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Advancements in Human Skin Models: From Basic Constructs to Disease-Specific Platforms for Drug Discovery and Therapeutics

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ABSTRACT

Skin serves as a major barrier to protect the body from physical, chemical, and pathological risks as well as adjust the transportation of two-way ions and nutrients. To improve the structure and function of skin as well as skin diseases, animal experience is often used, but the difference between surgery and physiology can cause poor animal data in clinical situations. *In vitro*, models such as the rebuilt epidermis of humans or the equivalent skin are valuable alternatives for animal experiments. There is now a greater demand for alternative *in vitro* platforms that replicate the structural and functional characteristics of natural skin due to ethical issues and genetic variations in traditional animal investigations. *In vitro* skin modelling has advanced significantly in recent decades; however, to replicate the pathological characteristics of diseased human skin, distinct repeatable bio-fabrication techniques are needed in comparison to those employed for healthy-skin models, the structural and functional features of healthy human skin to explain human skin modelling with disease markers. detail on how to replicate diseased human skin models *in vitro*, such as models for atopic, diabetic, skin-cancer, injured, and other pathological skin types. an outlook on diseased-skin modelling and its technical perspective for the further development of skin engineering.

Keywords: *in-vitro skin modelling, diseased human skin, bio fabrication techniques, skin engineering.*

1. INTRODUCTION

The largest organ in the body is the skin. With an exterior surface area of 1.8 m^2 in adults, skin makes up around 16% of our total weight. The epidermis and dermis are the two main layers of the skin, and there is also a third layer known as the hypodermis. Every layer has distinct compositions, structures, and cooperative roles. Every layer has a different mix of cells and matrices. (Runxuan Cai, 2022).The skin's complex, hierarchical, and stratified structure controls the movement of water and small metabolites out of the body while acting as a physical barrier to prevent xenobiotics from entering the body. Wounds that result from chemical or physical harm have the potential to seriously weaken the skin's protective layer and affect its physiological processes.In instances in which a considerable amount of the skin has been lost to injuries, it becomes critical to replace the impaired skin via grafts to protect water loss from the body as well as to mitigate the risk posed by opportunistic pathogens. (I. Risueno, 2021)

The epidermis and dermis are the two main layers of the skin, and there is also a third layer known as the hypodermis. Every layer has distinct compositions, structures, and cooperative roles. Every layer has a different mix of cells and matrices. Melanocytes, Merkel cells, Langerhans cells, keratinocytes, and inflammatory cells constituents of the epidermis. The epidermis supports temperature control, hydration, and defence against environmental harm. The thickness of the epidermis varies depending on the bodily part. (Elisabeth Hofmann, 2023).The eyelid's epidermis is the thinnest, measuring only 0.5 mm in thickness. The thickest epidermis, found on the palms or soles, is around 1.5 mm thick. Between the epidermis and hypodermis is the dermis. Fibroblasts, its primary cell type, generate elastin and collagen fibers that run parallel to the skin's surface. The skin has exceptional elastic qualities because to these matrix constituents. (Kristy Derr, 2018). The dermal layer also contains a variety of appendages, such as sweat glands, sebaceous glands, hair follicles, blood vessels, and sensory nerves. In the deepest layer, the hypodermis—also referred to as subcutaneous adipose tissue—connects the skin to the bone or muscle. The adipocytes that maintain energy balance are tightly packed in this highly vascularized tissue. It serves as a nutritional store and a mechanical pressure buffer. (Runxuan Cai, 2022). shown very high antiviral activity against many viruses infecting different crops [Verma *et al*., 1979 (a, b); Chaubey, 2014]. suspension in tap water (1:10). The pulp was strained through two folds of cheese cloth and the homogenate was clarified by centrifugation at 3000g for 15 minutes. The supernatants were used for experimental works. Tubers of “Kufri Ashoka”

Drug administration through the skin shows potential. For both localized and systemic administration, this calls for research on how medications affect and pass through the skin. Skin models of humans and animals have been used worldwide to study the toxicity and effectiveness of topical medications. Securing a significant quantity of removed skin, however, can occasionally be difficult and expensive.

