Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2021.39.006362

Cell Junctions Present in Reconstructed Human Skin Models

Christophe Capallere[1]*, Christelle Plaza[2], Catherine Serre[3] and Isabelle Imbert[4]

[1]*Leader of Tissue Engineering & Toxicology in vitro, Ashland Specialties, France*

[2]*Superior technician in Tissue Engineering & Toxicology in vitro, Ashland Specialties, France*

[3]*Engineer in Tissue Engineering & Toxicology in vitro, Ashland Specialties, France*

[4]*Group Leader Senior, Ashland Specialties, France*

***Corresponding author:** Christophe Capallere, Leader of Tissue Engineering & Toxicology *in vitro*, Ashland Specialties, 655 route du pin montard, 06904 Sophia Antipolis, France

ARTICLE INFO

Received: October 28, 2021

Published: November 09, 2021

Citation: Christophe Capallere, Christelle Plaza, Catherine Serre, Isabelle Imbert. Cell Junctions Present in Reconstructed Human Skin Models. Biomed J Sci & Tech Res 39(5)-2021. BJSTR. MS.ID.006362.

Keywords: Junction; Reconstructed Human Epidermis; Barrier Function; Full Thickness; Dermoepidermal Junction

ABSTRACT

The cohesion between cells and dermal matrix is essential to ensure both mechanical resistance and barrier function of the epidermis so the presence of epidermal junctions is crucial. Since several years, tissue engineering allowed the emergence of cutaneous reconstructed tissues allowing to better understand the role and the functions of the epidermis. In this study, both reconstructed human epidermis and full thickness models presented all differentiated layers of a native epidermis. Indeed, the four typical epidermal layers are well differentiated with the presence of columnar keratinocytes in the basal layer, spinous layer constituted of 2-3 layers of polyhedral keratinocytes resting on the basal layer, the *stratum granulosum*, with characteristic keratohyalin vesicles and finally, the *stratum corneum* which is formed by a superposition of anucleated and completely keratinized cells, the corneocytes, forming very elongated lamellae. The barrier function integrity is insured by cell junctions from the basal layer with hemidesmosomes, focal adhesions and dystroglycans, to the upper layers with GAP junctions, desmosomes, adherens junctions, tight junctions and finally with corneodesmosomes.

These different junctions can be observed by the detection of specific junction proteins involved in cell adhesion. In reconstructed human epidermis or full thickness tissue, at the dermoepidermal junction level, collagen XVII and β1 integrin involved in hemidesmosome or focal adhesion structure respectively were detected as well as the laminin 332 and the collagen IV. The claudin-1 and e-cadherin, detected by specific antibodies, were present in all suprabasal layers (spinous and granular) both in reconstructed epidermis and full thickness. The corneodesmosin was also detected in the *stratum corneum* in reconstructed human epidermis. These *in vitro* skin models are a perfect tool to study cell junction deficiency.

Abbreviations: DEJ: Dermoepidermal Junction; HD: Hemidesmosome; FA: Focal Adhesion; AJ: Adherens Junction; TAMP: Tight junction Associated MARVEL Protein; ZO: Zonula Occludens; MUPP1: MUlti PDZ Domain Protein; CDSN: Corneodesmosin; SDS: Sodium Dodecyl Sulfate; NHK: Normal Human Keratinocytes; BPE: Bovine Pituitary Extract; DMEM: Dulbecco's Modified Eagle Medium; FCS: Foetal Calf Serum; BSA: Bovine Serum Albumine; H&E: Hematoxylin & Eosin; PBS: Phosphate Buffer Saline; RHE: Reconstructed Human Epidermis; FT: Full Thickness; ECVAM: European Council of Validated Alternatives Method

Introduction

From several decades, reconstructed skin models have been developed both in the field of public research, in the cosmetics and pharmaceutical industries. All these approaches are based on the principle of isolation of primary cells, cultivation of cells in a monolayer and then specific differentiation of the tissue at the air-liquid interface in order to obtain a multi-layered and specialized tissues [1,2]. Since the first experiments carried out by Rheinwald and Green in 1975 [3] on the isolation and culture of human primary keratinocytes on a monolayer of fibroblasts, techniques have evolved towards a culture of keratinocytes without a nourishing sub-layer. The technical changes were more focused on the culture media in order to obtain a tissue close to the characteristics of the *in vivo* skin. As a result, the epidermis is now cultivated on de-epidermized dermis, inert filters or even more or less complex collagen matrices [1,4-8]. The growth and differentiation processes of the reconstructed epidermis, however, have changed little with a growth phase of keratinocytes in immersion and a differentiation phase induced with a medium concentrated in calcium ions [9,10].

These technical approaches improve the characteristics of reconstructed tissues with for example, better organization, structure, cohesion conferring them, a better integrity close to skin characteristics. The main role of the skin is to ensure a barrier function against external environmental stresses and to avoid water loss [11]. The maintenance of the skin integrity against stresses is due to the presence of numerous cutaneous junctions between cells and skin compartments [12,13]. In the skin, a stratified epithelium, from basal layer to *stratum corneum* we noticed the presence of focal adhesions (FA), dystroglycans and hemidesmosomes (HD) which ensure the adhesion to dermal compartment, cell-cell junction with GAP junctions, adherens junction, desmosomes then tight junctions in the granular layer and finally corneodesmosomes at the level of *stratum corneum* (Figure 1) [14]. The adhesion between the epidermis and the dermis is insured by a highly specialized zone called the Dermoepidermal Junction (DEJ).

