Psoriasis is a common chronic inflammatory disease affecting about 1%-2% of today’s Western population. Several subtypes of psoriasis are known, but herein we focus on psoriasis vulgaris which is most prevalent and phenotypically characterized by sharply demarcated plaques covered with white scales. The pathogenesis of psoriasis is complex and exact mechanisms of disease onset and manifestation remain unknown. However, it is known that psoriasis cannot be attributed to a single risk factor or insult but is likely caused by the interaction of environmental and genetic factors. Based on a number of genomewide association studies (GWAS) and meta-analyses thereof, more than 60 susceptibility loci have been identified that account for 20%-25% of psoriasis heritability. Although psoriasis has been regarded as an (auto) immune disease for a long time, an increasing body of evidence directs towards a combined role for the innate and adaptive immune system in combination with tissue specific epidermal factors.
1.1 | Psoriasis hallmarks

Psoriasis skin has several typical histological features that should be taken into account when developing in vivo or in vitro models to study disease pathogenesis or therapeutics. First, acanthosis (epidermal thickening) is prominent and caused by a rapid keratinocyte turnover. Normally, it takes about 30 days for a keratinocyte to migrate from the basal layer to the skin surface. In psoriasis, this process only takes about 6-8 days and this contributes to the excessive scaling of psoriatic skin. Moreover, premature differentiation and incomplete cornification of psoriatic keratinocytes result in the retention of nuclei in the cornified layer, better known as parakeratosis. The granular layer can be reduced or even be absent in lesional epidermis and the expression of late differentiation genes and proteins is affected by the inflammatory milieu. The pathogenesis of psoriasis is complex and a multitude of studies attempted to decipher the typical gene and protein expression signature of psoriatic skin and to distinguish it from other inflammatory diseases, like atopic dermatitis. Initially, studies appeared showing the contribution of one or two molecules to diseases pathology, whereas during the last years large-scale transcriptomics and proteomics studies have been performed comparing healthy, non-lesional and lesional skin and thereby identify disease markers (differentially expressed genes and proteins). 

1.2 | Epidermal disease markers

In general, there are a few very robust differentially expressed genes that are additionally validated at the protein level using specific antibodies and have been replicated in many cohorts. In this paragraph, we will highlight widely used markers that have been used for the development of in vitro 3D psoriatic skin models.

The hyperproliferative epidermis is key for the development of the important disease hallmarks acanthosis, and excessive scaling of the skin. The efficacy of therapeutics for psoriasis patients is therefore also determined by the normalization of the proliferation index leading to a decrease in epidermal thickness. Positive staining with the Ki-67 antibody is widely used as a marker for proliferative cells. In lesional psoriatic skin, the number of Ki-67-positive cells is dramatically increased indicating the rapid turnover of basal keratinocytes. The Ki-67 antigen is encoded by the MKI67 gene and was first identified in 1983. Nowadays, accurate cell proliferation assays can directly quantitate newly synthesized DNA by following the incorporation of a deoxyribonucleoside analog that contains a detectable tag (eg EdU proliferation kit). 

Cytokeratin-16 (CK-16) is related to hyperproliferation and abnormal epidermal differentiation and was found to be overexpressed in psoriatic plaques. It is expressed in the suprabasal layers, and disappearance of CK-16 protein is related with clearance of disease. CK-16 is absent in normal epidermis and the appearance of CK-16 in psoriatic skin is accompanied by the loss of CK-10, which is normally present in suprabasal keratinocytes of healthy skin. The expression of antimicrobial proteins (AMPs) is strongly induced in psoriatic epidermis, and this specific expression can therefore be valuable in distinguishing psoriasis from other inflammatory skin diseases, like atopic dermatitis. Robust markers for psoriatic disease are human beta-defensin 2 (hBD2 or DEFB4), skin-derived antileukoprotease (SKALP/elafin or PI3) and S100A7 (psoriasin). Defensins are small cationic proteins with antibacterial activity that can be subdivided into α-, β- and θ-defensins. hBD2 is absent in normal skin but has been found in psoriatic epidermis, where it is expressed and secreted from the keratinocytes in the granular layer. SKALP/elafin is a protein that is localized in the suprabasal layer of the epidermis up to the stratum corneum. S100A7 is one of more than twenty members of the S100 protein family and is barely expressed in healthy keratinocytes. Lesional psoriatic skin shows increased levels of this S100A7, hence its nickname “psoriasin”.

