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The role of tight junctions in skin barrier function and dermal absorption

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ABSTRACT

The skin protects our body from external assaults like pathogens, xenobiotics or UV irradiation. In addition, it prevents the loss of water and solutes. To fulfill these important tasks, a complex barrier system has developed which comprises the *stratum corneum*, tight junctions, the microbiome, the chemical barrier and the immunological barrier. These barriers do not act separately, but influence each other e.g. after external manipulation or in skin diseases. Especially the two mechanical barriers, i.e. *stratum corneum* and tight junctions, are of great interest for drug delivery, because they are the first interaction partners of drug delivery systems and play the major role in skin absorption. Tight junctions are of special interest, as they are centrally localized in this complex barrier system in the outermost viable layer - the *stratum granulosum* of the interfollicular epidermis and the companion cell layer of the hair follicle - and because they can react very quickly to stimuli. We summarize here our current knowledge about tight junction barrier function in mammalian interfollicular epidermis and hair follicles, and the interaction of tight junctions with other skin barrier components in health and disease. Furthermore, we discuss their relevance for drug delivery and provide examples for tight junction modulators.

1. Introduction

There exist several borders between the human body and the environment, e.g. the skin, the respiratory system, and the intestine. The skin ensures homeostasis by avoiding loss of water and nutrients with the aid of an inside-out barrier. Additionally, the outside-in barrier is in charge of the protection against environmental assaults like xenobiotics, UV radiation or microbes [1,2]. The outside-in barrier also plays a major role in skin absorption and is the main interaction partner with drug delivery systems. Furthermore, the skin is involved in thermoregulation, sensation, and metabolic processes [3,4].

Mammalian skin is composed of three parts, the epidermis, the dermis and the subcutis. Dependent on the body site, hair follicles and glands are interspersed (Fig. 1).

The interfollicular epidermis which is the major site of drug absorption (Fig. 1) is a stratified squamous epithelium. From inside to outside, it is composed of *stratum basale* (SB), *stratum spinosum* (SS), *stratum granulosum* (SG) and *stratum corneum* (SC) (Figs. 2, 3). Cell proliferation within the SB guarantees the continuance of the epithelium. After leaving the SB, a multi-step differentiation process escorts the keratinocytes continually outwards through the different layers of the SS which can be seen by the distinct expression of proteins indicating differentiation (e.g. keratin 1 and 10 or involucrin). In the SG, keratohyalin granules that are packed with pro-filaggrin and keratin intermediate filaments, as well as lamellar bodies that contain lipids and enzymes, are formed. In this layer, cells prepare for the final step of differentiation which provides protein-enriched anucleated corneocytes embedded in a lipid matrix and connected by corneodesmosomes – the SC [2,3,5,6]. Further cell types of the epidermis are melanocytes, which are involved in UV protection, Langerhans cells belonging to the skin immune system, as well as Merkel cells which serve, among others, as mechanoreceptors [3].

The dermis consists of collagen, elastic fibers and extrafibrillar matrix and provides stability and elasticity to the skin [3]. Furthermore,

Abbreviations: AD, atopic dermatitis; AMPs, antimicrobial peptides; BL, basal cell layer of HF; CaCo, colon carcinoma cell line; CCL, central cell layer of HF; cCPE, C-terminal domain of *Clostridium perfringens* enterotoxin; CD, cluster of differentiation; CL, companion cell layer of HF; Cldn, claudin; C, cortex; Cu, cuticle of hair shaft; Da, Dalton; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Flg, filaggrin; FITC, fluorescein isothiocyanate; HaCaT, human immortalized keratinocytes; He, Henle's layer; HF, hair follicle; hBD, human β-defensin; Hu, Huxley's layer; Icu, cuticle of IRS; IRS, inner root sheath; IgE, immunoglobulin E; IL, interleukin; IV, ichthyosis vulgaris; JAM, junctional adhesion molecule; KD, knock-down; KO, knock-out; M, medulla; MC, matrix cells; MDCK, Madin-Darby canine kidney; MHC, major histocompatibility complex; NHEKs, normal human epidermal keratinocytes; NISCH, neonatal ichthyosis sclerosing cholangitis; Ocln, occludin; ORS, outer root sheath; PAMPs, pathogen-associated molecular patterns; PKC, protein kinase C; PRR, pattern recognition receptor; SB, stratum basale; SC, stratum corneum; SG, stratum granulosum; siRNA, small interfering ribonucleic acid; SNP, single nucleotide polymorphism; SS, stratum spinosum; TER, transepithelial resistance; TEWL, transepidermal water loss; Th2, T-helper cell type 2; TJs, tight junctions; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; TRPV, transient receptor potential channels V; UV, ultraviolet; ZO, zonula occludens; ZOT, zonula occludens toxin.

