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The applied anatomy of human skin: A model for regeneration

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ABSTRACT

Autologous keratinocyte cultures and combinations of scaffolds, different cell types, solutions of macromolecules, or growth factors have contributed to the resurfacing of burns and large wounds. There are, however, significant limitations with these therapies. No tissue-engineered substitute can fully replace the split-thickness skin graft for permanent coverage of full-thickness skin loss in one step, and none contain a functional vascular plexus. Current research characterizes skin as more than a barrier with sensory function, but as an intricate biological factory participating in cell signaling, metabolism, and protein synthesis, and as a vital component of the nervous, immune and endocrine systems. This paper provides a comprehensive review of the structure and function of skin, highlighting the importance of regenerating an organ that will function physiologically.

1. Introduction

Skin has several functions. It provides a barrier to water loss and pathogens and protects against diverse forms of trauma, including thermal, chemical and ultraviolet radiation. Skin keeps us in touch with our environment through a host of nerve endings, regulates body temperature and enhances metabolic functions, as well as synthesizing vitamin D [1]. To fulfill this purpose, regenerated skin requires three integrated structural characteristics: 1. a layered interface, 2. epidermal appendages, and 3. mechanical stability.

2. Layered interface

Many organs have a series of layered interfaces (Fig. 1A); an avascular cellular epithelium that spontaneously regenerates, a basement membrane zone (BMZ) and stroma or vascular supporting connective tissue that does not regenerate [2]. In skin (Fig. 1B), these layers are referred to as an epidermis of stratified squamous epithelium, a BMZ and a fibrous neurovascular dermis which rests on a hypodermis or subcutaneous fat.

The epidermis is mainly composed of sheets of keratinocytes (Fig. 2) but also contains non-epithelial cells, including antigen-presenting dendritic Langerhans cells as well as melanocytes and Merkel cells. The epidermis is nourished by diffusion of intercellular fluids, from the dermal vasculature [3].

There are three distinct layers of nucleated cells. Approximately every 28 days, fully differentiated cuboidal basal keratinocytes with large nuclei, abundant organelles, and a phospholipid membrane migrate apically from the basal layer through the spinous and granular layers [4]. During this turnover process, an accumulation of keratin and lipids ensues which then undergoes terminal differentiation to form the stratum corneum.

Among their functions, keratinocytes proliferate to heal wounds, transport water and urea through aquaporins, receive melanin from melanocytes, control water permeability and participate in innate and adaptive immunity through antimicrobial peptide secretion and through the presence of Langerhans cells, respectively [5].

Of note, keratinocytes do not exist in isolation. Signaling pathways for growth and differentiation of skin through mitotic spindle orientation require molecular interactions between various cells [6]. The whole group is referred to as an interactome and gives the cells polarity, ensuring correct cellular orientation [7].

Reinforcing the epidermis is the dermis, that accommodates the vascular, neural, lymphatic and adnexa of the skin. The dermis provides a durable base that can absorb mechanical forces to prevent shear. It is described as having a superficial papillary zone, comprising relatively thin collagen fibers (Fig. 2), and a much thicker reticular dermis - a compact layer of thicker collagen fibers [8]. The primary cell type is the fibroblast, which produces the extracellular structural proteins, glycosaminoglycans, collagen and elastin fibers (Fig. 2), the latter enhancing the deformability of the dermis. Between the cells and fibers is the extracellular matrix, composed mainly of glycosaminoglycan/ proteoglycan molecules, which hydrate the tissue due to the high water binding capacity of hyaluronic acid [9].