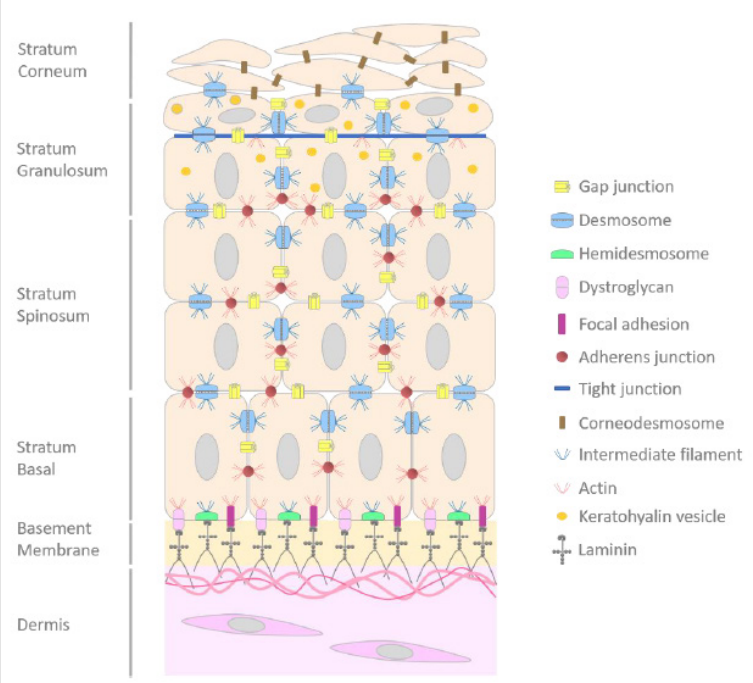

Figure 1: Schematic representation of cell-cell and cell-matrix junctions.

Note: Scheme of the epidermis and its junctions from basal layer to *stratum corneum*. Dermoepidermal junctions are also represented.

The DEJ confers a fine-tuned architecture to the skin useful for the maintain of the skin homeostasis. The DEJ regulates cell adhesion, cell differentiation and motility, and plays an important role in the communication between the epidermis and the dermis [15]. The DEJ also influences the basal keratinocyte polarity and defines the basal surface where proliferating epidermal cells are attached [16]. The DEJ is a highly complex structure composed to hemidesmosomes, focal adhesions and dystroglycans. Hemidesmosomes are found in different tissues such as the cornea, the skin [17] allowing the maintain of these tissues. HD have important role in cell adhesion, wound healing, tissue morphology allowing the maintenance of tissue structure and integrity. HD, half of desmosome, are small structure about less than 0.5µm consisting of a tripartite plaque with an inner and outer plaque separated by a less dense zone [18]. HD consist of α6β4 integrin, plectin (P1a), tetraspanin CD151, BPAG1 (or BP230) and collagen XVII (or BP180 or BPAG2) [19].

These junctions link anchoring intermediate filaments in the epidermal compartment and fibrils on the extracellular matrix side among which the following proteins are mainly found keratins K5, K14, collagen VII and IV and other proteins like laminin 332 (laminin 5) [20]. In addition to hemidesmosomes, focal adhesions are dynamic adhesions allowing also keratinocyte junctions to the extracellular matrix through the connection of α3β1 integrin transmembrane proteins to the actin cytoskeleton and on the opposite to laminin 332 to the extracellular matrix [21]. FA are involved in different processes like cell communication, proliferation, migration, apoptosis, spreading, wound healing and differentiation. The FA is a protein complex composed about more 50 proteins divided into three groups:

i) The structural components (talin, vinculin, kindlins also named FERMT1-3)

ii) The enzymatic components (Focal Adhesion Kinase (FAK), Integrin-Linked Kinase (ILK) and Tyrosine-Protein Kinase SRC-1 (SRC))

iii) Adaptors (paxillin, P130, LIMS1…) [21-23].

The dystroglycans, another complex present in DEJ, were shown as expressed by keratinocytes and fibroblasts in human skin [24] and localized in the epidermal basement membrane. The dystroglycans allow a closed-link with the actin cytoskeleton of epidermal basal keratinocytes and with the extracellular matrix in human skin [24,25]. The integrity of the epidermis compartment is ensured by cell-cell junctions present between epidermal cells in all layers including *stratum corneum*. Among all junctions, GAP junctions link the cytoplasm of two cells allowing intercellular exchange of ions and small molecules [26]. This intercellular communication is important for the maintenance of skin homeostasis, including keratinocyte growth and differentiation [27], regulation in melanogenesis [28]. In fact, GAP junctions are channels assembled from connexin subunits (26, 32 and 43) belonging to connexin family about 21 members. The assembly of 6 connexins forms an oligomer called connexon, transported to the plasma membrane [29].

The connexon docks with a connexon of adjacent cell and form a GAP junction channel. These GAP junctions are regrouped into GAP junction plaque. In addition to GAP junction, desmosomes form the intercellular junctions (0.2–0.5μm in diameter) allowing the link of intermediate filaments to the plasma membrane giving a resistance to mechanical stress in the skin and other tissues [30]. The desmogleins (Dsc1-3) and desmocollins (Dsg1-4), transmembrane proteins of the desmosome, belong to the cadherin family of calcium-dependent adhesion molecules. The cytoplasmic tails of desmosomal cadherins are associated with the desmosomal plaque proteins: plakoglobin and desmoplakin belonging to the armadillo and plakin family of linker proteins respectively [31]. The tethering of cytoskeleton is insured by interaction of desmoplakins with the keratin intermediate filaments giving rise to inner dense plaque [32,33]. A third cell junction complex is adherens junctions (AJs) which have conserved plasma-membrane structures that mediate cell–cell adhesions organized into two complexes of proteins: nectin/afadin and cadherin/catenin.