It is, however, important to note that these markers may already be expressed in normal 3D skin models as they are generally upregulated in activated epithelia (eg upon wounding) and in early-stage 3D organotypic cultures. Therefore, it is vital to screen for multiple disease markers in the healthy control skin models to determine the best markers for monitoring the effect of disease-associated cytokines or cells in the respective models. Transcriptome analysis of the in vitro psoriasis models and comparison to metatranscriptomic data available from skin from psoriasis patients, and other inflammatory skin diseases could be valuable, yet costly, to extensively characterize and validate the psoriasis-like features of the 3D skin models. The use of not just one marker but a panel of disease-associated genes and proteins is instrumental for the validation of novel psoriasis models, both in vivo and in vitro.

1.3 | Immune cells and cytokines

Many immune cell subsets have been identified in psoriasis skin, and about every type of immune cell (amongst others: neutrophils, macrophages, dendritic cells, B-cells, plasma cells, T cells) has been found to play a role in the disease pathogenesis. Major contributors to the disease are considered the CD4+ T helper (Th)-cells in the dermis. Th1, Th17 and Th22 cells and the cytokines derived thereof contribute to a complex inflammatory milieu where typically IFNγ, TNFα, IL-17, IL-22 and IL-23 prevail. Current therapeutic strategies are designed to neutralize these cytokines or interfere in the signalling pathways that they activate. The so-called biologics that target TNFα (etanercept, infliximab, adalimumab), IL12/23 (ustekinumab) and IL-17 (secukinumab, ixikizumab) and small molecules targeting the downstream JAK-STAT pathway (tofacitinib) are effective treatments for moderate to severe psoriasis patients. Still, current research on therapeutics for psoriasis is greatly facilitated by animal models developed to mimic human psoriasis disease. Although the field has advanced considerably, and the biology has changed the lives of many psoriasis patients, there is still an unmet need for newly developed drugs and identification of...
drug targets in the treatment of psoriasis. In-depth knowledge on the disease pathogenesis, in part facilitated by in vitro skin models, will facilitate future drug development studies.

2 | MODELS TO STUDY PSORIASIS

As human in vivo studies have strong limitations due to practical and ethical reasons, experimental models for psoriasis are needed that faithfully mimic the disease. In general, there are two types of experimental models, in vivo animal models and in vitro cell/tissue culture models. Murine models showing psoriasiform inflammation have been extensively used and they vary from genetic (spontaneous mutation, transgenic or knockout models) and "ligand" induced models, like the widely used imiquimod model. Multiple reviews have summarized the animals models used to study psoriasis research, and to date, many of these models are still used for preclinical studies on newly developed therapies. However, besides the strong societal urge to reduce the use of experimental animals, there are ample scientific reasons to refrain from animal models in psoriatic research. We have come to learn that the skin, immune system and microbiome of rodents is significantly different from humans. Over the last decade, we and others have strongly invested in the development of in vitro 3D human skin models to faithfully mimic in vivo healthy and diseased skin. Nowadays, in vitro 3D skin models are widely used to study skin biology, disease pathogenesis and therapeutics and should be considered as the gold standard in experimental dermatology research and superior to the conventional monolayer cultures. The aim of this review is to provide a concise overview on the different types of in vitro psoriatic models present to date, and a viewpoint on the quality of the models and presence or absence of psoriasis-associated cells, phenotypical hallmarks and gene/protein expression signatures therein. The field should strive to use 3D skin models with a high-quality epidermis that faithfully mimics native human skin. We therefore made a selection of papers that present a histological assessment of the reconstructed epidermis and we evaluated which features of psoriasis skin are present in the respective models. For a complete overview of all in vitro reconstructed skin models that are used to study psoriasis pathophysiology, we highly recommend a very recently published review by Desmet et al.