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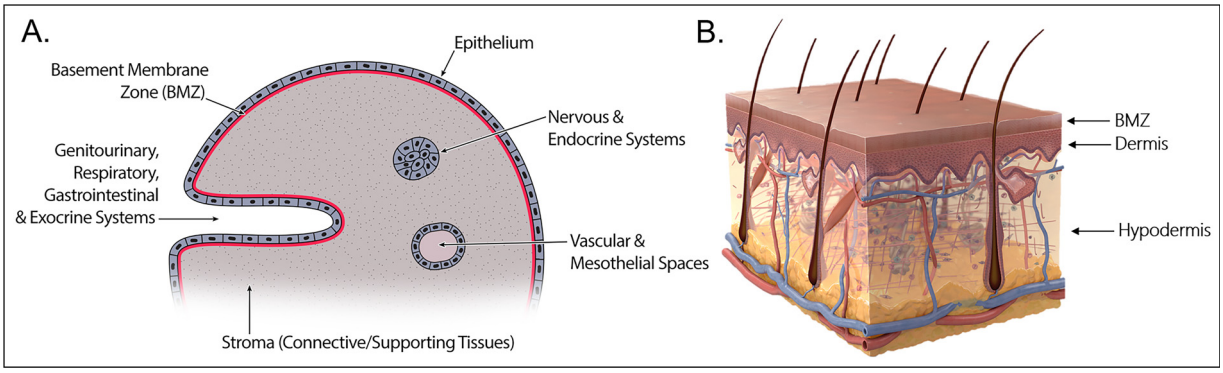

Fig. 1. (A) Simplified scheme of the triad of tissues seen in most organ systems. The BMZ is located between parenchymal cells (epithelia) that cover body surfaces and the space occupied by vascularized stroma (connective tissue) (2). (B) The ultrastructure of skin.