2. SKIN STRUCTURE AND FUNCTIONS

2.1 Epidermis

Although it contains other, less common cells like melanocytes, which produce the pigment melanin, Langerhans, which are involved in an immune response, or Merkel cells, which are involved in tactile sensation, the epidermis is the outermost stratified squamous keratinized layer of the skin made up of cells called keratinocytes. It controls the integrity and function of the underlying connective tissue and serves as a crucial barrier between the organism and its surroundings, shielding it from microbial, chemical, and physical harm. (I. Risueno, 2021). Secondary structures including sebaceous or sweat glands, nails, and hair follicles are created by the epidermis' constant renewal. Because of keratinocyte differentiation and cornification, the epidermis is further separated into four layers: the squamous layer (stratum spinosum), granular layer (stratum granulosum), cornified layer (stratum corneum), and basal layer (stratum germinatum). Type IV and VII collagens, laminin, and other proteins including fibronectin, perlecan, nidogen, and proteoglicans make up the basement membrane, also known as the dermo-epidermal junction, which permits proper material exchange. (Elisabeth Hofmann, 2023).

2.1 Dermis

The dermis is the bigger layer of the skin and provides important physical properties, such as flexibility, elasticity, and tensile strength.(Runxuan Cai, 2022)In contrast to the epidermis, the dermis is comparatively acellular. Elastic fibers (elastin) type I and type II collagens make up the majority of this intricate network of fibrous connective tissues. The primary purpose of elastin is to provide the skin resilience and suppleness, whereas collagen fibres primarily offer tensile strength. Because of its role in skin hydration, hyaluronic acid is also a crucial part of the dermal extracellular matrix (dECM). The composition, distribution, and arrangement of collagen, elastin, and hyaluronic acid are not consistent and are always changing and remodelling. The loss of skin elasticity brought on by aging is significantly correlated with the disarray and depletion of these key functioning proteins.(I. Risueno, 2021)

2.2 Hypodermis

The hypodermis, also known as the subcutaneous layer, is the deepest layer of skin made up of fat cells. It is situated between the skin and the body's subcutaneous structures, including muscles and bones, and directly underneath the dermis. (Runxuan Cai, 2022)

The hypodermis has more blood arteries and nerves than the dermis and is made up of loose, well-vascularized connective tissues that link the skin to nearby organs. It is mostly made up of macrophages, fibroblasts, and adipocytes. It is the thickest layer of skin and ranges in thickness from 1.9 to 7.1 mm, depending on the gender of the individual and the area of the body. (I. Risueno, 2021) It is regarded as an endocrine organ that contributes significantly to thermal homeostasis by providing buoyancy, energy storage, and hormone conversion.

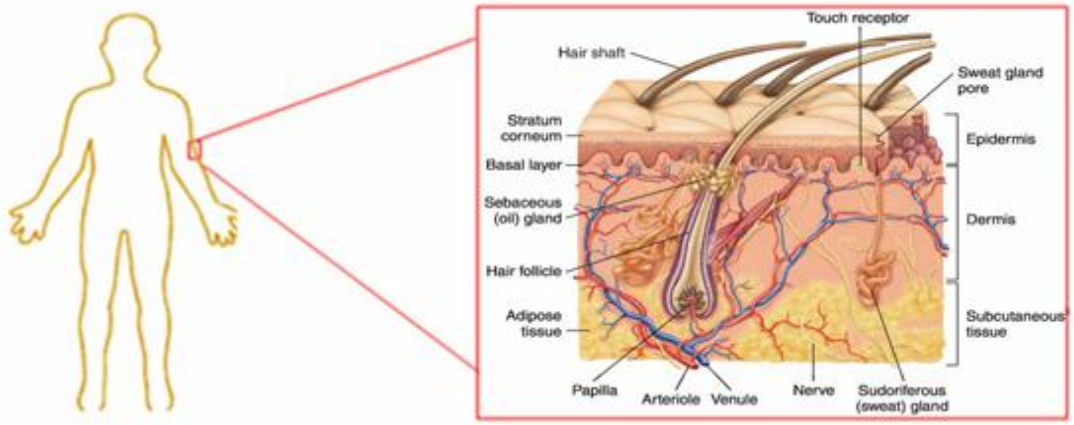

Fig 1. Structure of human skin. The skin layers include epidermis, dermis, and hypodermis (subcutaneous tissue). (Runxuan Cai, 2022)