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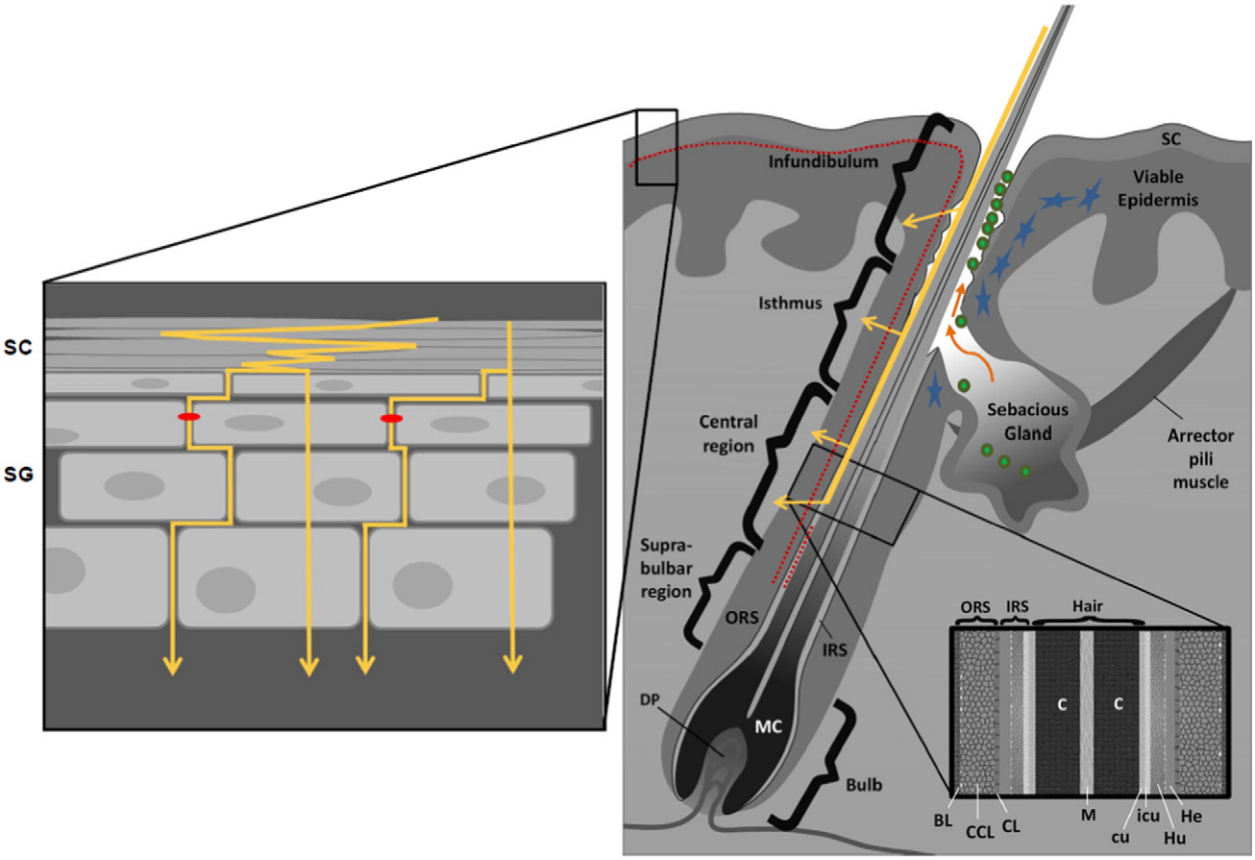