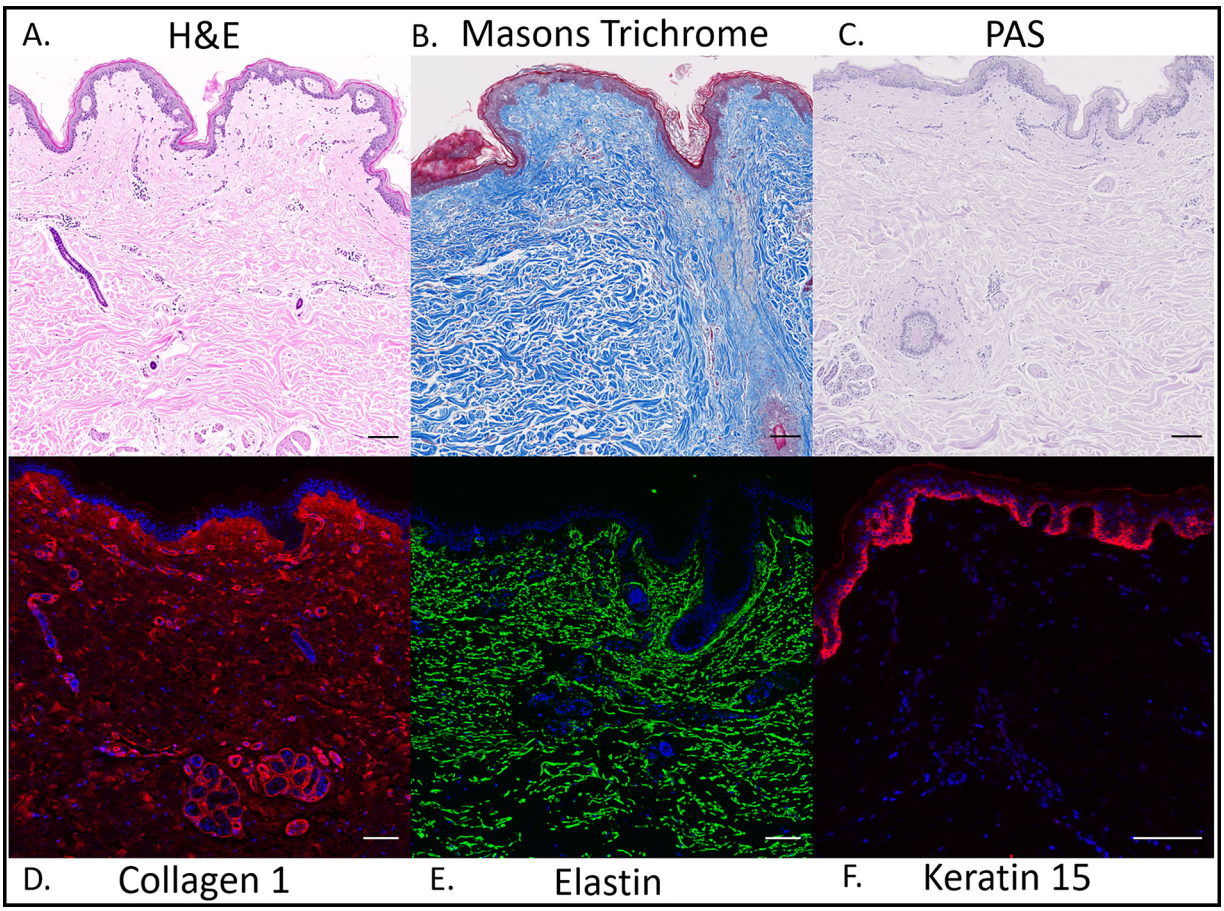

Fig. 2. Expression Trends of Full-thickness Skin – Epidermal layers, epidermal-dermal junction and mature vasculature of human native skin stained with H&E (A). Extracellular components (collagen) stained with Masson's Trichrome (B). Glycoprotein and glycolipid content present in connective tissue and basal lamina of epidermis and dermis (C). Collagen stain (red) in dermis of native human skin (D). Dermal elastin (green) content by fluorescent stain in human native skin (E). Keratin 15 (red) fluorescent stain in epidermis of human native skin (F).

3. Epidermal appendages

Hair follicles, sebaceous glands and sweat glands undergo constant cellular turnover to replace senescent cells and those exfoliated from the surface of the skin.

Efforts to regenerate skin from hair follicles was attempted as early as 1875 when Ernst Schweninger, Assistant Professor at the Institute of pathology in Munich, "grafted" hairs pulled out with their roots and placed these onto granulating wounds [10]. He observed that within 3–5 days epithelial islands formed around the hair roots. His investigations might have received more attention with the advantage of modern imaging technology (Fig. 3).

The hair shaft is about one-tenth of a millimeter wide and weighs only a few millionths of a gram. This organized and segmented structure originates from a major stem cell reservoir, in the outer root sheath below the sebaceous gland, called the follicular bulge [11]. Here the 7-transmembrane receptor, leucine-rich G-protein coupled receptor 5 (Lgr5) is a marker of the Wnt-regulated adult stem cell population. Expression mapping has also found $Lgr6^+$ epithelial stem cells, a close homolog of Lgr5, in a Wnt-independent niche in adult skin just above the bulge, in the isthmus region of the hair follicle [12]. Under physiological conditions, these follicular stem cells and their progeny

