Single-cell transcriptomics in human skin research: available technologies, technical considerations and disease applications

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Abstract
Single-cell technologies have revolutionized research in the last decade, including for skin biology. Single-cell RNA sequencing has emerged as a powerful tool allowing the dissection of human disease pathophysiology at unprecedented resolution by assessing cell-to-cell variation, facilitating identification of rare cell populations and elucidating cellular heterogeneity. In dermatology, this technology has been widely applied to inflammatory skin disorders, fibrotic skin diseases, wound healing complications and cutaneous neoplasms. Here, we discuss the available technologies and technical considerations of single-cell RNA sequencing and describe its applications to a broad spectrum of dermatological diseases.

KEYWORDS
scRNA- seq, single-cell sequencing, single-cell transcriptomics, skin research

1 | INTRODUCTION

The human skin is defined by a multilayer architecture based on diverse cell populations of mainly keratinocytes and fibroblasts, as well as various immune cells, melanocytes, adipocytes and endothelial cells that orchestrate events leading to wound repair of pathogenic infections and exposure to ultraviolet radiation and toxic compounds.1 The transcriptomic profile of skin can provide information on gene expression, non-coding regulatory elements and gene splicing, and therefore shed light on skin physiology and pathology. Earlier approaches such as bulk RNA-seq and microarrays provided information about the average transcriptome status
of an entire tissue or sample. Since the first single-cell RNA-seq (scRNA-seq) study conducted by Tang et al. the development of single-cell transcriptomics led to the revelation of gene expression variability amongst identical or distinct cell types. Importantly, scRNA-seq allowed researchers to uncover de novo cell populations and lineages that may be implicated in differentiation and development. Moreover, scRNA-seq can be used as a diagnostic tool in precision medicine by deciphering disease-related biomarkers and pathways. This approach can be very promising in drug development, in which cell-specific transcriptomic responses to treatment can be analysed.

The aim of this review is to summarize the available technologies and the technical considerations of single-cell transcriptomics, as well as provide a comprehensive compendium of scRNA-seq applications in dermatological research.

2 | AVAILABLE TECHNOLOGIES

Currently, available technologies of scRNA-seq library generation comprise of cell lysis, reverse transcription into first-strand cDNA, second-strand synthesis and cDNA amplification. These technologies can be broadly classified into two categories: full-length transcriptome sequencing and 3' or 5' end counting-based sequencing.

Smart-seq is a full-length transcription sequencing method based on a switching mechanism, in which nucleotides at the end of the RNA template are added, allowing the reverse transcriptase to synthesize the complementary cDNA strand. Smart-seq2, which is the most widely used smart-seq technology, provides higher sensitivity and efficiency in capturing RNA molecules. On the contrary, a popular 3' or 5' end counting-based approach is the droplet microfluidics technology known as Drop-seq, which encapsulates cells into independent microdroplets with unique barcoded beads. Each bead has a cellular barcode which is unique to each droplet, as well as unique molecular identifiers (UMIs) representing each RNA molecule. This protocol has been developed further and commercialized with the use of the Chromium instrument (10x Genomics), which is widely used in dermatological research projects. The 10x technology has also developed protocols which conduct immune cell mapping and profiling of specific developing leukocytes by screening the VDJ leukocyte-specific genes of T-cell immune receptors (TCR).

In another microfluidic technology, characterized as Seq-well, cells are incorporated into capture beads that are confined in subnanoliter wells and sealed with a semipermeable membrane. In the membrane, the beads are removed, followed by cell lysis and mRNA capture. The first commercially available Seq-Well microfluidic platform was the C1 by Fluidigm. Hughes et al. developed a new version of Seq-well, Seq-Well S, in which a randomly primed second-strand synthesis as a second oligonucleotide handle is established after reverse transcription. This method was applied to certain dermatological diseases and is suggested to be simpler, more compatible with fragile cells and able to manage more samples in parallel. Each of the methods above has its own benefits and drawbacks. Although Smart-seq provides a higher coverage amongst transcripts and alternatively spliced mRNA, Drop-seq enables more cells to be sequenced simultaneously, whereas Seq-well prevents cross-contamination between samples. A novel scRNA-seq technique is Smart-seq3, which incorporates full-length coverage and a 5' UMI tagging strategy. This protocol allows a dramatic increase of sensitivity and can estimate gene expression in a larger number of cells.

3 | TECHNICAL CONSIDERATIONS

The scRNA-seq experimental procedure consists of four main steps: sample preparation, cell enrichment, library preparation and data analysis (Figure 1). In regard to the isolation of single cells from skin samples, punch biopsies or larger specimens are obtained and dissociated via mechanical or enzymatic treatment. Because the various layers of the skin have different cell compositions and properties, single-cell dissociation can be quite challenging. Multiple single-cell dissociation approaches are available depending on whether the dermis, epidermis or both are needed for each experiment. For example, a research study that aimed to resolve the basal keratinocyte transition states isolated the epidermal tissue, while in a study aiming at profiling fibroblast subpopulations, the epidermis was discarded, and the dermis was processed.

Typically, whole-skin dissociation kits such as the gentleMACS (Miltenyi Biotec) dissociation system can be used. Nevertheless, some dissociation enzymes included in the kit favour the isolation of fibroblasts and might provoke the elimination of a subpopulation of immune cells or epidermal cells, abrogating the whole-cell map of the sample. Alternatively, one can incubate the whole skin with dispase, leading to dermal-epidermal dissociation. Keratinocytes, melanocytes and epidermal immune cells are further obtained by the application of trypsin and fibroblasts by digestion with collagenase. Mechanical means are also used, such as mashing, dicing or slicing, enhancing the whole process. Tissue dissociation can vary from 2 h to overnight. During incubation, the activity of each enzyme must be taken into consideration since longer incubations can negatively impact cell viability and induce mechanical stress or trigger immune activation to the cells.

An alternative approach for skin cell acquisition is the application of suction blistering, whereby an artificial blister is introduced to the skin, eliciting dermal-epidermal dissociation and the formation of a fluid skin sample combined with interstitial fluid. The liquid nature of the biopsy can enhance single-cell dissociation. A major limitation of this method is the inability to capture deeper dermal regions, and therefore, dermal, endothelial cell and macrophage (MΦ) information cannot be provided.

Another parameter to be considered is the possibility of samples being frozen prior to processing, allowing sample processing at different time points. However, reduction of cell numbers, and the
alleviation of the cell transcriptome, are two major considerations. Several cryoprotectant agents used in tissue preservation have facilitated post-thaw capture of an adequate number of cells, mainly fibroblasts. Another proposed method of preserving the sample is by freezing the single-cell solution after dissociation, which has shown satisfactory cell viability and integrity. However, working with fresh tissue when needed is preferred, and multiple freeze and thaw cycles should be avoided.