The AJs form extracellular adhesive contacts between cells, and intracellular links to the actin cytoskeleton. E-cadherin and the catenin family members including p120-catenin, β-catenin, and α-catenin are the main components of AJs [34]. Two types of cadherins are expressed in the epidermis: P-cadherin expressed in the basal layer and in hair follicles, and e-cadherin in all layers of the epidermis. AJs are involved in several processes such as cytoskeletal dynamics, cell polarity, cell adhesion, cell shape, division, growth, apoptosis and barrier function [35]. At the upper layer of the epidermis, another type of junctions is present. Indeed the tight junctions are localized in the granular layer, thus ensuring the barrier function, cell polarity and preventing epidermal water loss and solutes [36]. Tight junctions are protein complexes containing more than 40 proteins that form the semi-permeable mechanical connections between cells.

The tight junctions consist of three main type of structural transmembrane proteins that are common to all tight junctions: claudins belonging to a family of 26 members, Tight Junction-associated MARVEL proteins (TAMP) as occludin or tricellulin and junctional adhesion molecules (JAM-A, -B or -C) [37]. The tight junctions are linked to the cytoskeleton through protein adaptors called Zonula Occludens (ZO-1, -2, and -3) and MUPP1 (Multi-PDZ Domain Protein 1) forming the junctional plaque. Most of the proteins forming these junctions are found in the *stratum granulosum* including claudins 1, 4, 6, 7, 11, 12 and 18, occludin, ZO-1, ZO-2, MUPP-1 and cingulin [38]. And finally in the *stratum corneum*, composed of corneocytes responsible of the epidermis turnover and conferring a regenerating power of the skin, corneodesmosomes ensure the link to each other. Corneodesmosomes are a modified form of desmosome, indeed they are formed upon integration of corneodesmosin (CDSN) released by lamellar granules [39] during the conversion of desmosome to corneodesmosome in the *stratum corneum* of the epidermis [33]. CDSN glycoproteins embedded within the desmoglea (the intercellular space of desmosomes) to form the desmosomal plate [40].

Deposition of loricrin, a major component of the cornified cell envelope, begins at the desmosomal plaques in the cytoplasm of cell present in the upper layer of the *stratum granulosum* [39]. These junctions are degraded to allow the desquamation process by proteases as kallikreins and cysteine proteases (cathepsins) in contrast to protease inhibitors as LEKTI counterbalance to the *stratum corneum* formation [41]. In this article, we highlighted the presence of cell-cell junctions and cell-matrix junctions both in reconstructed epidermis and full thickness (combination of

dermis and epidermis) and the integrity of the barrier function demonstrated with the penetration of lucifer dye after chemical stress (SDS).

Material and Methods

Ethical Compliance

Samples were obtained from anonymous human healthy donors. Surgical residues were harvested according to French regulation (agreement DC 2021-4617) and procurement of written informed consent from the patient.

Cell Culture of Normal Human Keratinocytes and Fibroblasts

Normal human primary epidermal keratinocytes (NHKs) were isolated from surgery (circumcision). An enzymatic digestion was used to dissociate the epidermis from the dermis indeed the biopsies were incubated in the Dispase II (Sigma, France) at 4°C overnight. Then a second enzymatic digestion was used to separate the epidermal keratinocytes with Trypsin-EDTA (Sigma, France) at 37°C for 10 minutes from epidermis cut into small pieces. The cells were centrifugated and the pellet was taken in a specific medium complemented with BPE (Gentaur, France). Cells were placed at 37°C in a humidified atmosphere containing 5% of CO_2. In parallel, the dermis explant was placed in a Petri dish and incubated with DMEM with 1g/L of glucose (Lonza, Switzerland), 2mM L-glutamine (Lonza, Switzerland), Gentamycin (Euromedex, France) complemented with 20% FCS (Biowest, France). The explants were incubated at 37°C in a humidified atmosphere containing 5 % of CO_2. At the appearance of fibroblasts, the dermis explant was removed and the culture medium was replaced by DMEM with 1g/L of glucose (Lonza, Switzerland), 2 mM L-glutamine (Lonza, Switzerland), Gentamycin (Euromedex, France) complemented with 10 % FCS (Biowest, France) and incubated at 37°C in a humidified atmosphere containing 5% of CO_2.

Reconstruction of Epidermis

After keratinocyte isolation, the NHKs were seeded on a 0.5cm^2 inert polycarbonate membrane (Nunc, Thermo Fisher Scientific, USA) in a proprietary chemically-defined media and were placed at the air-liquid interface until 17 days at 37°C in a humidified atmosphere containing 5 % of CO_2.

Reconstruction of Skin Equivalent (Dermis and Epidermis)

After fibroblast isolation, dermal equivalents were prepared using a neutralized solution containing bovine type I collagen (Collagen Solution, USA) diluted in complete DMEM [(1g/L of glucose, 2mM L-glutamine; Lonza, Switzerland), 10 % FCS (Biowest, France) and gentamycin (Euromedex, France)]. The mixture was dispensed onto 12-well tissue culture plates and incubated 24 hours at 37°C to allow the polymerization. After polymerization, complete DMEM medium was added to each well. Dermal equivalents were maintained at 37°C in a humidified atmosphere containing 5 % of CO_2. After 4 days of contraction, the matrix was transferred in the proprietary chemically defined medium at the air-liquid interface. The NHKs were seeded on the dermal matrices and placed at 37°C under 5 % CO_2. After 3 days of culture, the full thickness was placed at the air-liquid interface at 37°C in a humidified atmosphere containing 5 % of CO_2.