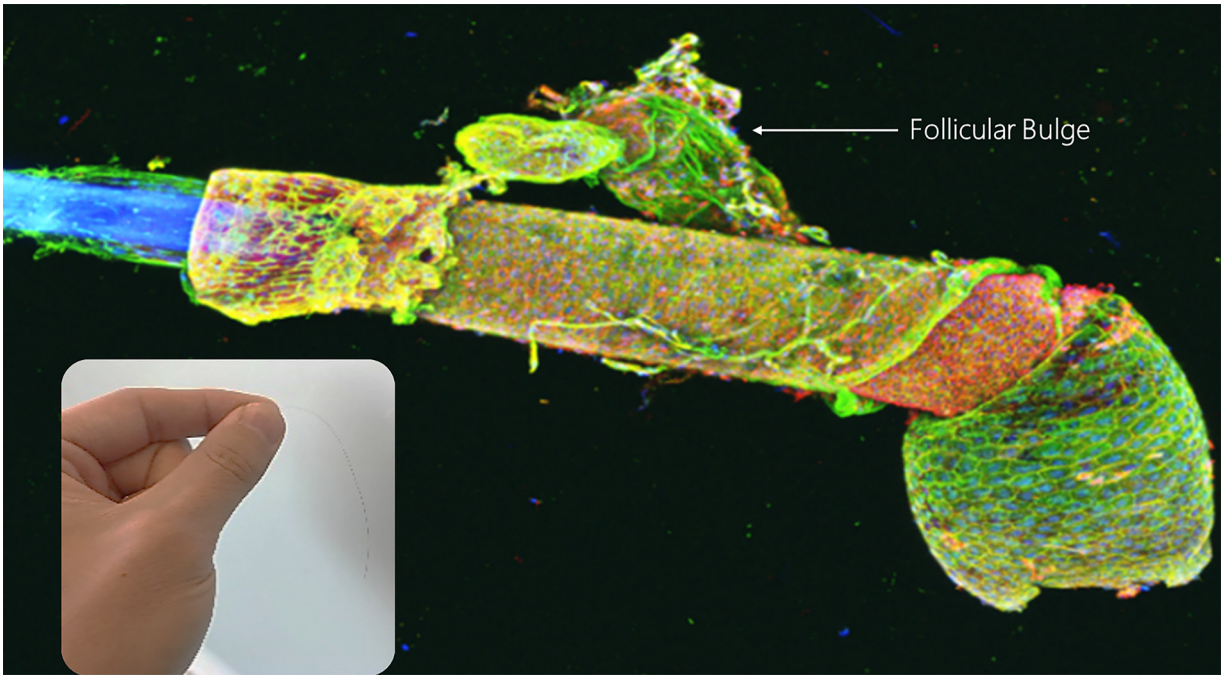

Fig. 3. Confocal microscopy of hair shaft. Left panel: full hair strand taken with digital photography.

contribute only to regeneration of the hair follicle, the isthmus and the sebaceous gland [13–15]. The interfollicular epidermis (IFE) is maintained by stem cells in the basal layer of the epidermis that give rise to short-lived progenitors that produce epidermal proliferative units, while sebaceous-gland cells are maintained by progenitors located above the bulge, which express the Blimp1 protein [16].

When the skin is injured, however, the follicular cells are actively recruited to produce all the cell lineages of skin including the IFE - a process requiring complex epithelial-mesenchymal interactions [12,17].

The development of epithelial culture systems facilitating the growth of human skin from isolated Lgr6$^+$ adult stem cells has led to a breakthrough in the resurfacing of full-thickness skin defects (Fig. 4) [18].

4. Mechanical stability

Thirdly skin must be mechanically stable. Prevention of shearing of the epidermis from the dermis is paramount. This is favored by several mechanisms. Neighboring keratinocytes, bound to each other by desmosomes, and a meshwork of tonofilaments provide a degree of epidermal stability. The epidermis is bound to the dermis by the interlocking of its epidermal projections into the dermis (*rete pegs*) and the complementary dermal capillary loops (*papillae*) projecting into the epidermis, a mechanism that increases the surface area for binding interactions between these layers [19].

More important, however, is the BMZ, a complex continuum of macromolecules which form a network providing a strengthened connection of the epidermis to the dermis [20]. Based on the electron microscopic appearance, the zone is the collective name for four distinct layers. Put simply, hemidesmosomes from the basal keratinocytes anchor keratinocytes to the basal lamina (basal lucida and densa) which is in turn bound to the anchoring plaques in the superficial papillary dermis by anchoring fibrils composed of type VII collagen [1].

5. Functions

5.1. Barrier function

5.1.1. Water homeostasis

The importance of the skin as a barrier is illustrated by the mortality associated with large surface area burns, where increased transepidermal water loss culminates in dehydration, renal failure and shock. Whereas other land-living animals such as insects have adapted by possessing a rigid cuticle coated with hydrocarbons, mammals have evolved a unique tough, flexible stratum corneum [21].