3. TYPES OF HUMAN SKIN MODELS

3.1 Basic skin constructs

Reinwald and Green originally observed the serial culture of human keratinocytes in monolayer culture over thirty years ago. Monolayer keratinocyte cultures are two-dimensional *in vitro* models that are often employed in pharmaceutical and dermatological research. They offer an easy and affordable tool to research medication interactions, wound healing processes, and keratinocyte biology. It is possible to separate Normal Human Epidermal Keratinocytes (NHEK) from adult normal human tissue from a variety of places, such as the face, breast, belly, and thighs, or from juvenile foreskin.(Julia Bajsert, 2024). NHEK from either a single donor or a pool of donors might be used for research. To provide the greatest circumstances for keratinocyte proliferation and differentiation, the culture's calcium concentration must be at its ideal level. (Elisabeth Hofmann, 2023)

3.1.1 Epidermal models

In vitro or *ex vivo* models of the epidermis are used to investigate skin biology, drugs penetration, toxicity, wound healing, and disorders of the skin. An ethical and regulated substitute for animal experimentation, these models replicate the composition and functionality of the human epidermis. There are a growing number of *in vitro* studies where researchers focus on the human skin or epidermis because of a number of practical restrictions in in vivo investigations of the human epidermis. *In vitro* procedures employing removed human or animal skin samples have been created for this purpose and are incorporated into OECD guideline 428. (I. Risueno, 2021)

3.1.2 Reconstructed human epidermis models

In vitro models of the human epidermis known as "reconstructed human epidermis" (RHE) are made of a polycarbonate membrane in which epidermal keratinocytes are cultivated at the air–liquid interface and successfully differentiate and cornify to create epidermal sublayers. Developed and validated as skin corrosion testing models by the European Centre for the Validation of Alternative Methods in the 1990s, the first RHE models were EpiSkin® (L'Oreal, Lyon, France) and EpiDerm® (MatTek Corp., Ashland, MA, USA). Histological features of RHE cultured in cdAOF (chemically defined animal origin-free) media were similar to those produced using human keratinocyte growth supplement, except that the basal keratinocytes were less cylindrical. Immunolocalization of involucrin in the basal layer and increased mRNA expression of several inflammatory-proliferative markers were also observed under cdAOF conditions. Other expected markers of keratinization were similarly expressed and immunolocalized in RHEs cultured in cdAOF media, and measurements of barrier function (transepithelial electrical resistance) showed results that were statistically equal to, or lower than those found in RHE cultured in human keratinocyte growth supplement. (Julia Bajsert, 2024)

The HSEs (human skin equivalent) are produced similarly to RHEs by briefly incubating them under submerged culture conditions prior to air-lift and the start of keratinocyte differentiation. Additionally, several standardized HSEs are offered for sale by various businesses. It was demonstrated that fibroblasts had a favourable effect on the differentiation and

proliferation of keratinocytes. Furthermore, it was demonstrated that a well-structured basement membrane depends on paracrine communication between fibroblasts and keratinocytes.

3.1.3 Organotypic skin models (full thickness model)

It is possible to develop complex biological tissues *in vitro* in a way that mimics some of their natural physiology and function by using an organotypic culture system (OCS). The epidermis and other skin constituents, including hair follicles, may be easily preserved *in vitro*, which is why OCSs have become widely used in dermatological research. OCS cells are able to perform complex behaviours, like growing new hairs, which necessitates intricate coordination of cell division, differentiation, and migration, in contrast to traditional "on-a-plastic" cultures, where individual dissociated cells rapidly lose all but a few of their original in vivo values. The capacity of OCSs to replicate the three-dimensional stratified space that skin cells typically inhabit and act in in vivo is its primary advantage over conventional "on-a-plastic" systems. In its most basic form, the skin is made up of a stratified epidermis on top of a collagen-rich stroma that is dominated by fibroblasts. (ji won oh, 2013)