2.1 | Conventional in vitro skin models

For the development of in vitro psoriatic skin models, one needs human keratinocytes to generate in vitro skin models. The development of human skin models rapidly evolved after the Rheinwald and Green laboratory described the isolation and clonal expansion of primary human keratinocytes using a mouse fibroblast feeder layer and their studies on keratinocyte differentiation in monolayer cultures. To study psoriasis pathogenesis, the addition of inflammatory mediators to the culture medium and co-culture with immune cells and/or fibroblasts has been reported. Drug screening models have also been developed by the addition of fetal calf serum or psoriasis-associated cytokines to keratinocytes and analysed for psoriasis-related gene and protein signatures. Simplicity, high throughput and reproducibility are major advantages of monolayer keratinocyte cultures. Yet, epidermal stratification is lacking in these models and important morphological features cannot be studied. To study psoriasis pathophysiology and for drug screening purposes, we therefore need models that better resemble the natural architecture and functions of human skin.

2.2 | Organotypic psoriasis skin models

Psoriatic 3D skin models can be constructed using several cell types or matrices depending on the purpose of the model and research question. The main building blocks to construct a psoriatic in vitro model are listed in Table 1, and herein, we have cited the studies that have developed and validated the models. In Table 2, the presence or absence of the most prominent psoriasis hallmarks and the gene/protein expression profile in each of these models are listed. The advantages, disadvantages and the main conclusion of the papers will be discussed in the paragraphs below.

2.2.1 | Patient-derived skin models

Mimicking a disease-specific phenotype in 3D organotypic models is a challenging task. Psoriasis is a multifactorial polygenic disease, and it is difficult to mimic all features of the disease in the in vitro environment. The study by Barker et al was the first to describe an in vitro skin equivalent model for psoriasis. Keratinocytes and fibroblasts from healthy volunteers and psoriatic patients were used to generate full-thickness skin equivalents. Although the morphology of the skin equivalents that time was quite poor, some striking differences were found between the psoriatic and normal skin equivalents. In the skin equivalent generated from psoriasis-derived cells, pro-inflammatory cytokine production and chemokine receptor expression were strongly induced and more proliferative keratinocytes were found. Based on this model, others have also used keratinocytes and fibroblasts from psoriasis patients in combination with cells from healthy volunteers to generate full-thickness skin equivalents. Herein, the presence of lesional keratinocytes and lesional fibroblasts induced a psoriatic phenotype characterized by epidermal thickening, increased involucrin expression and less filaggrin and loricrin expression. The commercially available psoriasis skin model from MatTek is generated from healthy foreskin keratinocytes and lesional psoriasis fibroblasts and shows morphological features of psoriasis, indicating that fibroblasts may be an important yet overlooked population in the development of psoriatic skin models.

The use of patient-derived skin cells also allows the functional analysis of genetic risk factors for psoriasis. The pathogenesis of psoriasis has a strong genetic component and GWAS studies identified a multitude of genes associated with disease development. Knowledge on the gene and protein function of these risk factors
provides important insight in the understanding of the pathogenesis and pathophysiology of psoriasis. One example of such studies is the recent identification of a novel function of the Late Cornified Envelope (LCE) genes. The deletion of the LCE3B/C genes is an epidermis-specific risk factor, and we found the LCE3B/C_del to result in a strong increase in the expression of the neighbouring gene, LCE3A. Furthermore, we found that LCE proteins, and LCE3A in particular, show broad-spectrum antimicrobial activity. This discovery opens up a complete new avenue for research on the possible involvement of the microbiome in the pathogenesis of psoriasis. The use of patient-derived skin cells to generate an in vitro psoriasis model seems attractive; however, the amount of patient-derived cells from a limited number of biopsies hampers large-scale production. Genome editing techniques, like CRISPR-Cas9, may be important tools for future studies on genetic risk factors for psoriasis by introducing single or multiple risk factors in normal keratinocytes. This approach would require the availability of human immortalized keratinocyte cell lines as extensive subculturing is required to obtain genome edited clonal cell line. The aneuploidy of HaCaT cells renders them less suitable for genome editing studies. The recently described N/TERT cell line may provide a good alternative as the cells are diploid and faithfully mimic primary keratinocytes in their response to cytokines and can be successfully used for the development of organotypic skin models. The most recently, two immortalized keratinocyte cell lines, N/TERT-1 and N/TERT-2G, were described as a useful alternative for primary keratinocytes in 3D equivalents. Herein, Th1 (TNFα, IL-1α, IL-6) or Th17 (IL-17 and IL-22) cytokines were used to stimulate epidermal equivalents to mimic a psoriasis phenotype. The phenotype was comparable to that of primary keratinocyte models. All-trans retinoic acid (ATRA) treatment, an anti-psoriatic drug that acts...
primarily at the level of the keratinocyte, restored the expression and morphology to that of normal skin. Therefore, the N/TERT cell lines are considered a valuable tool to study the biology as well as treatment options of psoriatic skin.\[58\]