An unbiased view of the cellular composition of the sample and the projection of its cellular heterogeneity is revealed when one captures all desired cells. Therefore, quality check and specific cell isolation are performed in the cell enrichment step. Obtaining a pure single-cell solution without cell debris, fragmented, or dead and apoptotic cells can reduce artifacts during downstream analysis. Quality check can be done manually by using an inverted microscope and micropipettes, or by applying negative charge in a patch pipette, a method known as micromanipulation. However, these methods are low-throughput and very laborious. High throughput methods such as fluorescence-activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) are predominantly used. FACS has an improved sensitivity even when a cell expresses low levels of the specific labelled markers with an antibody or with a viability dye such as DAPI. A limitation of this method is the relatively large number of cells needed as a starting material and the added stress exerted on the cells during the sorting process. On the contrary, MACS uses enzymes, antibodies and peptides conjugated to magnetic beads for sorting, requiring less time and equipment, but lacks sensitivity and specificity. Sorting methods can also be used to isolate specific cell populations of interest, which are further analysed as a distinct sample. For example, a study outlining the vascular endothelial cell heterogeneity in human skin has used both FACS and MACS to enrich for endothelial cells, whereas another study focused on the dendritic cell signalling in psoriasis by using FACS for dendritic and Mφ cell isolation.

4 | APPLICATIONS IN SKIN RESEARCH

4.1 | Healthy skin

Different studies have dissected the heterogeneity of dermal fibroblasts with scRNA-seq (Table 1). Tabib et al. reported two major fibroblast subpopulations largely defined by SFRP2 and FMO1 expression, as well as five minor subsets with distinct gene expression profiles. Immunostaining and gene ontology analyses revealed different cell morphologies and suggested specialized functions, such as extracellular matrix protein localization for SFRP2+ cells and stress response for FMO1+.
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|----------------------------|------------------------|--------------------------------------|---------------------------|-----------|
| 1            | Identifying major fibroblast populations | Healthy skin from dorsal mid-forearm | 6 healthy individuals | 3h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 8522 | Tabib et al, 2018 |
| 2            | Human fibroblast subpopulations | Healthy abdominal skin | 1 healthy individual | 1h in dispase II, epidermis peeled off, dermis overnight in Miltenyi whole-skin dissociation kit | FACS for CD90+ | Smart-Seq2 | 184 | Philipeos et al, 2018 |
| 3            | Skin fibroblast heterogeneity | Surplus trunk skin of female donors removed during abdominoplasty. | 3 healthy female donors | 2.5h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | FACS to exclude dead cells and debris | 10x Genomics | N/A | Vorstandlechner et al, 2020 |
| 4            | Skin immune cells during foetal skin development | Healthy foetal skin | 6 foetal surgical discards | 40–60 min in collagenase IV | FACS for CD45+ | 10x Genomics | N/A | Xu et al, 2021 |
| 5            | T lymphocytes of foetal skin | Healthy foetal trunk and adult human surgical discards | 9 foetal skin samples, 9 adult skin samples | Overnight in dispase II, 90 min in liberase 3 or Overnight in collagenase P/DNase I or overnight with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | FACS to exclude dead cells and debris, CD3+ | 10x Genomics | 1506 | Reitermaier et al, 2022 |
| 6            | αβγδT cells | Foetal skin | 3 foetal skin samples | 3h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | FACS to exclude dead cells and debris, CD3+ | 10x Genomics | N/A | Reitermaier et al, 2021 |
| 7            | Human melanocyte | Healthy surgical discards from adult and foetal skin | 22 healthy individuals | 14h in dispase II, dermal/epidermal separation, epidermis 3 min in trypsin | FACS for KIT+ melanocytes | Smart-seq2 | 9719 | Belote et al, 2021 |
| 8            | Myeloid cells | Healthy skin from dorsal mid-forearm | 10 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 27869 | Xue et al, 2020 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|-----------------------|----------------------------------------|-----------------------------|-----------|
| 9            | Basal stem cell transition states | Human neonatal foreskin | 5 neonatal foreskin | 2h in dispase, dermal-epidermal separation, epidermis 15 min in trypsin | FACS to exclude dead cells and debris | Droplet-enabled single-cell RNA sequencing | 17 553 | Wang et al, 22 2020 |
| 10           | Vascular endothelial cell heterogeneity in human skin | Surgical skin tissue discards from healthy individuals | 10 healthy individuals | Overnight with dispase II, dermis 40 min in collagenase type 4/ DNase I | MACS for CD31+ CD45− cells and FACS for endothelial cells | 10x Genomics | N/A | Li et al, 35 2021 |
| 11           | Eosinophilic fasciitis | Healthy skin (T helper 2 cell–fibroblast niche) | 3 healthy individuals | Mechanical dissociation, overnight in collagenase XI/DNase | FACS for CD45+, PDPN+, CD45, -CD31-ECadherin-CD235a−, viable cells | 10x Genomics | N/A | Boothby et al, 95 2021 |
| 12           | Ageing | Eyelid skin from blepharoplasty | 9 human female individuals | 1h in collagenase IV/ dispase/trypsin cocktail | FACS to exclude dead cells and debris | 10x Genomics | 35 678 | Zou et al, 44 2021 |
| 13           | Ageing | Skin from a defined, Caucasian sun-protected skin | 2 young donors, 3 old donors | 1h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 15 457 | Solé-Boldo et al, 45 2020 |
| 14           | Dermal sheath cells and ageing | Skin from upper forearm | 3 young and 4 old female donors | 1h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 72 –048 | Ahelrs et al, 96 2022 |

**Inflammatory skin disorders**

| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|-----------------------|----------------------------------------|-----------------------------|-----------|
| 15           | AD and psoriasis | Skin from the lower back close to lesions | 5 healthy skin samples, 4 AD skin samples, 3 psoriasis skin samples | 1h with dispase II, dermal-epidermal separation, collagenase IV overnight | FACS for CD45− and CD45+ | 10x Genomics | 17 000 | Reynolds et al, 47 2021 |
| 16           | AD and psoriasis | Lesional and non-lesional skin | 2 AD skin samples, 2 psoriasis skin samples | Overnight with collagenase/ DNase/10% FCS/RPMI/Pen Strep/L-glutamine | FACS for CD45+, CD3−CD19 | Smart-seq2 | N/A | Nakamizo et al, 36 2021 |
| 17           | AD | Suction blisters and skin biopsies from AD patients | 8 AD patients, 7 healthy individuals | Suction blisters: 10 min in trypsin Biopsies: collagenase IV (time not specified) | FACS for CD45+ and CD45− | 10x Genomics | 17 –160 | Rojahn. et al, 27 2020 |