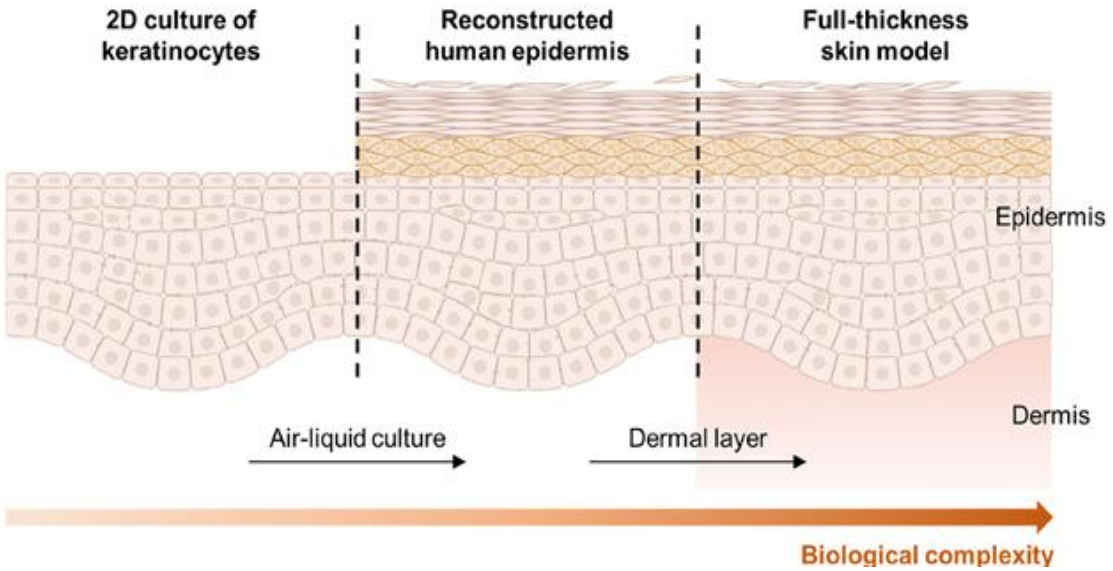

Fig 2. Advancement of an in vitro human skin model based on the tissue-engineering approach (Minjun Ahn, 2023)

3.2 ADVANCED 3D SKIN EQUIVALENTS

A typical additive method for creating volumetric items is three-dimensional (3D) printing, which involves merely layering pertinent elements on top of one another. It can create more complicated geometries than older technologies with an ever-expanding array of materials, and it usually has minimal fixed setup costs and fast speed, which appeal to many engineers. (Ahn Minjun, 2023)More recently, a new in-house reconstructed human epidermis (RHE) model for *in vitro* skin irritation assays was developed with high similarity to human epidermis. This system was further compared with a full-thickness skin mimetic model to evaluate the performance in animal tests for skin irritants, and both models revealed capacity to correctly classify the tested compounds according to their corrosive nature. 3D skin equivalents have been described as a model composed of fibroblasts and keratinocytes grafted on a viscose rayon support to test potential skin irritants.(Tânia Moniz, 2020).

3.2.1 Organoid based approach

Using three-dimensional growth conditions, organoid technology includes cultivating pluripotent stem cells and other cell types *in vitro* and causing them to self-organize. This approach results in the creation of constructs that may mimic certain physiological processes and have the three-dimensional structure of real organs. It offers an innovative experimental approach for examining skin conditions. Advances in tissue regeneration and repair are accelerated because it makes it possible to replicate diseased conditions *in vitro* and provides insights into tissue healing mechanisms. (Runji Zhou, 2025)

3.2.2 Bioprinting technologies

A new bioengineering approach called three-dimensional (3D) bioprinting has a significant chance to increase production and enhance the accuracy of conventional tissue engineering techniques(Kristy Derr, 2018).New and more accurate human skin models are still needed, even with the significant advancements in *in vitro* lipid- or cell-based models. Thus, it has been stated that bioprinted skin may be produced in accordance with the latest developments in 3D bioengineering

technology. The skin is not an exception to the numerous tissue and organ models that have been created. Nowadays, bioprinting is seen as a potential technique for creating skin-like materials since it enables the creation of multilayered and multicellular systems. The architecture of the skin model may be regulated with excellent reproducibility thanks to these novel techniques, which include computer-controlled deposition of skin cells and matrix polymers in spatially controlled patterns(Tânia Moniz, 2020).Furthermore, by employing numerous printing heads loaded with different cell-rich bioinks, 3D bioprinting technology makes it possible to create a heterogeneous skin model that includes the dermis and epidermis. In order to create physiologically biomimetic skin models, this technique spatially regulates the deposition of bioinks that include cells along with the necessary proteins, growth factors, and other bioactive compounds (Ahn Minjun, 2023).