Histology and Immunohistochemistry

For histological analysis, the reconstructed human epidermis and skin equivalent were fixed in the 10% formalin buffer (Sigma, France). After successively dehydration, the tissues were then embedded in paraffin. Paraffin section (4µm) were deposited on glass slides, deparaffinized and then successively rehydrated in xylene baths, alcohol of different percentages and water. The Hematoxylin & Eosin staining (H&E) staining was performed by placing the sections in a hematoxylin bath for 3 minutes. The sections were rinsed with water for 5 minutes at room temperature. The slides were then placed in an eosin bath for 2 minutes. The tissues were then dehydrated in successive baths of absolute alcohol and xylene. After mounting a coverslip with Eukitt* (O. Kindler), the photos were acquired with a Qimaging* Retina 2000R Fast1394 camera and processed by using the Q-Capture Pro 7 (QImaging, England) acquisition software.

For immunohistochemistry (IHC), the reconstructed human epidermis and skin equivalent were fixed in the 10% formalin buffer (Sigma, France), then embedded in paraffin. Paraffin sections (4µm) were deposited on glass slides, deparaffinized and rehydrated with successive bath of xylene, absolute alcohol and water. The exposure of the antigens was realized by treatment of the sections with 0.01 M citrate buffer pH6 and 0.25 % pepsin or 0.05 % trypsin (Zymed, Invitrogen, Thermo Fisher Scientific, USA) for 15 minutes at 37°C. After the fixation, the saturation of nonspecific sites was performed with 5% BSA buffer (Sigma, France) for 30 minutes, primary antibody was applied at room temperature for 1 hour (Table 1). The sections were rinsed in PBS and the secondary antibody (Alexa Fluor Donkey anti-rabbit, Alexa fluor Goat anti-mouse, Alexa Fluor Goat anti-rat, (Invitrogen, Thermo Fisher Scientific, USA) was deposited at room temperature in dark for 1 hour. Finally, the slides were incubated with 0.3µM 4',6'diamidino-2-phenylindole (DAPI, Molecular Probes, USA) for 5 minutes at room temperature in the dark and mounted with Fluoromount-G® (Electron Microscopy Sciences, USA). The photos were taken with a Qimaging EXI blue camera coupled to Volocity acquisition software (Improvision, England).

Table 1: Antibody characterics used for immunohistochemistry.

Primary antibody	Secondary Antibody	Material	Antigen retrieval / Reconstructed tissue	Provider
Claudin-1	Rabbit polyclonal	Paraffin	Citrate buffer & trypsin	Abcam
E- Cadherin	Rabbit polyclonal	Paraffin	Citrate buffer & trypsin	Abcam
β1 Integrin	Rat monoclonal	Paraffin	Unmasking	BD Biosciences
Corneodesmosin	Rabbit polyclonal	Frozen	Unmasking	Abcam
Collagen XVII	Rabbit polyclonal	Paraffin	Citrate buffer & pepsin	Abcam
Laminin V	Mouse polyclonal	Paraffin	Citrate buffer & pepsin pepsin	Chemicon
Collagen IV	Mouse polyclonal	Paraffin	Citrate buffer & pepsin	Chemicon

Barrier Integrity Assay

To observe the barrier integrity, a Sodium Dodecyl Sulfate (SDS) (Sigma, France) stress (concentration 0.1 to 0.75 % w/v diluted in PBS 1X) was applied topically for 3 hours. Then epidermises were rinsed 3 times with PBS 1X and then Lucifer yellow 1mM was applied topically for 2 hours to visualize the diffusion of this passive dye throughout the epidermis. Epidermises were rinsed 3 times with PBS 1X then tissues were fixed with formalin solution neutral buffer 10 % and embedded in paraffin. Paraffin sections (4µm) were deposited on glass slides, deparaffinized and rehydrated with successive bath of xylene, absolute alcohol and water. The slides were incubated with 0.3µM 4',6'diamidino-2-phenylindole (DAPI, Molecular Probes, USA) for 5 minutes at room temperature in the dark and mounted with Fluoromount-G® (Electron Microscopy Sciences, USA). The photos were taken with a Qimaging EXI blue camera coupled to Volocity acquisition software (Improvision, England).

Results

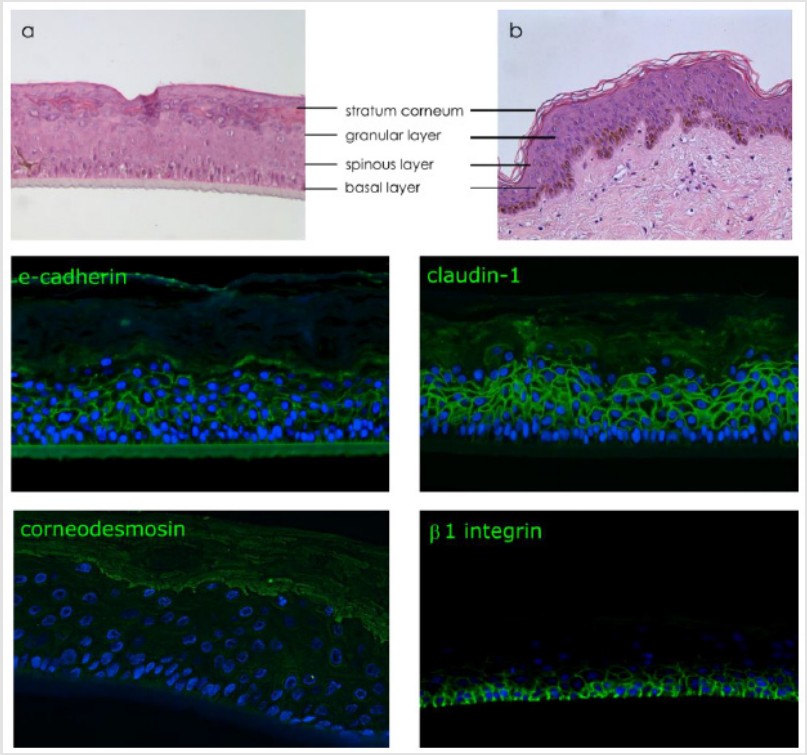

Figure 2: Characterization of reconstructed human epidermis.