A simplistic view of the structure of the stratum corneum is the *Brick and Mortar* hypothesis, which depicts 10–20 layers of brick-like corneocytes, held together by corneodesmosomes (rivets), embedded in a hydrophobic extracellular lipid matrix (mortar), and enclosed in a protein envelope (Fig. 5) [22].

Eighty percent of the corneocytes is keratin, whose filaments are aligned into disulfide cross-linked macrofibres under the influence of filaggrin [23]. This protein is secreted as a precursor pro-filaggrin, released from the keratohyalin granules of the granular layer. The tortuous pathway between the stacked corneocytes is filled with “mortar” lipids, which are secreted into the intercellular space from the lamellar body secretory system of the granular layer as an intercellular lipid membrane bilayer consisting of three class of lipids: ceramides, cholesterol and free fatty acids in an equimolar ratio (Fig. 5) [24]. Using a water-soluble tracer injected into the dermis, an upward movement of water has been shown to occur between the epidermal cells, but efflux is blocked at the stratum granulosum/stratum corneum interface. From here on, water is lost in vapor form, but it is also trapped within the corneocytes, plasticizing the keratin. Thus, the lipids seal in the moisture but enables transepidermal water loss [25].

The cornified cell envelope is formed from the cross-linking of proteins loricrin and involucrin, also produced from the keratohyalin granules, following the action of calcium-dependent epidermal transglutaminase [26].

The stratum corneum also contains a battery of lipolytic and proteolytic enzymes involved with processing of probarrier lipids, mediating desquamation of corneocytes, and degradation of profilaggrin to filaggrin, which is further broken down into a blend of amino acids