4. DISEASE SPECIFIC SKIN MODELS

Treatments for skin conditions can be applied directly to the skin. Although it is evidently crucial to evaluate the impact of drugs on skin conditions, there is a limited supply of excised human skin that has been diseased, mostly because of the growing regulatory limits on the usage of both people and animals. Models of healthy skin are unable to capture the changed physiological and morphological traits brought on by illness. As a result, creating models of skin diseases is a difficult task. One potential approach to creating a design that incorporates the necessary illness features is to alter the existing mimetic models for healthy skin. Skin pigmentation changes, photodamage (photodermatitis), inflammatory conditions (psoriasis and atopic dermatitis), cutaneous wounds, and skin cancer (melanoma) have all been represented by models(Tânia Moniz, 2020).Three-dimensional modelling has demonstrated a great deal of promise in reproducing in vivo cell fates. The choice between a 2D and 3D model can have a big influence on a study's cell survival, mechanical responses, differentiation, and proliferation. According to the Nuremburg Code, preclinical studies must use animal models; nevertheless, there are concerns over the accuracy of these models when it comes to simulating human systems. Many cases of notable disparities in the display of symptoms seen in animal models and people have been reported in transgenic animal models intended to replicate human genetic disorders.(Diana Nicole Stanton, 2022).

4.1 Skin cancer models

The skin is exposed to UV radiation because it is essential for shielding the internal organs from it, but this exposure can lead to skin cancer, including melanoma. Over the past few decades, melanoma, one of the most aggressive and lethal types of skin cancer, has become more common.(Ahn Minjun, 2023). Melanoma and other common skin malignancies, including squamous cell carcinoma and basal cell carcinoma, have been simulated in mice using skin models. In order to investigate the possible efficacy of anti-cancer medications, SKH1 hairless mice's skin is subjected to UVB irradiation in research that detailed a squamous cell carcinoma mouse model. The impact of several medications in the treatment of squamous cell carcinoma was also examined using this mouse model. A hairless mouse model that spontaneously develops cutaneous malignant melanoma has been reported, and other mouse models were created to evaluate the impact of anti-tumour signalling inhibitors on the pathways of basal cell carcinoma. Additionally, xenograft models were used to evaluate the activity of possible anti-melanoma medications.

The creation of *in vitro* skin cancer mimetic models can be challenging as it involves integrating different tumor entities into a three-dimensional skin system to mimic interactions between cells and the extracellular matrix. A 3D model of human skin was created using cultured melanocytes as sample examples, and a metastatic melanoma was created using collagen type I, normal human-derived dermal fibroblasts, normal human-derived epidermal keratinocytes, and melanoma cells (A375). A skin squamous carcinoma mimetic model was later proposed, which included normal human-derived dermal fibroblasts, normal human-derived epidermal keratinocytes, collagen type I, and squamous carcinoma cell lines (SCC12B2 and SCC13 cell lines). (Tânia Moniz, 2020).

4.2 Psoriatic models

An example of a psoriasis model is the creation of epidermal VEGF receptor knockout mice, which are utilized to pinpoint a particular function of epidermal VEGF in preserving permeability barrier homeostasis. These mice might be regarded as an intriguing model since they also exhibit an elevated proinflammatory state due to the overexpression of IL-1α in their epidermis. Mice lacking the IL-1 receptor antagonist showed signs of an inflammatory response akin to those seen in psoriatic skin in humans, and these mice have been used as a viable model for psoriasis.It has been reported that cell-based *in vitro* systems, which are often created inside, may replicate compromised skin. The majority of models, such as those created to represent psoriasis, have been created to resemble inflammatory skin conditions(Tânia Moniz, 2020).