(Continues)
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|------------------------|---------------------------------------|---------------------------|-----------|
| 18           | AD                       | Non-lesional and lesional skin taken from the extremities of AD patients | 9 patients with AD, 7 healthy individuals | Frozen tissue cryopreserved in FBS + 10% DMSO Dissociation in liberase 2x for 15 min, trypsin for 15 min | N/A | 10x Genomics | 39 042 | He et al, 2020 |
| 19           | AD                       | Lesional AD skin | 3 normal human skin samples, 4 AD skin samples | 40 min in collagenase | FACS for viable CD45+, CD3− | 10x Genomics | N/A | Alkon et al, 2021 |
| 20           | AD                       | Suction blisters of Caucasian patients with spontaneous remission from previous moderate-to-severe AD | 4 healed AD patients, 4 healthy individuals, 4 AD patients | N/A | FACS for CD45+ | 10x Genomics | 14 538 | Rindler et al, 2021 |
| 21           | AD                       | Suction blisters from AD skin treated and non-treated with dupilumab | 19 AD patients | Enzymatically digested epidermis (not specified) | FACS for CD45+, CD45− | 10x Genomics | 47 997 | Bargert et al, 2021 |
| 22           | Psoriasis                | Lesional psoriatic skin | 3 psoriasis patients, 3 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 24 259 | Gao et al, 2021 |
| 23           | Psoriasis                | Epidermis of truncal skin, neonatal foreskin and psoriatic skin | 9 normal skin samples (3 Scalp, 3 Truncal, 3 Neonatal), 3 Psoriatic truncal epidermis | Dermal-epidermal separation, 2h in dispase, 15 min in Trypsin | FACS to exclude dead cells and debris | 10x Genomics | 92 889 | Cheng et al, 2018 |
| 24           | Psoriasis                | Psoriatic skin | 13 psoriasis patients, 5 healthy individuals | 3h in dispase II, dermal/epidermal separation | N/A | 10x Genomics | N/A | Kim et al, 2021 |
| 25           | Psoriasis and cancer     | Lesional psoriatic skin | 11 patients with psoriasis, 5 healthy individuals | Overnight with Collagenase IV/DNase | FACs for CD45+CD3+CD8+ | Smart-Seq | 4 575 | Liu J et al, 2021 |
| 26           | Langerhans cell subsets in psoriasis | Epidermis of foreskin trunk, and scalp tissue | 25 psoriasis patients, 25 healthy individuals | Overnight in dispase II, dermal/epidermal separation, epidermis for 15 min in | FACs for viable CD45+HLA-DR+CD207+ | 10x Genomics | 3704 | Liu X et al, 2021 |
| 27           | Granuloma                | Lesional skin from granulomas | 3 granuloma patients, 3 normal skin samples | Dispase II followed by liberase | FACS to exclude dead cells and debris | 10x Genomics | 19 766 | Wang et al, 2021 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|----------------------------|------------------------|--------------------------------------|--------------------------|-----------|
| 28           | Granuloma                | Granulomas from patients with antimicrobial responses in human leprosy | 5 patients with reversal reactions, 5 patients with disseminated lepromatous leprosy | 1h with dispase II, dermal/epidermal separation, epidermis 30 min in trypsin dermis 2h in collagenase II/DNAse | MACS for CD1a+ | Seq-Well S² | 21318 | Ma et al, 98 2021 |
| 29           | Systemic Sclerosis       | Dorsal mid-forearm systemic sclerosis skin | 4 systemic sclerosis patients, 4 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | FACS to exclude dead cells and debris | Smart-Seq2 | N/A | Apostolidis et al, 54 2018 |
| 30           | Systemic sclerosis       | Systemic sclerosis skin | 12 patients with systemic sclerosis, 10 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi) | N/A | 10× Genomics | N/A | Tabib et al, 55 2021 |
| 31           | Systemic Sclerosis       | Lesional and non-lesional systemic sclerosis skin | 27 patients with systemic sclerosis | Enzymatically digested (not specified) | N/A | 10× Genomics | N/A | Gaydosik et al, 56 2021 |
| 32           | Systemic Sclerosis       | Mid-forearm skin | 12 systemic sclerosis patients, 10 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 65 199 | Xue et al, 57 2021 |
| 33           | Localized Scleroderma    | Fresh and frozen localized sclerodermas | 3 patients with localized scleroderma | Frozen tissue in cryostore 3h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 14 901 | Mirizio et al, 99 2020 |
| 34           | Cutaneous lupus erythematosus (CLE) | Lesional and sun-protected non-lesional CLE skin | 7 patients with active CLE, 14 healthy individuals | Overnight with dispase, dermal/epidermal separation, epidermis 1h in trypsin, dermis 1.5h in collagenase | N/A | 10× Genomics | 46 540 | Billi et al, 63 2021 |
| 35           | Dermatomyositis and lupus erythematosus | Lesional and non-lesional skin from a dermatomyositis, lupus erythematosus patients | 2 dermatomyositis patients, 2 lupus erythematosus patients, 2 healthy individuals | Overnight in dispase, dermal-epidermal separation, epidermis 1h in trypsin/DNase I, demis 2h in collagenase I, V | N/A | 10× Genomics | N/A | Tsoi et al, 100 2020 |
| 36           | Lupus erythematosus      | Frozen and fresh healthy skin of patients with lupus nephritis | 17 patients with lupus nephritis | Frozen tissue in cryostore for 1h, dissociation in liberase for 15 min | N/A | Fluidigm C1 | 4019 | Der et al, 65 2019 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|-------------|--------------------------|------------------|------------------|----------------------------|-----------------------|--------------------------------------|---------------------------|-----------|
| 37          | Lupus erythematosus      | Non-lesional, non-sun-exposed skin | 12 patients with lupus nephritis, 5 healthy individuals | 15 min in Liberase TL | FACS for CD4+/CD14+ | Fluidigm C1 | N/A | Der et al. 2017 |
| 38          | Erythema migrans         | Erythema migrans lesions | 10 erythema migrans patients | 3h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 70000 | Jiang et al. 2021 |
| 39          | Hidradenitis suppurativa | Axillary lesions and surgical excisions from hidradenitis suppurativa patients | 3 hidradenitis suppurativa patients, 1 healthy individual | Overnight with dispase II/ collagenase II cocktail | N/A | 10× Genomics | 33624 | Mariottoni et al. 2021 |
| 40          | Hidradenitis suppurativa | Excisional skin from patients with severe hidradenitis suppurativa | 9 patients with severe hidradenitis suppurativa | Overnight in dispase, dermal/epidermal separation, epidermis for 1h in trypsin/ DNase I, dermis 1.5h in collagenase II, collagenase V | N/A | 10× Genomics | 30636 | Gudjonsson JE et al. 2020 |
| 41          | Human vitiligo           | Suction blisters of lesional and non-lesional human vitiligo skin | 10 individuals with active vitiligo, 7 healthy individuals | Suction blisters | N/A | Drop-seq | 32405 | Gellatly et al. 2021 |
| 42          | Human Papillomavirus infection | Warts from the chest and elbow and normal skin | 1 immunosuppressed patient | Overnight in dispase, epidermis peeled off, epidermis 2 min in trypsin | FACS to exclude dead cells and debris | 10× Genomics | 15105 | Devitt et al. 2021 |