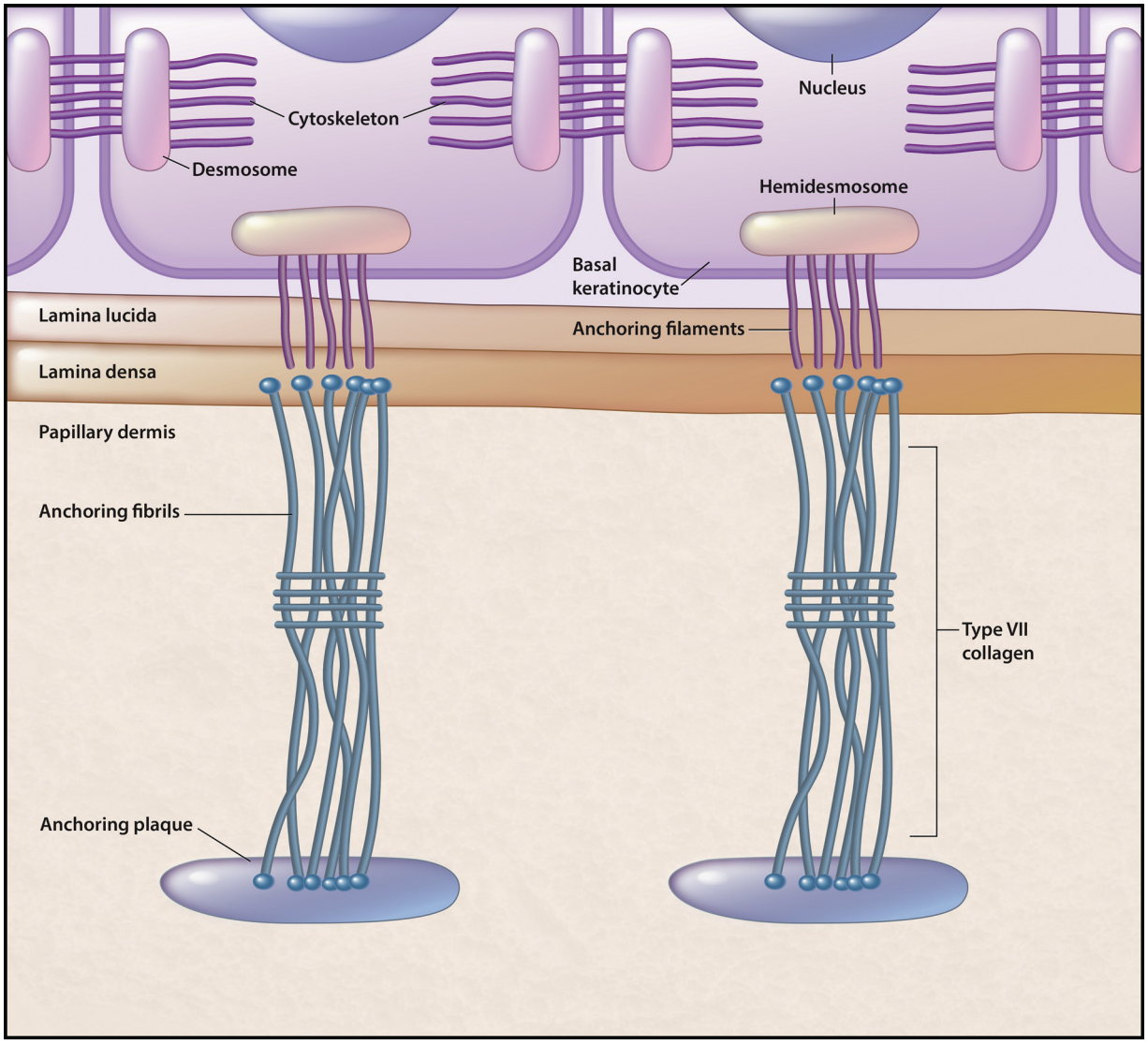

Fig. 4. Microstructure of the basement membrane zone of skin.

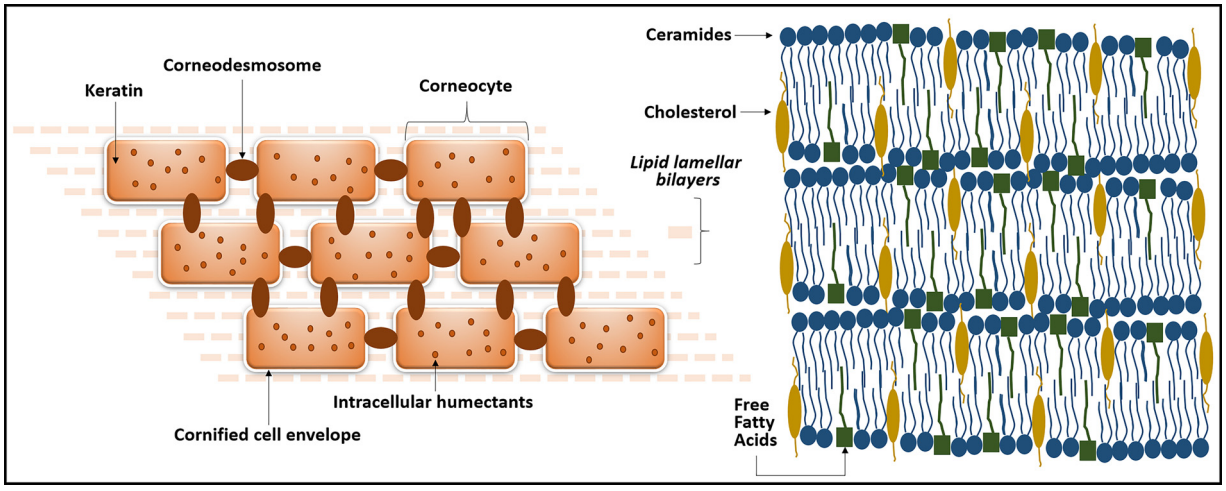

Fig. 5. Arrangements and structure of the stratum corneum.

Table 1
Antimicrobial peptides (innate immune defenses) present in the layers of the skin [28,30,40–49,32,50–58,33–39].