Note:

a) Histological observation by H&E of reconstructed human epidermis

b) Native skin.

Immunodetection of e-cadherin and claudin-1 presents in all epidermal layers, corneodesmosin localized in the upper layers (granular layer and *stratum corneum*) and β1 integrin expressed specifically in basal layer. Nucleus are stained in blue by DAPI.

The reconstruction of cutaneous tissues such as epidermis or more complex tissue (dermis combinate with epidermis for example) is not only a layering of keratinocytes but a cellular stratification with the presence of specialized keratinocytes with specific functions and characteristics according to the corresponding epidermal layer such as basal, spinous, granular layers or *stratum corneum*. To investigate the integrity of the tissue due to the presence of cell junctions, immunodetections of specific proteins related to junctions were realized on these reconstructed tissues. Claudin-1 (a major protein involved in tight junction), e-cadherin (a main protein involved in cell-cell junction), β1 integrin (protein involved in cell-matrix adhesion and more specifically to collagen fiber) and corneodesmosin (a protein involved in the stratification of *stratum corneum*) were selected. Prior to realize immuno-detection of specific proteins, a histological observation of the human reconstructed tissue by hematoxylin and eosin (H&E) staining was performed to compare the structure of reconstructed epidermis to native human skin (Figures 2a & 2b).

The structure of reconstructed epidermis is similar to native skin with the presence of all differentiated layers; basal layer with columnar keratinocytes, spinous layer, granular layer with the presence of keratohyalin vesicles and finally a *stratum corneum* constituted of several layers of corneocytes. The detection of β1 integrin is located to the basal keratinocytes, this is in adequation with the function of β1 integrin to anchor the epidermis to the extracellular matrix at the dermoepidermal junction. The detection of both claudin-1 and e-cadherin were localized at the membrane of cells in the suprabasal layers. Detection of these proteins are less pronounced in basal layer. Corneodesmosin was expressed in the lastgranular layer of the epidermis and all layers of the *stratum corneum* where this protein is progressively proteolysed during corneocyte maturation and desquamation. The presence of specific proteins involved in the cohesion of epidermis seems to confirm the efficiency of barrier function of epidermis in the reconstructed tissues.

To validate this epidermal function, a passive dye was topically applied on the top of the RHE to observe the diffusion after a chemical stress by SDS at various concentrations (Figure 3). Treatment with SDS, a well know surfactant, disrupted cell junctions resulting in a deep penetration of dye Lucifer yellow inside the epidermis. This diffusion is dependent to the concentration of SDS, indeed at lowest concentration (0.1 %) the lucifer yellow was only present in the *stratum corneum*, and diffusion was not observed in viable cell layers. But on the opposite, at the highest tested concentration (0.75 %), lucifer yellow penetrated completely inside the reconstructed epidermis (Figure 3). After the reconstruction and the characterization of the *in vitro* epidermis, a more complex tissue combining dermal and epidermal compartment was studied. Prior to detect the presence of specific cell junction proteins, a histological staining by H&E was performed to compare the structure of full thickness to native skin (Figure 4).

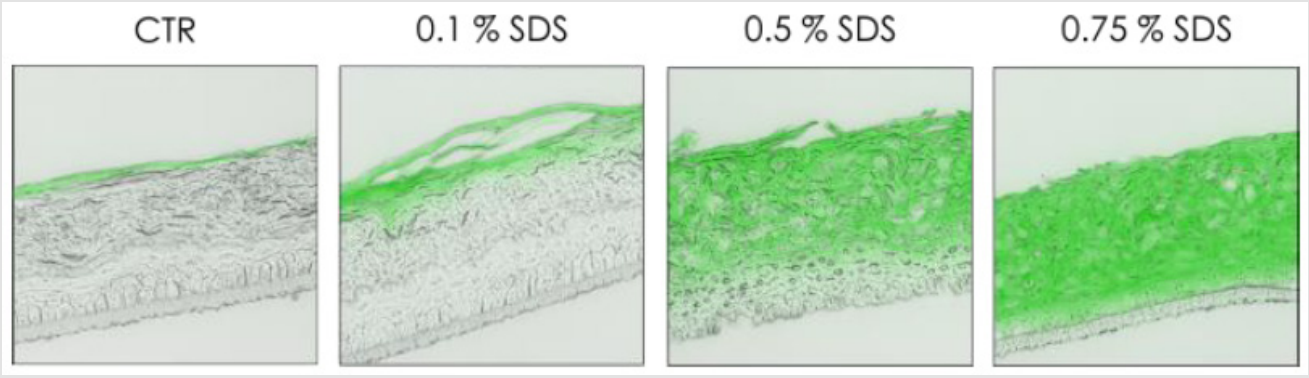

Figure 3: Diffusion of passive dye after chemical stress (SDS).

Note: Observation of the passive diffusion of lucifer yellow applied topically (2h) after chemical stress induced by SDS (3h) at various concentration 0.1%, 0.5% and 0.75% on RHE at day 17.