| 43          | Psoriasis, eczema, AD, erythrokeratodermia variabilis | Epidemic from lesional skin from patients with psoriasis, AD, erythrokeratodermia variabilis | 3 psoriasis patients, 1 AD patient, 1 Erythrokeratodermia variabilis patient, 3 healthy individuals | 2h in dispase, dermal-epidermal separation, 15 min in trypsin | FACS to exclude dead cells and debris | 10× Genomics | 59502 | Harirchian et al. 2019 |
| 44          | Acne, alopecia areata, granuloma annulare (GA), leprosy, and psoriasis | Skin from acne, alopecia areata, granuloma annulare, leprosy, and psoriasis patients | 4 patients with acne, 1 patient with alopecia, 2 patients with granuloma annulare, 4 patients with leprosy, 5 patients with psoriasis, 3 normal skin samples | 1h with dispase II, dermal/epidermal separation, Epidermis: 30 min in trypsin/ DNase Dermis: 2h in collagenase II/DNase | MACS for CD1A+ | Seq-Well S³ | 38274 | Huges et al. 2020 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|-----------------------|--------------------------------------|---------------------------|------------|
| 45           | Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DiHS/DRESS) | Lesional skin of patient with refractory DiHS/DRESS | 1 patient with refractory DiHS/DRESS, 5 healthy individuals | 3h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | FACS to exclude dead cells and debris | 10x Genomics | 18 218 | Kim et al, 24 2020 |
| 46           | Keloids | Keloids | 4 patients with keloids | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10x Genomics | 28 064 | Liu. X et al, 68 2021 |
| 47           | Keloids | Mature keloids and normal scars | 3 patients with keloids, 3 healthy individuals | 2h with dispase II, dermal-epidermal separation, dermis 2h in collagenase IV | N/A | 10x Genomics | 40 655 | Deng et al, 67 2021 |
| 48           | Keloids | Keloids and normal skin | 2 patients with keloids, 3 patients with normal skin | Keloids: 60 min in liberase Normal skin: 1h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | LUNA-FL dual fluorescence cell counter to exclude dead cells and debris | 10x Genomics | 35 424 | Shim et al, 101 2022 |
| 49           | Pressure ulcers | Skin from the excision site of patients with ulcers | 5 patients with spinal cord injury with grade IV pressure ulcers, 4 healthy individuals | Overnight with dispase, dermal/epidermal separation, epidermis 10 minutes in trypsin | N/A | Smart-seq2 | 1170 | Li et al, 71 2021 |
| 50           | Diabetic foot ulcers | Foot and forearm skin; healed and non-healed ulcers of patients with Diabetes Mellitus | 10 non-diabetic patients, 10 diabetic patients with no foot ulceration, 11 diabetic patients with foot ulcers, 4 non-diabetic patients with arm biopsy, 2 diabetic patients with no foot ulcers giving arm biopsies, 5 diabetic patients with foot ulcers giving arm biopsies | Overnight with dispase II, 90 min in Collagenase | N/A | 10x Genomics | 94 325 | Theocharidis et al, 70 2022 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment | Transcriptomic platform and chemistry | Number of transcribed cells | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|-----------------------|---------------------------------------|---------------------------|-----------|
| 51           | Diabetic foot ulcers     | Ulcers and foot skin from healthy and diabetic patients | 4 diabetic patients’ ulcers, 4 diabetic patients’ foot skin, 4 healthy individuals | 2h in collagenase P/dispase II/ DNase I cocktail | N/A | 10× Genomics | 9878 | Theocharidis et al, 2020 |
| 52           | Hypertrophic scars       | Skin from hypertrophic scars | 3 resected scar tissue, 3 healthy skin samples | 2.5h with whole-skin dissociation kit (Miltenyi) | FACS to exclude dead cells and debris | 10× Genomics | N/A | Vorstandlechner et al, 2021 |
| 53           | Cutaneous B-cell and T-cell lymphoma | Skin from a brownish plaque on the left flank and purple tumor on the left abdomen | 1 cutaneous follicle centre lymphoma patient | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 8654 | Joniak et al, 2021 |
| 54           | Cutaneous T-cell lymphoma | Lesional skin from advance stage Cutaneous T-cell lymphoma patients | 5 patents with Cutaneous T-cell lymphoma, 5 healthy individuals | 2h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 14 119 | Gaydosik et al, 2019 |
| 55           | Cutaneous T-cell lymphoma (mycosis fungoides) | Lesional and non-lesional skin from flat skin and plaque/tumor lesions of mycosis fungoides patients | 11 mycosis fungoides patients, 3 healthy individuals | 1h with whole-skin dissociation kit (Miltenyi), followed by gentleMACS | N/A | 10× Genomics | 47 172 | Rindler et al, 2021 |
| 56           | Mycosis fungoides        | MF lesion         | 1 mycosis fungoides patient | 30 min in collagenase IV | FACS for viable CD45+CD3+CD4+ T helper cells, other CD45+ and CD45−cells | 10× Genomics | 4512 | Rindler et al, 2021 |
| 57           | Squamous cell carcinoma (SCC) | SCC tumor | 10 SCC patients | 30 min in trypsin, frozen 10%DMSO/ DNase I SCC-13 media/30 min in collagenase I | FACS for CD45+ | 10× Genomics | 50 009 | Ji et al, 2020 |
| 58           | Basal cell carcinoma (BCC) | BCC tumor | 4 BCC patients | Overnight in dispase II/ collagenase IV cocktail, 15 min in trypsin | N/A | 10× Genomics | N/A | Guerrero-Juarez et al, 2021 |
| 59           | Basal cell carcinoma (BCC) | BCC tumor | 4 BCC patients | 1h in collagenase, 15 min in trypsin | FACS for ItgA6+ | 10× Genomics | N/A | Yao et al, 2020 |
| Study number | Condition/research focus | Skin sample type | Number of donors | Tissue dissociation method | Single-cell enrichment method | Transcriptional platform and chemistry | Reference |
|--------------|--------------------------|------------------|------------------|---------------------------|-----------------------------|---------------------------------------|-----------|
| 60           | Melanoma                 | Melanomas        | 31 melanoma patients | 10 min in collagenase P/ DNase I | FACs for viable CD45+ cells | Smart-Seq2, 10 × 2987 | Jerby-Aronn et al, 2016 |
| 61           | Melanoma                 | Melanomas with lymphoid tissue metastasis | 10 melanoma patients | 10 min in collagenase P/ DNase I | FACs for viable CD45+ cells | Smart-Seq2 | Tiross et al, 2016 |
| 62           | Langerhans cell histiocytosis (LCH) | LCH lesional skin | 4 patients with multi-system disease, 3 patients with single-system disease | 2h with whole-skin dissociation cocktail | FACs for CD207 viable cells | N/A | Jalbert et al, 2019 |
| 63           | Paget's disease | Epidermal cells of Paget's Disease skin | 1 extra-Mammary Paget's Disease patient | 2h with whole-skin dissociation kit (Miltenyi) followed by gentleMACS | FACs to exclude dead cells and debris | N/A | Halbritter et al, 2020 |
| 64           | Cutaneous neurofibroma at the globular stage | Cutaneous neurofibroma | 3 cutaneous neurofibroma samples | 22h with whole-skin dissociation kit (Miltenyi) | FACs for viable CD45+ cells | 10x Genomics | Song et al, 2021 |
| N/A          |                          | Melanoma patient | Melanoma patient | N/A | N/A | N/A | Brosseau et al, 2016 |