2.2.3 | Co-culture psoriatic models with immune cells

As the interaction between keratinocytes and immune cells is vital in the pathophysiological process and development of psoriasis, researchers attempted to study skin and immune cell interaction in the context of psoriasis using conventional monolayer cell co-cultures.\[49-51\] In these conventional models, there is a direct interaction possible between immune cells and keratinocytes or in case of transwell cultures, the cells are physically separated by a plastic membrane.

The incorporation of T cells in normal human skin equivalents allowed us to study this interaction in a tissue micro-environment.\[62\] In the 3D skin T-cell model, activated CD4+ T cells secreting a psoriasis-associated repertoire of pro-inflammatory cytokines (IFN\(\gamma\), IL-6, IL-8, TNF\(\alpha\), IL-22, IL17) were added to full-thickness skin equivalents grown on de-epidermized dermis. This led to an inflammatory and activated phenotype of the epidermis as shown by the induction of psoriasis-markers: hBD2, SKALP/elafin, S100A7, CK-16 and others. Similar effects were observed when T cells were polarized towards a Th1 or Th17 subtype.\[62\] Important to note is the absence of morphological hallmarks like acanthosis, parakeratosis or hyperproliferation in this model. One major asset of this model would be the use of patient-derived keratinocytes and immune cells and this could be an interesting window of opportunity for future studies.

Besides T cells, many other immune cells have been implicated in the pathogenesis and disease progression of psoriasis. However, inclusion of immune cells in 3D skin models is still limited and challenging. Recently, the successful integration of Langerhans cells (LCs, MUTZ-3 cell line) into a full-thickness 3D normal skin model was reported.\[63\] LCs remained functionally active and showed in vivo like mechanisms with regard to cell activation and migration in the 3D skin model. As LCs may be important players in psoriasis,\[64\] and CD1a on LCs was recently shown a potential therapeutic target for skin inflammation,\[65\] the future incorporation of LCs in psoriasis skin models would provide a relevant research tool to study the involvement of LCs in psoriasis under controlled in vitro conditions.