Fig. 1. Schematic overview of the skin including putative penetration pathways. BL: basal cell layer of HF, C: Cortex, CCL: central cell layer of HF, CL: companion cell layer of HF, cu: cuticle of hair shaft, He: Henle's layer, Hu: Huxley's layer, icu: cuticle of IRS, IRS: inner root sheath, M: Medulla, MC: matrix cells, ORS: outer root sheath, SC: *stratum corneum*, SG: *stratum granulosum*; Yellow arrows: putative paracellular and transcellular penetration pathways. Red dots: tight junctions, blue stars: Langerhans cells, orange arrows: sebum, green circles: microbiota. Modified from [7].

immune cells including macrophages, lymphocytes (T and B cells), and mast cells (Fig. 2) are present. Blood vessels and lymphatic vessels in the dermis are important for nourishment and immune response as well as waste removal from dermal and epidermal cells. They are also important for the systemic uptake of topically applied substances. Dermis and epidermis are separated by a basement membrane.

The subcutis, the lowermost part of human skin, is important for fat storage and thermal isolation [4].

Skin barrier function is executed by several components: the microbiome, the SC, tight junctions (TJs), the chemical barrier and the immunological barrier (Fig. 2). Concerning drug delivery and skin absorption, mainly the mechanical barriers – SC and TJs – play a role. However, also the other barriers influence the microenvironment and can interact with active ingredients and carriers and have therefore to be taken into account. Excellent reviews on the microbiome, the SC, the chemical and the immunological barriers have been published recently [8–13]. Thus, we will describe these barriers only briefly in this review and will concentrate on TJs.

TJs are cell-cell junctions that are present in simple and multi-layered epithelia as well as in endothelia. They consist of transmembrane proteins (proteins of the claudin (Cldn) family, TJ-associated marvel proteins (e.g. occludin (Ocln)), and junctional adhesion molecules (JAMs)), as well as TJ plaque proteins (e.g. zonula occludens (ZO) proteins 1–3, MUPP-1 and cingulin). They are connected with the actin filament cytoskeleton (Fig. 3). The main function of TJs is sealing of the paracellular pathway to restrict the movement of molecules within the intercellular space. Furthermore, TJs are involved in differentiation, proliferation, cell polarity and signal transduction processes of cells [14–17].

In this review we will describe composition and barrier function of TJs as well as their interaction with the other skin barrier components in interfollicular epidermis (Section 2.1) and in the hair follicle (Section 2.2). We distinguish between healthy skin (Section 2) and skin diseases (Section 3), because TJs are clearly altered in several skin diseases which presumably has an impact on skin absorption due to altered TJ barrier function and due to their interaction with SC barrier function. In addition, it is expectable that skin diseases with impaired SC barrier increase accessibility of TJs to topically applied drugs and therefore increases their importance for drug delivery (Section 4). Relevance of TJs for dermal absorption in health and disease and TJ modulators will be described in Section 4. Mathematical modelling of dermal diffusion is a valuable tool to understand skin absorption and drug delivery processes. It will also help us in future to better understand the role of tight junctions in these processes for molecules of different size, polarity and charge as well as in health and disease and is also briefly summarized in Section 4.

2. Tight junctions in healthy skin

2.1. Tight junctions in the interfollicular epidermis

In healthy interfollicular epidermis, functional TJs are located in the SG (Figs. 1-3). However, immunostaining of the epidermis elucidated that only a few TJ proteins are restricted to the SG (e.g. Ocln, cingulin) while some TJ proteins are additionally present in the upper SS (e.g. ZO-1, ZO-2, Cldn-4, -6, -18) or in all living layers (e.g. Cldn-1, -7, JAM-A, MUPP-1) (Fig. 3). This hints for further, TJ-independent tasks of TJ proteins reviewed in [15,18].