Moreover, Philippeos et al. used flow sorting to capture CD90+ mesenchymal cells from human dermis, which clustered into five subpopulations after sequencing. These included CD39+CD26+ papillary dermis fibroblasts, characterized by the expression of specific minor collagens such as COL6A5 and COL23A1, CD36+ cells located in the lower dermis and representing preadipocytes and three additional groups representing cells throughout the reticular dermis. According to another publication, skin fibroblasts should be classified not only based on their anatomical location but also their transcriptome. The six fibroblast clusters which were identified did not overlay with previously established markers of papillary and reticular cells and showed inter-cluster similarities at the transcriptional level. In addition, they were predicted to perform specific functional roles like regulation of TNF-α or p38/MAPK signalling and the DPP4+ cell population was shown as a key player of extra-cellular matrix genes, suggesting it could be a therapeutic target in fibrotic diseases.

Considerable efforts have been made in recent years to characterize different cell types in embryonic and foetal human skin. To unravel the developmental dynamics of immune cells in human skin, CD45+ hematopoietic cells from foetal skin specimens at distinct gestational points were profiled with scRNA-seq. Mφ origins and transcriptional changes were explored, establishing yolk sac- or hematopoietic stem cell-derived populations. DCs, innate lymphoid, natural killer and T cells were also described in detail. The second trimester was identified as a critical time point where most of skin immune cells differentiate to a mature state, a process accompanied by metabolic reprogramming and involvement of cell type-specific transcription factors. Two manuscripts by Reitermeier et al. characterized foetal skin T lymphocytes, revealing a previously undescribed double-positive αβγδ functional T-cell subset with a potential role in protecting from intrauterine infections. By integrating scRNA-seq with flow cytometry, in situ immunofluorescence and TCR repertoire profiling, they provided a comprehensive atlas of T cells during development. Finally, Belote et al. performed targeted scRNA-seq to comprehensively characterize melanocytes across different anatomical sites, skin tones, sexes and developmental stages and defined transcriptional programs and gene expression signatures that could be applied in melanoma prognosis.

A transcriptomic map of Mφ and dendritic cells (DCs) was built by performing unbiased scRNA-seq on healthy human skin. Three Mφ and six DC populations were described, including an unreported LAMP3+ mature DC and a proliferating progenitor DC subpopulation, as well as a TREM2+ Mφ subpopulation resembling Mφ present in neurodegenerative diseases. Wang et al. defined the heterogeneity of the epidermal compartment in healthy human skin, reporting at least four basal stem cell populations in neonatal interfollicular epidermis. These cells with distinct localization were characterized by specific marker gene expression, such as proto-oncogene PTTG1 and epigenetic modifiers HELLS or UHRF1, and were shown to have different roles in homeostasis including terminal differentiation and proliferation. Cutaneous vascular endothelial cells were recently surveyed using scRNA-seq, identifying five major subtypes and...
specifying IGFBP3 and RBP7 as arteriole and SELE and MT2A as venous markers, respectively.\textsuperscript{35} Postcapillary and capillary endothelial cells demonstrated enhanced inflammatory-associated gene expression pointing to an immunomodulatory role of the dermal vasculature.\textsuperscript{35}

Analysing eyelid skin from healthy subjects across distinct ages, Zou et al. resolved the cellular composition of human skin in young, middle-aged, and older individuals and defined molecular alterations associated with ageing.\textsuperscript{44} Epidermal basal cells were classified into six proliferating or quiescent subsets. Augmented chronic inflammation and attenuated basal cell self-renewal pathways were prominent characteristics of aged skin. Fibroblasts exhibited the highest level of transcriptional variability out of all cell types during ageing and growth-controlling transcription factor HES1 was an important driver of senescence in fibroblasts.\textsuperscript{44} An additional study aiming to delineate age-related effects on fibroblasts, collected sun-protected tissue from young and old male subjects for scRNA-seq analysis.\textsuperscript{45} Four major fibroblast populations were described and categorized as secretary-reticular, secretary-papillary, pro-inflammatory and mesenchymal according to their anatomical location and predicted functional role, but these identities were blurred in aged cells. Notably, aged fibroblasts also displayed a decreased number of interactions with other cell types.\textsuperscript{45}

### 4.2 Inflammatory skin disorders

Atopic dermatitis (AD) has been one of the most extensively studied cutaneous diseases with scRNA-seq. Harirchian and colleagues analysed epidermal cells to demonstrate that IL-17A induced targets of A20, an NFκB inhibitor and contributor to different skin rashes, share a similar overexpression in keratinocytes not only from AD, but also from psoriasis and erythrokeratoderma variabilis.\textsuperscript{46} This highlights the role of keratinocytes in inflammatory skin disorders and suggests A20 skin upregulation as potential treatment.\textsuperscript{46} An AD study combining a suction blistering technique, which allows simultaneous proteomic profiling of interstitial fluid, alongside traditional biopsies, showed enrichment of myeloid cells and upregulated proteins of DC or Mφ origin in AD samples compared to controls.\textsuperscript{27} In addition, He et al. profiled both lesional and non-lesional AD specimens and reported a novel COL6A5+ COL18A1+ lesional fibroblast subpopulation expressing CCL2 and CCL19 cytokines.\textsuperscript{30} The presence of a unique lesional DC population enriched for the CCL19 receptor CCR7 underscored a potential important role of fibroblast and immune cell communication.\textsuperscript{30}