### TABLE 2 3D psoriasis models: morphological hallmarks and expression signatures

| Reference         | Cellular components               | AC | PK | HP | Keratinocyte expression                        |
|-------------------|-----------------------------------|----|----|----|-----------------------------------------------|
| Barker\[55\]     | Epidermis: NS/PS KCs, Dermis: collagen NS/PS Fibs | no | no | yes| ↑ protein TNF\(\alpha\), IFN\(\gamma\), CXCR2, IL-8 |
| Boniface\[40\]   | Epidermis: NS KCs, Immune: cytokines | yes | no | no | ↑ mRNA KRT6, CXCL5, PDGF-A, S100A7/8/9 ↓ mRNA IVL, LOR, FLG, Hsp, calmodulin-related protein NB-1, HO-1 ↑ protein S100A7 ↓ protein FLG, CK-10 |
| Sa\[61\]         | Epidermis: NS KCs, Immune: cytokines | yes | yes | no | ↑ mRNA KRT16, S100A7, CXCL1/8/20, CCL2, DEF4B ↑ protein KRT16, S100A7, STAT3, pY-STAT3, IL-8, MIP3\(\alpha\) |
| Nograles\[60\]   | Epidermis: NS foreskin KCs, Dermis: collagen, NS Fibs, Immune: cytokines | yes | yes | no | ↑ mRNA DEFB4, CCL20, CXCL8, S100A7 |
| Tjabringa\[59\]  | Epidermis: NS, Dermis: DED, Immune: cytokines | no | no | no | ↑ mRNA SKALP/Elafin, DEFB4 ↑ protein SKALP/Elafin, hBD2, CK-16, TNF\(\alpha\), IL-8 ↓ protein CK-10 |
| Jean\[56\]       | Epidermis: NS/PS KCs, Dermis: NS/PS Fibs | yes | yes | yes| ↑ protein IVL ↓ protein FLG, LOR, laminin |
| van den Bogaard\[62\] | Epidermis: NS KCs, Dermis: DED, Immune: T cells | no | no | no | ↑ mRNA DEFB4, SKALP/elafin, LCE3A, KRT16, S100A7/8 ↑ protein IL-6, IL-8, IL-23, CXCL10, hBD2, CK-16, SKALP/elafin, IVL ↓ protein FLG |
| Smits\[58\]      | Epidermis: N/TERT KCs, Immune: cytokines | no | yes | no | ↓ mRNA FLG, LOR ↑ mRNA IVL, SKALP/Elafin, hDB2 |

AC, Acanthosis; DED, de-epidermized dermis; Fibs, fibroblasts; HP, Hyperproliferation; KCs, keratinocytes; NS, normal skin; PK, Parakeratosis; PS, psoriatic skin.

Of note: the MatTek psoriasis model is not included in this table as no peer-reviewed publications are available on the development of this model. For more information, we refer to the website of MatTek: https://www.mattek.com/products/psoriasis/
2.2.4 | 3D skin models including endothelial cells

In 2014, Ayata et al.[66] included endothelial cells within the dermal compartment of their self-assembly psoriasis skin model to generate branch-like capillary structures. Although epidermal psoriasis features were not studied herein and differences with regard to the formation of the capillary structures were minor, "vascularized" models may enable the study on the contribution of pro-angiogenic factors in lesional psoriatic skin and may facilitate anti-angiogenic drug development.

3 | THE PERFECT IN VITRO PSORIASIS SKIN MODEL: ARE WE THERE YET?

For more than three decades both academic and industry-related research groups have dedicated their time and effort to the development of in vitro skin models for psoriasis. Many approaches have been published, all with the same goal of generating the perfect 3D skin model that faithfully mimics psoriasis skin. But what determines a perfect model? Is that the use of patient-derived cells, the presence of all cells and immunological factors involved in the psoriasis pathophysiological process, or a model that is amenable for medium to high-throughput analysis and suitable for drug screening assays? The answer lies within the specific aims of the study, read-out parameters needed to answer the research question and preferences of the scientist leading the project. This viewpoint and the recent review on all in vitro psoriasis skin models present to date,[36] guides researchers in finding the best model present to date for their research project. However, we also must admit that the psoriatic phenotype of the available models still needs improvement to completely replace in vivo experimental models of psoriasis. We would advocate that efforts should be directed to identify relevant keratinocyte mitogens and to incorporate these in the 3D skin models. This could be achieved by addition of these mitogens (or combinations thereof), or by application of other cells (immunocytes, endothelial cells, fibroblasts) that are a source of these elusive mitogens. Yet, the later may be more difficult to achieve.