4.3 Wounded skin model

External damage to the skin, including the epidermis and/or dermis, caused by physical stimuli such burns, radiation exposure, and blunt trauma is one of the most common skin illnesses. Through haemostasis, growth, re-epithelialization, and remodelling by skin-constituting cells, normal skin aids in wound healing in the lesion; if not, the injured skin's compromised barrier functions may result in a secondary infection. It is possible to assess wound-healing processes using woundedskin modelling. Dermal fibroblasts and/or epidermal keratinocytes were cultured on two-dimensional culture plates to create the simplest models of injured skin. This technique, known as the *in vitro* wound scratch assay, used mechanical (pipette tip,

cell scraper, metallic micro-indenter, and toothpick), optical, electrical (electric cell-substrate impedance monitoring), and heat instruments to produce a cell-free area in a confluent cell monolayer.

Even while 2D-based bio fabrication techniques for models of damaged skin have given us a basic grasp of how wounds close through cell migration and proliferation, 2D systems are not very good at simulating the structural similarities of the skin or real 3D-based physiological responses in vivo. Additionally, compared to cells cultivated in 2D circumstances, those cultivated in 3D have notably distinct morphologies, cell–matrix/cell–cell interactions, and migratory behaviours. In light of this, several attempts have been undertaken to create three-dimensional (3D) models of damaged skin that have a bilayer structure with dermal and epidermal compartments(Ahn Minjun, 2023).

4.4 Diabetes-skin model

High blood glucose levels are a symptom of diabetes mellitus, also commonly referred to as diabetes. The hormone insulin moves blood glucose into cells in a healthy body so that it can be stored or used as fuel. However, a body with diabetes is either unable to use insulin efficiently or does not produce enough of it. The American Diabetes Association advises that skin issues are the first apparent symptom of diabetes mellitus. Patients with diabetes generally have a number of skin issues, including fungal infections, diabetic dermopathy, rash, blistering, and skin itching. These are outcomes of impaired blood circulation owing to elevated sugar levels in the blood stream. Skin-cell processes such as cell division, extracellular matrix remodelling, and self-healing (re-epithelialization) are all hampered by a reduced blood supply.(Ahn Minjun, 2023). Kim et al. developed an *in vitro* skin equivalent for type 2 diabetes. The conventional technique of therapeutic development for type 2 diabetes was to employ animal models. However, there were a lot of unavoidable issues with animal models.

In order to produce an artificial skin model for type 2 diabetes research, this study employed 3D bioprinting. By using 3D cell printing of the primary skin layer and physiological phenomena found in genuine patient skin, they were able to replicate the skin of patients with type 2 diabetes. The majority of the cells used in this investigation were from donors with type 2 diabetes and normal human cells. The skin models were made using methods based on extrusion and inkjet. To create the structure of artificial skin equal to or more comparable to that of the diabetic skin model, they added perfusion vascularized subcutaneous tissue of diabetes using 3D printing, allowing the features of diabetic skin to become more important. In the artificial diabetes models, adipocyte hypertrophy, inflammatory response, and sluggish re-epithelialization were seen. (Runxuan Cai, 2022).

4.5 Atopic dermatitis model

One of the most widely used in vivo models for atopic dermatitis is the NC/Nga mice. When these animals are kept in normal surroundings, they acquire skin lesions on their own that are quite similar to those that humans have. The flaky tail mouse is another in vivo model of atopic dermatitis, exhibiting abnormalities in the genes responsible for the development of atopic-like skin lesions. The significance of this receptor as a possible therapeutic target for atopic dermatitis was further demonstrated by the results of experiments employing histamine H4 receptor knockout mice, which were created as a model for the condition. Atopic dermatitis characteristics in several of these models, however, differed from those of the real condition.

Furthermore, mimetic models of *in vitro* atopic dermatitis were described, such as the 3D RHE model, which was utilized to examine the expression of filaggrin in atopic patients' epidermis. To simulate atopic dermatitis, a different impaired rebuilt epidermis model has been created. To simulate atopic dermatitis, a 3D model comprising human keratinocytes, memory-effector CD45 (RO+) T lymphocytes, collagen type I, fibronectin, and human foreskin fibroblasts was also published. A full-thickness skin model that resembles photodermatitis illness has been developed to investigate the relationship between UV light exposure and the development of wrinkles and discoloration process(Tânia Moniz, 2020).