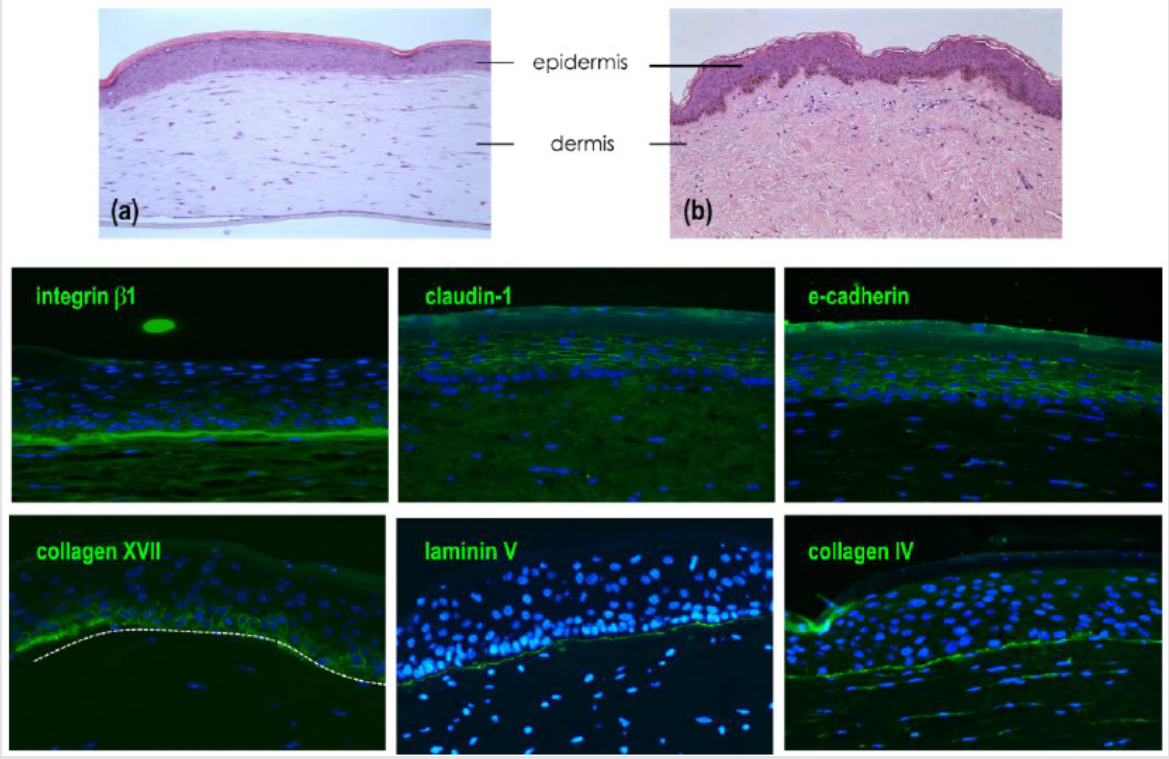

Figure 4: Characterization of skin full thickness (FT) model.

Note:

a) Histological observation by H&E of *in vitro* FT model

b) Compared to native skin.

Immuno-detection of markers expressed in full thickness. Detection of proteins expressed in dermal epidermal junction (β1 integrin, collagen XVII, laminin 332 and collagen IV) and cell-cell junction in epidermal compartment (claudin-1 and e-cadherin).

As we observed a similar structure of reconstructed human epidermis to the epidermis of native skin, the observation of the epidermal compartment in the full thickness model is similar with the presence of all differentiated layers; basal layer with columnar keratinocytes, spinous layer, granular layer with the presence of keratohyalin vesicles and finally a *stratum corneum* (Figures 4a & 4b). The global structure of reconstructed tissue is similar to native skin (except for the presence of dermal papilla) but due to the early stage of reconstruction (day 10), the stratum appeared thinner than the epidermal stratum observed in RHE at day 17. Cohesion of the epidermal compartment with the dermal compartment is fully present all along the tissue. The detection of β1 integrin is localized all along the dermoepidermal junction, collagen XVII, laminin 332 and collagen IV were also expressed at the DEJ. As observed on reconstructed epidermis alone, the detection of both claudin-1 and e-cadherin are localized in the membrane of cells constituting suprabasal layers, the detection of these proteins are less pronounced in the basal layer.

Conclusion

Reconstruction of *in vitro* skin models from human cells is a real advantage for the research field and also it has been a revolution for toxicological studies to avoid the use of animals. These RHE are "real skin" and not only a superposition of cells which allowed the validation of these models by ECVAM (European Council of Validated Alternatives Methods) to use them for several toxicological studies such as skin irritation or corrosion (respectively OECD TG439 (2021) and 431 (2019), [42,43]). To obtain these validations, the "*in vitro* skin" must have similar properties to native skin and more precisely in term of barrier function. This barrier function is ensured by the *stratum corneum* with its structure in "bricks and mortar" (bricks symbolize corneocytes and mortar the intercellular lipids). In addition of the *stratum corneum*, to ensure this barrier function, cohesion between epidermal cells in the viable layers is essential. To ensure the tissue cohesion, there is several types of epidermal junctions such as GAP junctions, tight junctions (essentially located

in the *stratum granulosum*), desmosomes, corneodesmosomes (in the *stratum corneum*) and hemidesmosomes, focal adhesions, dystroglycans in the basement membrane.

In this study, both reconstructed human epidermis and full thickness models presented all differentiated layers of a native human epidermis. Indeed, four typical epidermal layers are well differentiated with the presence of columnar keratinocytes in the basal layer, spinous layer constituted of 2-3 layers of polyhedral keratinocytes above the basal layer and the *stratum corneum*. The cells of the epidermal layers come from the migration of cells of basal layer. In the *stratum spinosum*, cells are provided with spicules or thorns, hence their name of spiny cells. These spines are in fact desmosomes to which microfilaments are attached. The third layer, the *stratum granulosum*, with characteristic cytoplasmic keratohyalin vesicles comprising loricrin, trichohyalin and profilaggrin (the precursor of filaggrin), contributes to the formation of interfibrillar cement by keratin filament aggregation. Thus, the function of keratohyalin vesicles is to stabilize the tonofibrils at the level of the corneocytes, by contributing to the formation of the insoluble matrix of the *stratum corneum* that leads to keratinization.