One important aspect that requires attention is whether we should continue to strive for complexity by generating models including keratinocytes, fibroblasts, immune cell subsets, nerves, microbiota or vasculature. As every cell type has its own culture requirements, and immune cells for example typically do not function well in a culture medium designed for keratinocytes and vice versa, perhaps should we honour the beauty of simplicity and design a research pipeline by combining simple mono-cultures in a multi-step approach? For such purpose, the skin-on-a-chip technology could provide a standardized, dynamic, and medium to high-throughput model. This technology enables a dynamic cell culture on a microfluidic device that can be constructed of several layers separated by transparent, porous membranes to allow cellular communication and interaction to study inflammation and drug treatment.[67,68] Ultimately, in vitro models for psoriasis will certainly refine and reduce experimental animal models but perhaps may never fully replace them.

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CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

EB initiated and supervised the study. HN and EB wrote and edited the manuscript.

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REFERENCES

[1] E. Christophers, Clin. Exp. Dermatol. 2001, 26, 314.
[2] L. C. Tsoi, S. L. Spain, J. Knight, E. Ellinghaus, P. E. Stuart, F. Capon, J. Ding, Y. Li, T. Tejasvi, J. E. Gudjonsson, H. M. Kang, M. H. Allen, R. McManus, G. Novelli, L. Samuelsson, J. Schalkwijk, M. Stahle, A. D. Burden, C. H. Smith, M. J. Cork, X. Estivill, A. M. Bowcock, G. G. Krueger, W. Weger, J. Worthington, R. Tazi-Ahnini, F. O. Nestle, A. Hayday, P. Hoffmann, J. Winkelmann, C. Wijmenga, C. Langford, S. Edkins, R. Andrews, H. Blackburn, A. Strange, G. Band, R. D. Pearson, D. Vukcevic, C. C. Spencer, P. Deloukas, U. Mrowietz, S. Schreiber, S. Weidinger, S. Koks, K. Kingo, T. Esko, A. Metspalu, H. W. Lim, J. J. Voorhees, M. Weichenthal, H. E. Wichmann, V. Chandran, C. F. Rosen, P. Rahman, D. D. Gladman, C. E. Griffiths, A. Reis, J. Kere, Collaborative Association Study of Psoriasis (CASP); Genetic Analysis of Psoriasis Consortium; Psoriasis Association Genetics Extension; Wellcome Trust Case Control Consortium 2; R. P. Nair, A. Franke, J. N. Barker, G. R. Abecasis, J. T. Elder, R. C. Trembath, Nat. Genet. 2012, 44, 1341.
[3] L. C. Tsoi, S. L. Spain, E. Ellinghaus, P. E. Stuart, F. Capon, J. Knight, T. Tejasvi, H. M. Kang, M. H. Allen, S. Lambert, S. W. Tost, S. Weidinger, J. E. Gudjonsson, S. Koks, K. Kingo, T. Esko, S. Das, A. Metspalu, M. Weichenthal, C. Ennerback, G. G. Krueger, J. J. Voorhees, V. Chandran, C. F. Rosen, P. Rahman, D. D. Gladman, A. Reis, R. P. Nair, A. Franke, J. N. Barker, G. R. Abecasis, R. C. Trembath, J. T. Elder, Nat. Commun. 2015, 6, 7001.
[4] X. Zuo, L. Sun, X. Yin, J. Gao, Y. Sheng, J. Xu, J. Zhang, C. He, Y. Qiu, G. Wen, H. Tian, X. Zheng, S. Liu, W. Wang, W. Li, Y. Cheng, L. Liu, Y. Chang, Z. Wang, Z. Li, L. Li, J. Wu, L. Fang, C. Shen, F. Zhou, B. Liang, G. Chen, H. Li, Y. Cui, A. Xu, X. Yang, F. Hao, L. Xu, X. Fan, Y. Li, R. Wu, X. Wang, X. Liu, M. Zheng, S. Song, B. Ji, H. Fang, J. Yu, Y. Sun, Y. Hui, F. Zhang, R. Yang, S. Yang, X. Zhang, Nat. Commun. 2015, 6, 6793.
[5] M. Kamsteeg, P. A. Jansen, I. M. van Vlijmen-Willems, P. E. van Erp, D. Rodijk-Olthuis, P. G. van der Valk, T. Feuth, P. L. Zeeuwen, J. Schalkwijk, Br. J. Dermatol. 2010, 162, 568.
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