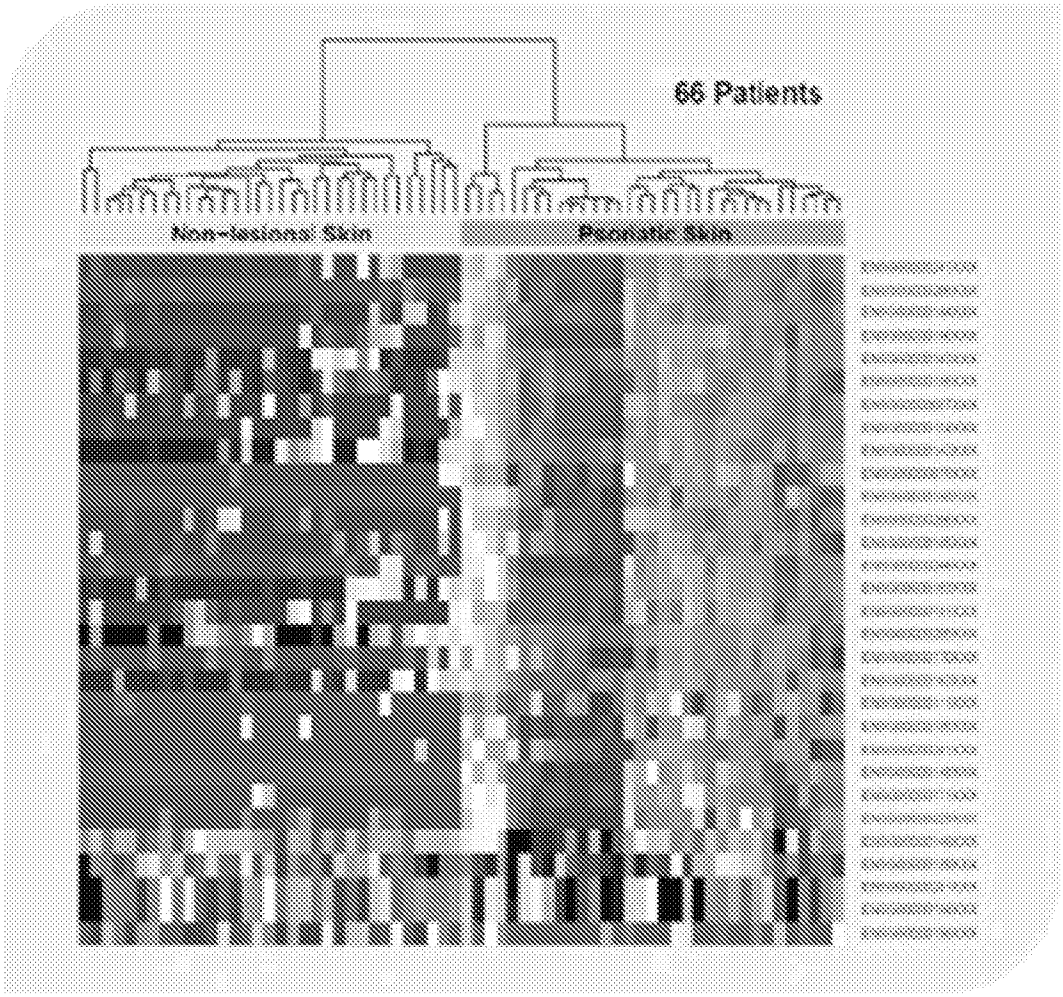


FIG. 11

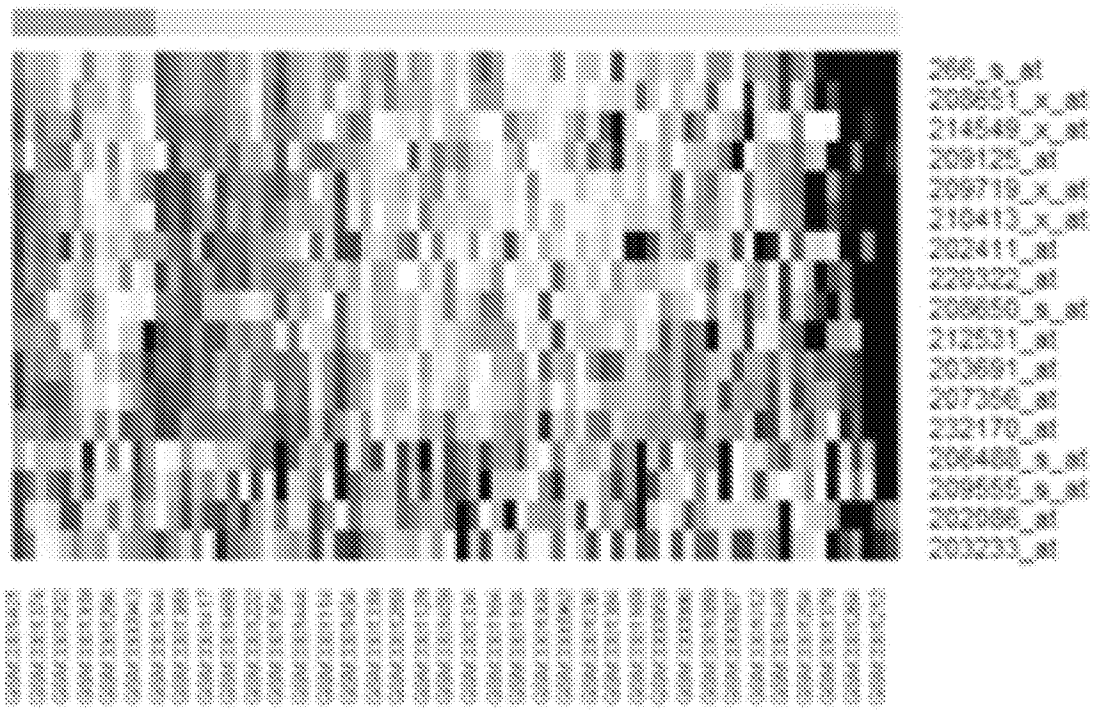

206_s_at
236651_x_at
214546_x_at
209125_at
209719_x_at
210413_x_at
202411_at
228322_at
236656_s_at
212531_at
205691_at
207268_at
232178_at
236468_s_at
209555_s_at
202286_at
202233_at