A recent study integrating flow cytometry and scRNA-seq with published skin data sets examined the Mφ and DC landscape in AD and psoriasis and identified IL-1B and IL-23 producing CD14+ DC3s as potential inflammatory modulators in psoriasis.\textsuperscript{36} Furthermore, in an effort to characterize tissue-resident immune memory in AD patients treated with IL-4Rx blocker dupilumab, Bangert et al. employed scRNA-seq and proteomics to discover persisting immune cell populations after a year of clinical remission.\textsuperscript{6} These included LAMP3+ CCL22+ mature DCs, CRTH2+ CD161+ T helper cells and CRTAM + cytotoxic T cells with a cytokine receptor repertoire suggestive of an epidermal alarmin cross-talk.\textsuperscript{6} In a seminal publication from the Hanifia laboratory, more than half a million single cells from developing and adult healthy skin, as well as psoriatic and AD skin were profiled, to establish a skin atlas with unique cell populations enriched in disease such as F13A1+ Mφ, migratory DCs and a subset of vascular endothelial cells expressing inflammatory cytokines and leukocyte adhesion molecules.\textsuperscript{47} The gene signatures of these Mφ and endothelial cells bore striking similarities to their foetal counterparts, indicating a re-emergence of developing cell states in AD and psoriasis pathogenesis and offering new insights for targeted therapeutic interventions.\textsuperscript{47} A new single-cell sequencing method, called second-strand synthesis, was recently developed for enhanced gene detection and transcript capture and its efficiency was demonstrated in samples from five different inflammatory skin conditions: acne, alopecia areata, granuloma annulare (GA), leprosy and psoriasis.\textsuperscript{35} Findings include an overrepresentation of Tregs and an IRF4+ DC population in psoriasis, enrichment of proliferating endothelial cells in acne and immature cytotoxic T-cell clusters in leprosy and GA, together with unique fibroblast and Mφ populations.\textsuperscript{18} GA immunopathogenesis was also recently investigated and CD4+ T-cell-derived IFN-γ and IL-21, as well as Mφ secreted oncostatin M, were found to be elevated in GA.\textsuperscript{48} As all these cytokines are involved in the JAK-STAT signalling pathway, the authors postulated that JAK inhibitor treatment could be an effective therapeutic strategy for GA and proceeded to demonstrate improvement in five patients after treatment with JAK1/3 inhibitor tofacitinib.\textsuperscript{48}

Numerous scRNA-seq studies have focused on characterizing psoriatic skin. Gao et al. uncovered an immunoregulatory role of skin resident epidermal and mesenchymal cells, which express major histocompatibility complex genes and can activate DCs via secretion of LIF, IL-6, IL-17B, IL-36 and CD58 cytokines to contribute to disease progression.\textsuperscript{49} Other reports only analysed the epidermal component and discovered a CD1C+CD301A+ myeloid DC population,\textsuperscript{50} or used a technique to capture emigrating immune cells from skin biopsies in order to increase the number of sequenced leukocytes without harsh enzymatic digestion, sorting or activation of characteristically plastic cell populations.\textsuperscript{51} This methodology revealed four distinct T17 cell subsets with a uniquely enriched IL-17F+ IL-1+ population and a subset of immature DCs expressing IL-23A and IL-36G in psoriasis.\textsuperscript{51} Liu and colleagues comprehensively charted the highly heterogeneous CD8+ T-cell populations in psoriatic lesions and highlighted the increased expression of CXCL13 amongst T17 cell subsets, showing it could function as a biomarker of disease severity with a comparable or greater accuracy than IL-17A.\textsuperscript{52} Finally, in a scRNA-seq study characterizing Langerhans cells, two steady-state (LC1 and LC2) and two activated subsets were revealed. LC2, which were more likely to be activated, bore similarities to monocytes, expressed immunosuppressive genes and were more abundant in psoriatic lesions.\textsuperscript{53}

The Lafyatis laboratory used scRNA-seq to gain insights into vasculopathy of systemic sclerosis (SSc) by characterizing cutaneous
endothelial cells and revealed genes APLN and HSPG2, which are mediators of Apelin/Elabela-APLN and TGF-β signalling and could potentially serve as biomarkers of pathogenesis.\textsuperscript{54} The authors further explored the disease by analysing myofibroblast populations, which are the driver cell type of fibrosis, the most prominent manifestation of SSc on the skin.\textsuperscript{55} They showed that SSc myofibroblasts arise from a SFRP2\textsuperscript{hi} DPP4\textsuperscript{+} progenitor fibroblast population in two steps: an initial global shift of SFRP2\textsuperscript{hi} WiFi1\textsuperscript{+} to SFRP2\textsuperscript{hi} PRSS23\textsuperscript{+} WiFi1\textsuperscript{−} fibroblasts, only a subset of which subsequently differentiate into myofibroblasts also expressing SFRP4 and FNDC1.\textsuperscript{56} Additional reports focused on mapping either T lymphocyte heterogeneity in SSc skin, revealing a unique CXCL13\textsuperscript{+} T-cell subpopulation possibly promoting B-cell responses and autoantibodies production;\textsuperscript{57} or myeloid cells, identifying enriched FCGR3A\textsuperscript{+} Mφ and FCN1\textsuperscript{+} monocyte-derived DC subsets associated with severe skin SSc.\textsuperscript{58}

In axillary lesions from hidradenitis suppurativa (HS) patients, a chronic inflammatory follicular occlusion condition, monocytes and Mφ exhibited similar transcriptomic profiles to diabetic foot ulcer cells.\textsuperscript{59} They also overexpressed a series of markers associated with Fc signalling, metabolic activity, type I and II interferon stimulation and were more polarized towards the M1 phenotype.\textsuperscript{60} In addition, excisional samples from patients with severe HS analysed with scRNA-seq and proteomics, unmasked activation of the immune complement system together with B-cell and plasma cell as crucial pathways contributing to HS pathogenesis.\textsuperscript{59}

ScRNA-seq has also been proven effective in mapping the immune response of bacterial infection lesions such as erythema migrans and leprosy granulomas, or human papillomavirus (HPV) positive lesions. Samples of erythema migrans, a skin rash and initial sign of Lyme disease, were analysed with scRNA and B-cell receptor sequencing. Increased numbers of B cells with MHC class II upregulated genes, as well as memory B cells with IgM receptors, were found pointing to local antibody production at the skin infection site.\textsuperscript{61} By combining scRNA-seq with spatial transcriptomics, Ma et al. constructed the cellular network of the antimicrobial response in leprosy granulomas.\textsuperscript{58} Mφ were predominantly located at the centre of the granuloma and were surrounded by lymphocytes and distinct fibroblast subpopulations. Successful antimicrobial response was regulated by IFN-γ and IL-1β and was orchestrated not only by immune cells but also keratinocytes, fibroblasts and endothelial cells.\textsuperscript{62} Incorporation of common epithelial HPV genotypes with their human counterparts during the mapping step, along with scRNA-seq in warts of an immunosuppressed patient, allowed the detection of the alpha papillomavirus HPV78 in basal and suprabasal keratinocytes and in hair follicle stem cells and could be applied for identifying HPV transcripts with malignancy potential in specific cells.\textsuperscript{63}