4.6 Psoriatic skin model

A significant number of genetically modified mice were created to serve as models for skin conditions; specifically, several of these animals have been investigated as in vivo psoriasis models. The creation of mice lacking the epidermal VEGF receptor, for instance, is regarded as a psoriasis model and is utilized to pinpoint a particular function for epidermal VEGF in preserving permeability barrier homeostasis. Since these factors are crucial for the development of epithelial cells, psoriasis-like skin has been observed in mice with proteins knocked down. These mice can be regarded as an intriguing model since heightened proinflammatory conditions are caused by over-expression of IL-1α in the mouse epidermis. Mice lacking the IL-1 receptor antagonist had an inflammatory response akin to that seen in psoriatic skin in humans, and these mice have been regarded as a valuable psoriatic model.(Tânia Moniz, 2020).

4.7 Skin ageing

The process of skin aging occurs when the quality of the skin declines with age as a result of the combined impacts of environmental factors, hormonal deficiencies, photoaging, and chronological aging. There are four different types of skin aging, including intrinsic ageing, which is characterized by unblemished, pale, drier, less elastic skin with fine wrinkles. This occurs within the tissue itself through reductions in dermal mast cells, fibroblasts, and collagen production. In this case, there is a decrease in the number of fibroblasts that synthesize collagen and vessels that supply the skin, which increases laxity

and consequently causes wrinkles. Excessive sun exposure and other exogenous substances, such pro-oxidants and antioxidants, affect cell turnover through neuro-endocrine-immune biological responses, which results in extrinsic aging.

The pathophysiology can be explained by the theory of cellular senescence or deprivation of the cellular DNA repair capacity and loss of telomeres, point mutations of extranuclear mitochondrial DNA, oxidative stress, chromosome abnormalities, single-gene mutations, reduced sugar, chronic inflammation, and many other factors. According to research, approximately 3% of ageing factors are intrinsic, and extrinsic factors account for the majority of skin aging(Manupriya Chaudhary, 2019).

4.8 Vitiligo skin model

A common skin condition called vitiligo is brought on by the depletion of melanin produced by epidermal melanocytes. The condition is typified by small, white spots on the skin that enlarge with time. Inflammatory cells, such as CD4 + and CD8 + T lymphocytes, are usually seen around the borders of the lesions, and the histological characteristic of vitiligo is the complete lack of melanin and functional melanocytes in the lesions. In order to evaluate possible treatments for vitiligo patients and get a better understanding of the mechanisms driving melanocyte loss, it might be helpful to replicate the physiological characteristics of vitiligo *in vitro* to guarantee a uniform distribution of melanocytes inside the dermis. A 3D bioprinting method created by scientists can create an FT skin model with skin pigmentation. Following the printing of many hydrogel-containing fibroblast layers, melanocytes and keratinocytes were then printed across the dermis to cause skin pigmentation. The bioprinted skin model showed that the keratinocytes differentiated into the stratum corneum and that the dermal and epidermal layers were properly stratified. Despite accurately depicting the intricate structure of the skin, the 3D vitiligo model is rarely utilized due to its significant reliance on specialized knowledge and experimental variances. However, by lowering the number of experimental variables, 3D bioprinting technology makes it possible to create *in vitro* vitiligo models consistently(Ahn Minjun, 2023).

5. CHALLENGES AND FUTURE PERSPECTIVE

Due to current shortcomings in conventional skin equivalent techniques, new platforms that replicate human physiological settings have been developed, making it possible to create more realistic in vitro testing models. novel techniques have been created to mimic the dynamic component seen in human individuals, in addition to novel cell kinds and biological components found in natural skin to increase their dermatological performance. By creating perfusable human skin counterparts, which primarily entail inserting a microvasculature in the dermal compartment to allow the injection of medications or culture media to examine their diffusion and subsequent effects, several research have evaluated this problem. Skin tissue engineering has been improving lately, and new strategies have been created to improve its functionality. One of its primary limitations is that it still has the skin equivalent of huge size, which raises the expense of manufacture and upkeep and prevents high-throughput research. The development of skin-on-a-chip technology is therefore gaining traction as a way to lower manufacturing costs by performing real-time monitoring of a large number of specimens in a highly automated manner(I. Risueno, 2021).