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061033

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61B 5/15; A61B 5/00; A61B 10/00; A61B 10/02; A61M 5/00; A61M 37/00 (2022.01)
CPC - A61B 5/15; A61B 5/00; A61B 10/00; A61B 10/02; A61M 5/00; A61M 37/00 (2022.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2018/0001071 A1 (3M INNOVATIVE PROPERTIES COMPANY) 04 January 2018 (04.01.2018) entire document	1-6, 8, 15, 16, 18, 28 --- 9, 10, 12-14, 17, 19, 20, 23, 26, 27
Y	US 2004/0146611 A1 (ARIAS et al) 29 July 2004 (29.07.2004) entire document	9
Y	US 2017/0145489 A1 (MINDERA CORPORATION et al) 25 May 2017 (25.05.2017) entire document	10, 14, 20, 23, 26
Y	US 2007/0023386 A1 (KRAVITZ et al) 01 February 2007 (01.02.2007) entire document	12, 13
Y	US 2019/0184366 A1 (VERNDARI INC) 20 June 2019 (20.06.2019) entire document	17, 27
Y	US 2008/0039805 A1 (FREDERICKSON et al) 14 February 2008 (14.02.2008) entire document	19
A	US 2019/0388473 A1 (RUBIUS THERAPEUTICS INC) 26 December 2019 (26.12.2019) entire document	1-28
A	US 2008/0108958 A1 (CARTER et al) 08 May 2008 (08.05.2008) entire document	1-28
A	US 2017/0050010 A1 (GEORGIA TECH RESEARCH CORPORATION) 23 February 2017 (23.02.2017) entire document	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 March 2022	APR 06 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061033

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 29-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-28

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/061033

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-28, is drawn to a microneedle device comprising: a microneedle region comprising (i) a microneedle base substrate comprising a first base substrate surface and a second base substrate surface.

Group II, claims 37-39, 85-101, 103, 104-107, 108-129 is drawn to a method of preparing a biological sample from a subject comprising: contacting skin of the subject with a microneedle device, wherein the microneedle device comprises a plurality of nucleic acid probes coupled to a microneedle.

Group III, claims 40-64, 65-84, 85-101, 102, 103, 104-107, 108-129, is drawn to a method of treating a subject with an autoimmune disease of the skin comprising: collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA.

The inventions listed as Groups I, II and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature of the Group I invention: a microneedle region comprising (i) a microneedle base substrate comprising a first base substrate surface and a second base substrate surface, wherein the first base substrate surface and the second base substrate surface are positioned on opposite sides of the microneedle base substrate; and (ii) a plurality of microneedles protruding from the first base substrate surface; and a support substrate adjacent to the microneedle base substrate, the support substrate connected to or integral with the microneedle base substrate as claimed therein is not present in the invention of Groups II and III. The special technical feature of the Group II invention: the microneedle device comprises a plurality of nucleic acid probes coupled to a microneedle; applying pressure to the microneedle device such that the microneedle device penetrates the skin of the subject; allowing the microneedle device to penetrate the skin of the subject for no more than 10 minutes to obtain a ribonucleic acid (RNA) sample hybridized to the nucleic acid probes, wherein the RNA sample comprises a population of RNA fragments comprising about 700 bases or more and a population of RNA fragments comprising about 150 bases to about 200 bases, and wherein a ratio of the population of RNA fragments comprising about 700 bases or more to the population of RNA fragments comprising about 150 bases to about 200 bases is greater than 1; and removing the microneedle device from the skin of the subject, the microneedle device comprising the RNA sample hybridized to the nucleic acid probes after removal of the microneedle device from the skin of the subject as claimed therein is not present in the invention of Groups I or III. The special technical feature of the Group III invention: collecting a sample comprising RNA derived from skin from the subject, wherein the subject has not been administered the autoimmune therapeutic drug within 7 days prior to the collecting the sample comprising RNA; determining an expression level of at least one gene based on the RNA; predicting that a subject with the autoimmune disease of the skin will be responsive to the autoimmune therapeutic drug with a positive predictive value greater than 80% based on the expression level of the at least one gene; and based on the predicting in (c), treating the subject with the autoimmune therapeutic drug as claimed therein is not present in the invention of Groups I or II.

Groups I, II and III lack unity of invention because even though the inventions of these groups require the technical feature of collecting a RNA sample from a subject using a microneedle device, this technical feature is not a special technical feature as it does not make a contribution over the prior art.

Specifically, US 2019/0388473 to RUBIUS THERAPEUTICS, INC. teaches collecting a RNA sample from a subject using a microneedle device ("Applicator" refers to any device used to connect to a subject. This includes, e.g., needles, cannulae, catheters, and tubing, para. 0520. The term "biological sample" refers to any type of material of biological origin isolated from a subject, including, for example, DNA, RNA, lipids, carbohydrates, and protein. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, para. 0526).

Since none of the special technical features of the Group I, II or III inventions are found in more than one of the inventions, unity of invention is lacking.