The cellular composition and molecular drivers in cutaneous lupus erythematosus lesional and non-lesional skin were reported by Billi et al.\textsuperscript{64} Normal appearing skin in lupus patients was revealed as a highly type I interferon enriched environment that universally affects the gene expression of all skin cell types, while in lesional skin, accumulated CD16\textsuperscript{+} DCs arose as potent disease contributors.\textsuperscript{65} In other studies, focused on lupus nephritis, skin scRNA-seq was leveraged to determine whether skin biopsies could be utilized as renal disease biomarkers.\textsuperscript{66} IFN-inducible genes, including IFI6, STAT1 and IFITM1, were indeed upregulated in keratinocytes of patients with lupus nephritis indicating a systemic response to IFN.\textsuperscript{67} Expanding on their previous report, the authors processed more samples and included paired renal and skin biopsies from the same individuals to confirm augmented expression of type I interferon response pathway genes in lupus patients.\textsuperscript{68} They also stratified patients as responders and non-responders to treatment and found that non-responders’ tubular epithelial cells and keratinocytes overexpressed fibrosis-associated extracellular matrix genes.\textsuperscript{69}

To better understand the initiation and progression of vitiligo, Gellaty et al. employed suction blistering and scRNA-seq on affected and unaffected skin in vitiligo patients along with healthy individuals’ skin.\textsuperscript{70} They demonstrated the inability of lesional regulatory T cells (Tregs) to suppress autoreactive CD8\textsuperscript{+} T-cell-mediated depigmentation as they effectively do in non-lesional skin and also identified the CCR5/CCL5 axis as pivotal for the cross-talk between effector CD8\textsuperscript{+} T cells and Tregs. In both animal model and patient samples, the chemokine receptor CCR5 appeared to influence Treg function by promoting their proximity to CD8\textsuperscript{+} T cells to suppress them.\textsuperscript{71}

### 4.3 Fibrotic skin diseases and impaired wound healing

Dysregulated cutaneous wound repair can lead to the development of keloids, abnormal fibroproliferative growths with excessive accumulation of collagen and other extracellular matrix components. To gain insights into keloid pathogenesis and aetiology, Deng et al. compared dissociated dermis of normal scar tissue with that of keloids using scRNA-seq.\textsuperscript{72} They found four major fibroblast subpopulations, of which the mesenchymal group was significantly more abundant in keloids. The fibroblasts in this group overexpressed osteogenesis and chondrogenesis-related secretory proteins, POSTN and COL11A1, and were involved in collagen overproduction.\textsuperscript{67} Furthermore, in another comparative investigation, keloid lesional skin was analysed together with adjacent normal tissue and different signalling pathways were identified as important disease mediators in fibroblasts and vascular endothelial cells.\textsuperscript{68} TGF-β signalling molecules SMAD3 and TWIST1 were reported as upregulated in fibroblasts, while the Ephrin-Eph pathway was activated in both fibroblasts and endothelial cells. Notably, the pathway for negative regulation of transcription of PTEN, one of the most commonly mutated tumor suppressor genes, was activated, indicating cell growth pathway overlap between keloids and some cancers.\textsuperscript{73} Vorstandechnner et al. studied mature hypertrophic scars from resection surgeries and discovered that serine proteases DPP4 and PLAU could potentially be implicated in scar formation.\textsuperscript{74} Pharmacologic inhibition or knockdown of either gene prevented TGF-β induced fibroblast to myofibroblast differentiation and protease inhibitor
BC-11 or Sitagliptin treatment led to better collagen alignment in mouse scars.78

Chronic wounds, such as diabetic foot ulcers and pressure sores, are on the opposite end of the healing spectrum and characterized by failure to progress to an orderly and timely course of repair. ScRNA-seq analysis of diabetic foot ulcers, diabetic non-ulcerated and healthy foot skin revealed multiple fibroblast subpopulations and the ones derived from diabetic skin exhibited an injury-associated gene expression profile suggesting that prolonged exposure to stressors such as inflammation and hyperglycaemia impacts the cells even before the development of a wound.69 IL-13 and IFN-γ expression were inhibited in ulcers and both molecules were predicted as upstream regulators in multiple cell types, which could be translated therapeutically by targeted activation for improved healing.69 A substantial increase of the sample size in a subsequent report allowed the comprehensive mapping of the diabetic wound healing ecosystem and comparison between patients who healed and those who did not heal their ulcers.70 A subtype of fibroblasts that was uniquely present in the wounds of healers was identified and corroborated with spatial transcriptomics and immunostaining. These cells were enriched for extracellular matrix and inflammation-associated genes, including CHI3L1, MMP1, MMP3 and IL-6. In addition, healing ulcers also contained elevated numbers of classically activated or M1 macrophages also contained elevated numbers of classically activated or M1 macrophages, which are important mediators of intercellular communication including cancer-associated fibroblasts and endothelial cells. Several cell types were also revealed to be involved in immunosuppressive mechanisms, including DC inhibition, Treg recruitment and T-cell exhaustion.29

Cutaneous neurofibromas are benign peripheral nerve tumors and a clinical presentation of the genetic syndrome neurofibromatosis type 1. ScRNA-seq was employed to profile the matrisome gene expression of the tumor microenvironment cells, revealing the absence of collagen I myofibroblasts and elevated expression of collagen VI by fibroblasts instead.74

Rindler et al. discerned disease progression in primary cutaneous T-cell lymphoma by performing scRNA-seq and T-cell receptor sequencing on skin samples from patients with different stages of mycosis fungoides (MF), the most frequent type of this malignancy.75 Lesion progression correlated with downregulation of tissue-resident memory T-cell markers CXCR4 and CD69, heat shock protein HSPA1A, immunoregulatory molecules ZFP36 and TXNIP, and interleukin receptor IL7R.75 Furthermore, because malignant cells can spread from the skin in later stages, the authors profiled with scRNA-seq and simultaneous V-D-J sequencing the tumor microenvironment of skin, blood and lymph node in a patient with advanced MF.76 They found skin tissue-resident memory T cells that could switch to a more central memory-like phenotype in circulation and could explain their migratory behaviour.76 A single patient’s samples with concurrent MF and primary cutaneous follicle centre lymphoma (PCFCL)—the most common cutaneous B-cell lymphoma—appearing in separate lesions were analysed with scRNA-seq and combined T-cell and B-cell receptor sequencing.77 Two co-occurring clonal malignancies were unveiled, with the T-cell clone expressing Th2-related markers while the PCFCL lesions exhibited a more Th1 skewed gene expression profile and this was reflected in the tumors micromilieu.77 Finally, Gayodosik and colleagues employed scRNA-seq on lymphocytes purified from skin biopsies of advanced stage cutaneous T-cell lymphoma patients and confirmed a large inter- and intratumor T-cell gene expression heterogeneity.78 Patient-specific enriched cell subpopulations and markers were outlined, demonstrating the efficiency of scRNA-seq as a diagnostic and informational tool for personalized medicine. Additionally, a T-cell population with a common proliferating and resistance to apoptosis gene expression signature was described.78