The cohesion of the spinous layers is also ensured by different junctions such as tight junctions evidenced by claudin-1 and desmosomes that are conversed in corneodesmosomes evidenced by corneodesmosin. Finally, the *stratum corneum* is formed by a superposition of anucleated and completely keratinized cells, the corneocytes, forming very elongated lamellae. All epidermal cells are firmly attached to each other, thus causing a mechanical cell coupling inducing resistance of the epidermis to mechanical stresses and a part of communication between cells are ensured by the presence of GAP-type junctions. The barrier function integrity can be observed by the detection of specific junction proteins involved in cell-cell adhesion. Claudin-1 (tight junction) and e-cadherin (adherens junctions), detected by specific antibodies, were presents in all suprabasal layers (spinous and granular) both in reconstructed epidermis and full thickness. It is interesting to notice that the detection of these proteins was absent or very weak in the basal layer.

This lower detection is due to the presence of proliferating cells and stem cells which initiate the renewal of the epidermis, indeed basal keratinocytes expressed high levels of β1 integrins and lower levels of e-cadherin and claudin-1. Presence of these two proteins is crucial because e-cadherin favors the incorporation of claudin-1 in the structure of tight junction and a decrease of claudin-1 plays a central role in dermatitis atopic [44]. The cohesion of the epidermal compartment to the dermis is also ensured by several proteins forming complex such as hemidesmosomes, focal adhesions or dystroglycans. Our *in vitro* model presented proteins belonging at the DEJ demonstrated by the presence of collagen XVII (hemidesmosomes), laminin 332, collagen IV and β1 integrin (focal adhesion). The study of the cell-cell junctions and cell-matrix junctions are very important to better understand skin diseases. The development of *in vitro* models deficient in specific proteins can be a perfect tool in this investigation.

Indeed, for example hemidesmosome are very important bonds because mutations in the genes encoding these proteins induce serious pathologies such as bullous dermatosis which results from the loss of interaction between plectin and collagen XVII [45], in mice the deficiency of β1 integrin induced a resistance to skin scleroderma resulting in reducing dermis thickness [46]. To develop specific *in vitro* models, the combination between molecular biology and tissue engineering allows to modify genetically the cells then to reconstruct human tissues deficient in the expression of specific proteins involved in cell-cell junctions or cell-matrix junctions to mimic diseases.

Acknowledgment

This paper has been written thanks to the bibliographical research of Catherine Serre focused on epidermal junction and her availability.

References

1. Prunerias M, Régnier M, Woodley D (1983) Methods for cultivation of keratinocytes with an air-liquid interface. J Invest Dermatol 81: 28s-33s.
2. Bernstam LI, Vaughan FL, Bernstein IA (1986) Keratinocytes grown at the air-liquid interface. *In Vitro* Cell Dev Biol 22(12): 695-705.
3. Rheinwald JG, Greene H (1975) Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 6(3): 331-343.
4. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T (1981). Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. Science 211(4486): 1052-1054.
5. Boyce S, Hansbrough J (1988) Biologic attachment, growth, and differentiation of cultured human epidermal keratinocytes on a graftable collagen and chondroitin-6-sulfate substrate. Surgery 103(4): 421-431.
6. Tinois E, Tiollier J, Gaucherand M, Dumas H, Tardy M, et al. (1991) *In vitro* and post-transplantation differentiation of human keratinocytes grown on the human type IV collagen film of a bilayered dermal substitute. Exp. Cell Res 193: 310-319.
7. Ponec M, Weerheim A, Kempenaar J, Mulder A, Gooris G, et al. (1997) The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. J. Invest Dermatol 109(3): 348-355.
8. El Ghalbzouri A, Siamari R, Willemze R, Ponec M (2008) Leiden Reconstructed human epidermal model as a tool for the evaluation of the skin corrosion and irritation potential according to the ECVAM guidelines. Toxicol *in Vitro* 22(5): 1311-1320.
9. Mak VHW, Cumpstone MB, Kennedy AH, Harmon CS, Guy RH, et al. (1991) Barrier Function of Human Keratinocyte Cultures Grown at the Air-Liquid Interface. Journal of Investigative Dermatology 96(3): 323-327.

10. Rosdy M, Pisani A, Ortonne JP (1993) Production of basement membrane components by a reconstructed epidermis cultured in the absence of serum and dermal factors. Br J Dermatol 129(9): 227-34.

11. Elias PM (2008) Skin barrier function. Current allergy and asthma reports 8(4): 299-305.

12. Agner T (2016) Skin Barrier Function. Indian J Med Res 147: 117-120.

13. Basler K, Bergmann S, Heisig M, Naegel A, Zorn Kruppa M, et al. (2016) The Role of Tight Junctions in Skin Barrier Function and Dermal Absorption. Journal of Controlled Release 242: 105-118.

14. Green KJ, Jaiganesh A, Broussard Joshua A (2019) Desmosomes: Essential contributors to an integrated intercellular junction network. F1000Res, p. 1-16.

15. Hashmi S, Marinkovich MP (2011) Molecular organization of the basement membrane zone. Clin Dermatol 29(4): 398-411.

16. Serre C, Plaza C, Meyrignac C, Capallere C, Botto JM (2019) The dermoepidermal junction: a key cutaneous structure for a healthy skin. Development of a bioinformatic interaction network and relevant bioengineered skin models. EURO COSMETICS 5: 16-20.