New and more accurate human skin models are still needed, even with the significant advancements in *in vitro* lipid- or cell-based models. Thus, it has been stated that bioprinted skin may be produced in accordance with the latest developments in 3D bioengineering technology. The skin is not an exception to the numerous tissue and organ models that have been created. Nowadays, bioprinting is seen as a potential technique for creating skin-like materials since it enables the creation of multilayered and multicellular systems. The architecture of the skin model may be regulated with excellent reproducibility thanks to these novel techniques, which involve computer-controlled deposition of skin cells and matrix polymers in spatially controlled patterns.

Several 3D printing methods, including electrospinning, micro extrusion, inkjet printing, and laser-assisted bioprinting, might be used to biofabricate intricate human skin models. The kind of biomaterials selected for the mimetic model typically dictates the choice of the best printing method. The range of printing processes has given rise to several choices for fine-tuning the model's structure to suit the intended use(Tânia Moniz, 2020).

Our knowledge of the complex structure of the skin barrier and illnesses has also grown as a result of the investigation of *in vitro* models in many skin conditions (psoriasis, atopic dermatitis, skin cancer, etc.). Furthermore, by using these models in cancer research, scientists have been able to comprehend the intricate molecular processes that underlie the development of treatment resistance in cancer. In addition to improving our knowledge of the biology of cancer, these models provide a valuable platform for preclinical therapeutic research.It is significant that scientists and clinical professionals embrace these *in vitro* models and take advantage of their potential, not only to clarify the uncharted potential for skin but also to reinforce current therapies, given the current intersection of cutting-edge technology and skin drug discovery. In light of the latest developments in *in vitro* models, this review offers guidance to researchers on how to use these tools in their preclinical studies to minimize the usage of animals. By using these models, medicinal regimens might be developed more accurately while causing less harm to animals. This might enhance knowledge of disease models, which would eventually raise the likelihood of creating successful treatments and enhancing patient outcomes in general(Mohammad Imran, 2024).

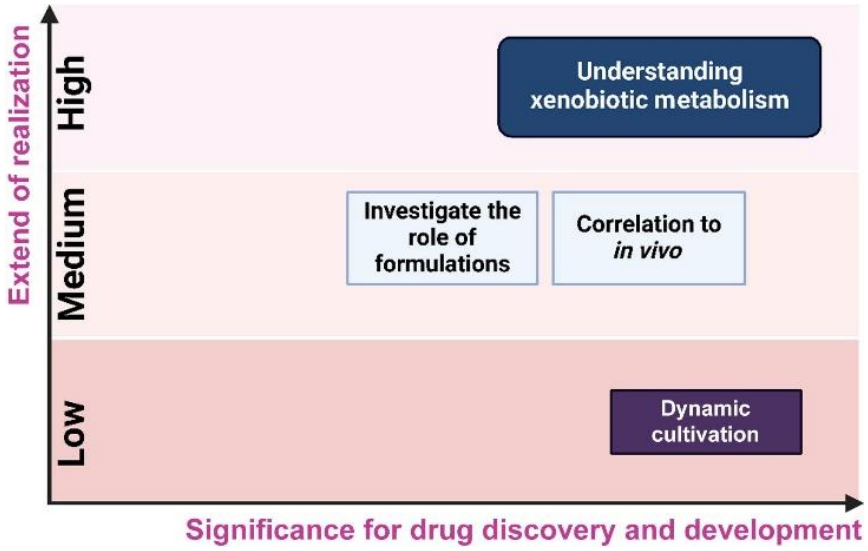

Fig 3. Further design and development of 3D in vitro models with respect to their significance in drug discovery and development (Mohammad Imran, 2024).

6. CONCLUSION

The design and development of human skin models has advanced quickly from simple 2D structures to intricate 3D and disease-specific models, opening up new avenues for personalized treatment, toxicological testing, and drug discovery. These developments increase prediction accuracy and decrease the need for animal testing by improving the physiological relevance of *in vitro* systems. Standardization, scalability, vascularization, and immune component integration continue to be obstacles despite advancements. Future work should concentrate on integrating bioprinting, bioinformatics, and stem cell technologies to develop completely functional skin systems that may be used in clinical translation as well as research.

Conflict of interest: None

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