The first study to harness the technology of scRNA-seq in human skin samples examined metastatic melanoma from 19 tumors with diverse clinical and therapeutic backgrounds.79 Tirosh et al. observed drug-resistant malignant cell subpopulations that existed before treatment and were further enriched as a result of MAP kinase-targeted treatment, a finding systematically validated in a
number of melanoma cell lines.\textsuperscript{79} Cell-cell interactions were inferred by deconvolution of bulk RNA-seq melanoma profiles and a notable set of genes highly correlating with T-cell infiltration was particularly upregulated in cancer-associated fibroblasts. T lymphocyte diversity was also profiled and potential biomarkers to separate cytotoxic and exhausted T cells were suggested.\textsuperscript{77} Furthermore, Jerby-Amon et al. sought to decipher immune checkpoint inhibitor (ICI) resistance in melanoma and established a prognostic program that could inform therapeutic approaches.\textsuperscript{80} Anti-PD-1 and anti-CTLA-4 treatments were predicted as effective, while CDK4/\textit{\^}i could reverse the resistance and resulted in improved ICI response \textit{in vivo}. An association between immunosuppressive gene expressing M\textsubscript{\text{ip}} and T-cell abundance was also noted.\textsuperscript{80}

In extramammary Paget’s disease, a form of malignant intra-epidermal adenocarcinoma characterized by the appearance of Paget cells, scRNA-seq of one patient’s epithelium provided new understanding on disease pathogenesis.\textsuperscript{81} KRT6C+ keratinocytes were uniquely found in diseased epidermis, while a novel cell surface marker (CD166/ALCAM) was reported in Paget cells which would allow flow sorting and more in depth study of these cells in future.\textsuperscript{81} Activation of mTOR signalling either through PTEN or HER2 was highlighted as an aberrant pathway in the disease and topical treatment of patients with mTOR inhibitor rapamycin effectively mitigated symptoms.\textsuperscript{81} Langerhans cell histiocytosis (LCH) is another relatively rare neoplasm, with lesions appearing mostly in skin and bones and predominantly affecting paediatric patients, which was recently mapped with scRNA-seq.\textsuperscript{82} Two LCH subpopulations with characteristics of progenitor cells were identified and the authors posited the existence of a cell hierarchy commencing from these cells and leading to the more differentiated four states that were discovered. These four subsets expressed marker genes similarly to different immune cell types, including DCs and mature Langerhans cells, but also extracellular matrix destruction genes such as matrix metalloproteinases and aminopeptidases. The JAK/STAT signalling pathway was suggested as a master driver of the differentiation with the participation of NFkB, AP-1, IRF8 and BATF3.\textsuperscript{83} Merkel cell carcinoma lesions profiled with scRNA-seq showed a unique \( \gamma \delta \) T-cell subpopulation with a 13-gene pro-inflammatory signature, which could serve as a prognostic biomarker.\textsuperscript{83}

### 5 | Conclusions and Perspectives

Single-cell-based screening methods can provide a more comprehensive depiction of the multiple molecular features and gene regulation of a cell. By combining transcriptomics with proteomics and epigenomics, scientists can elucidate regulatory elements and transcription factors that affect gene expression, methylation, protein abundance and chromatin accessibility.\textsuperscript{84} The 10x platform enables the combination of scRNA-seq with other complementary technologies such as ATAC-seq, which uses a prokaryotic transposase to tag accessible regulatory regions with sequencing adaptors\textsuperscript{85} or CITE-seq,\textsuperscript{86} which measures cell surface protein levels by using oligonucleotide-labelled antibodies.\textsuperscript{87} This method can be particularly useful in better characterizing cutaneous immune cell populations that participate in multiple inflammatory signalling pathways. For instance, Liu et al. integrated scRNA-seq with CITE-seq data, identifying a strong correlation for transcript- and protein epitope-defined APC and T cells in response to skin inflammation.\textsuperscript{88} Despite their high accessibility, antibody-based approaches lack specificity and precision in protein targeting. Therefore, single-cell proteomics technologies, which combine chromatographic and mass spectrometry methods, have been developed. One of these technologies is SCoPE-MS, in which single cells are labelled with tandem mass tags (TMTs) and analysed together by liquid chromatography-tandem mass spectrometry.\textsuperscript{89} A new high-throughput single-cell proteomics version has been reported, called proteoCHIP, in which cells are isolated and pooled in nanowells placed on microscopic slides. This method has identified 2000 protein groups across 158 multiplexed single cells.\textsuperscript{90} These proteomic technologies can provide a more precise understanding of protein structure and function, which is required in the investigation of the many structural proteins that are present in the skin.\textsuperscript{91}

The spatial distribution of each cell in the organism can be interrogated by a method classified as spatial transcriptomics, with the aid of fluorescent probes conducting single-cell \textit{in situ} hybridization or by oligonucleotide barcoding prior to sequencing.\textsuperscript{23} This feature can be particularly useful in the dermatologic context since the relationships between gene expression and ultrastructural tissue regions can be deconvoluted, providing information about tumor microenvironment interactions.\textsuperscript{59} Cell fate during development and repair,\textsuperscript{23} and spatial composition of inflammation.\textsuperscript{92} For instance, spatial transcriptomics has been combined with ATAC-seq and scRNA-seq, providing information about the space and time of gene expression in mouse fibroblasts during the wound healing process.\textsuperscript{8} Other technologies facilitating the incorporation of multiple-omic approaches, include single-cell triple-omics sequencing (scTrio-seq),\textsuperscript{93} which combines genetic, epigenetic and transcriptomic profiling, as well as genome and transcriptome sequencing (G&T-seq).\textsuperscript{94} These technologies provide high-throughput omic readouts that could be applied to molecular medicine and dermatology research.

Going forward, with a multitude of -omics modalities and computational frameworks for integration being introduced on a regular basis, different areas of dermatological science stand to benefit from the combination of particular technologies. For example, the analysis of inflammatory skin conditions via scRNA-seq in conjunction with T-cell and B-cell sequencing and focused immunoproteomics, either with a method like CITE-seq or SCoPE-MS, could provide deeper insights into immune cell population activation state and reveal disease-associated cell subtypes. On the other hand, in skin cancers, the combination of scRNA-seq with spatial transcriptome sequencing could be advantageous in unravelling localized gene expression differences between the tumor microenvironment, tumor-adjacent tissue and healthy tissues, as well as in mapping the trajectory and cross-talk between malignant and non-malignant...
cells at distinct stages of cancer development, invasion and metastasis. As single-cell technologies evolve even further, we anticipate gaining an increasingly better understanding of the pathobiology of certain skin diseases and identifying key cell populations that play important roles in disease progression. Such progress offers scope to target therapeutically biologic pathways with new or repurposed drugs and develop better treatments for skin diseases.

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The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
G.T. and A.O. involved in conceptualization. A.O., S.T. and G.T. involved in investigation and writing—original draft. A.O. involved in supervision. G.T., A.V., J.A.M. and A.O. involved in writing—review & editing. S.T. involved in visualization.

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