17. Borradori L, Sonnenberg A (1999) Structure and Function of Hemidesmosomes: More Than Simple Adhesion Complexes. J Invest Dermatol 112(4): 411-8.

18. Walko G, Castanon M, Wiche G (2015) Molecular architecture and function of the hemidesmosome. Cell and Tissue Research Volume 360(3): 529-544.

19. Owaribe K, Kartenbeck J, Stumpp S, Magin TM, Krieg T, et al. (1990) The hemidesmosomal plaque. Characterization of a major constituent protein as a differentiation marker for certain forms of epithelia. Differentiation 45(3): 207-220.

20. McGrath J, Uitto J (2016) Structure and Function of the Skin (9th Edn.)., Rook's Textbook of Dermatology.

21. Hatzfeld M, Magin TM (2019) Crosstalk between Hemidesmosomes and Focal Adhesions: A Primer for Wound Healing, Blistering Skin Disease, and Skin Aging. J Invest Dermatol 139(9): 1854-1856.

22. Lo H (2006) Focal adhesions: What's new inside. Developmental Biology 294(2): 280-291.

23. Duperret EK, Ridky TW (2013) Focal adhesion complex proteins in epidermis and squamous cell carcinoma. Cell Cycle 12(20): 3272-3285.

24. Herzog C, Has C, Franzke CW, Echtermeyer FG, Schlötzer-Schrehardt U, et al. (2004) Dystroglycan in Skin and Cutaneous Cells: β-Subunit Is Shed from the Cell Surface. Journal of Investigative Dermatology 122(6): 1372-1380.

25. Durbeej M, Henry MD, Ferletta M, Campbell KP, Ekblom P (1998) Distribution of dystroglycan in normal adult mouse tissues. J Histochem Cytochem 46(4): 449-57.

26. Beyer E, Berthoud V (2017) Gap junction structure: unraveled, but not fully revealed. Research 6: 1-10.

27. Meşe G, Richard G, White TW (2007) Gap Junctions: Basic Structure and Function. Journal of Investigative Dermatology 127(11): 2516-2524.

28. Divya Padma, Kapaettu Satyamoorthy, Kumar MR Bhat (2015) Role of gap junctions between keratinocyte and melanocyte in melanogenesis. Frontiers in Biology 10: 495-502.

29. Churko JM, Laird DW (2013) Gap Junction Remodeling in Skin Repair Following Wounding and Disease. Physiology 28(3): 190 -198.

30. Kowalczyk AP, Green KJ (2013) Structure, Function and Regulation of Desmosomes. Prog Mol Biol Transl Sci 116: 95-118.

31. Delva E, Tucker DK, Kowalczyk AP (2009) The Desmosome. Cold Spring Harb Perspect Biol 1(2): a002543.

32. Kowalczyk AP, Stappenbeck TS, Parry DA, Palka HL, Virata ML, et al. (1994) Structure and function of desmosomal transmembrane core and plaque molecules. Biophys Chem 50(1-2): 97-112.

33. Garrod D, Chidgey M (2008) Desmosome structure, composition and function. Biochimica et Biophysica Acta 1778(3): 572-587.

34. Hartsock A, Nelson WJ (2008) Adherens and Tight Junctions: Structure, Function and Connections to the Actin Cytoskeleton. Biochim Biophys Acta 1778(3): 660-669.

35. Brandner JM, Haftek M, Niessen CM (2010) Adherens Junctions, Desmosomes and Tight Junctions in Epidermal Barrier Function. The Open Dermatology Journal 4: 14-20.

36. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, et al. (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 156(6): 1099-1111.

37. Vermette D, Hu P, Canarie MF, Funaro M, Glover J, et al. (2018) Tight junction structure, function, and assessment in the critically ill: a systematic review. Intensive Care Medicine Experimental 6(1): 37.

38. Brandner JM, Zorn Kruppa M, Yoshida T, Moll I, Beck LA, et al. (2015) Epidermal tight junctions in health and disease. Tissue Barriers 3: 1-2.

39. Ishida Yamamoto A, Eady RA, Watt FM, Roop DR, Hohl D, et al. (1996) Immunoelectron microscopic analysis of cornified cell envelope formation in normal and psoriatic epidermis. J Histochem Cytochem 44(2): 167-175.

40. Raknerud N (1975) The ultrastructure of the interfollicular epidermis of the hairless (hr/hr) mouse. III. Desmosomal transformation during keratinization. J Ultrastruct Res 52(1): 32-51.

41. Marek Haftek (2014) Epidermal barrier disorders and corneodesmosome defects. Cell Tissue Res 60(3): 4 83-90.

42. (2019) OECD. Test No. 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method, OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France.

43. (2021) OECD. Test No. 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method, OECD Guidelines for the Testing of Chemicals, Section 4; OECD Publishing: Paris, France.

44. Bergmann S, Von Buenau B, Vidal (2020) Claudin-1 decrease impacts epidermal barrier function in atopic dermatitis lesions dose-dependently. Sci Rep 10(1): 2024.

45. Natsuga Wataru K (2017) Loss of interaction between plectin and type XVII collagen results in epidermolysis bullosa simplex. Human mutation 38(12): 1666-1670.

46. Liu S, Kapoor M, Denton CP, Abraham DJ, Leask A (2009) Loss of β1 integrin in mouse fibroblasts results in resistance to skin scleroderma in a mouse model. Arthritis & Rheumatism 60(9): 2817-2821.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2021.39.006362

C Capallere. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php