Antimicrobial Peptide	Abbreviation	Structure	Secreted By	Antigen Target and Function	Source
Alpha Melanocyte Stimulating Hormone	α-MSH		Sebaceous glands	Has antimicrobial influences against two major and representative pathogens: *Staphylococcus aureus* and *Candida albican.*	Singh M (2009); Singh M (2014)
Calprotectin	S100A8/S100A9		Neutrophils	Bacterial flagellin induces upregulation of Calprotectin. Antibacterial and antifungal properties are from its ability to sequester manganese.	Abtin A (2010); Brophy (2015)
Cathelicidin	CAP18		Epithelial cells	Rapidly destroys lipoprotein membranes of microbes enveloped in phagosomes after fusion with lysosomes in macrophages.	Kościuczuk EM (2012)
Dermcidin	PIF		Sweat glands	Has a broad spectrum of activity and no homology to other known antimicrobial peptides. Activity remains over a broad pH range.	Schittek B (2001); Song C (2013)
Elafin/ SKALP	ESI		Keratinocytes	Elastase-specific inhibitor: has anti-microbial activity against Gram-positive and Gram-negative pathogens.	Simpson AJ (1999); Pfundt R (1996)
Histone 4	H4		Sebaceous glands	Directly kill microbes and has as antimicrobial activity against *S.aureus* and *Propionibacterium acnes.*	Lee DY (2009)
Human α-defensin 1	DEFA1		Neutrophils	Plays a role in phagocyte-mediated host defense against viral hemorrhagic septicemia rhabdovirus, adenoviruses, and fungi.	Ericksen B (2005); Zou G (2007); Aldred PM (2005)
Human α-defensin 3	DEFA3		Neutrophils	Exhibits strong antimicrobial activity against gram-negative and gram-positive bacteria and fungi, including *Burkholderia cepaci*.	Kocsis AK (2009); Zhao J (2018)
Human β-Defensin 1	HBD-1		Epithelial cells	Provides resistance to microbial colonization in epithelial layers and exhibits diverse host antimicrobial utility.	Ali RS (2001); Jaeger SU (2013)
Human β-Defensin 2	HBD-2		Epithelial cells	Exhibits potent antimicrobial activity against Gram-negative bacteria and *Candida*. Contributes to infrequency of Gram- infections in skin.	Ali RS (2001); Schröder JM (1999)
Human β-Defensin 3	HBD-3		Keratinocytes	Exhibits antibacterial activities towards Gram-positive and Gram-negative bacteria, and acts as a chemo-attractant.	Dhople V (2006)
Lactoferrin	LTF		Sebaceous glands	Multifunctional iron glycoprotein that exerts a broad-spectrum primary defense activity against bacteria, fungi, protozoa and viruses.	Orsi N (2004); Yen CC (2011); van der Strate BW (2001)
Lysozyme C	LYZ		Sebaceous glands	A typical antibacterial protein, exibits muramidase activity against the Gram-positive bacteria *Micrococcus luteus* and *Bacillus subtili.*	Kajla MK (2010); Nester EW (2007)
Psoriasin	S100A7		Epithelial cells	Expression is induced in skin wounds through activation of the EGFR. A key factor against *E. coli* by disrupting their cell membranes.	Lee KC (2007); Bulet P (2004)
Ribonuclease A Family Member 7	Rnase 7		Stratum corneum	Described as a main player in the unusually high resistance of human skin against infections.	Harder J (2002)
Secretory leukocyte protease inhibitor	SLPI		Epithelial cells	Protects epithelial surfaces from attack by endogenous proteolytic enzymes. SLPI has antibacterial, antifungal and antiviral activity.	McNeely TB (1995); Doumas S (2005)

collectively called the NMF, or Natural Moisturizing Factor, which plasticizes keratin [27].

5.2. Immunological barriers

5.2.1. Innate immunity

Skin is an active immunological organ, and dysfunctional innate defenses have serious clinical implications. Products of the stratum corneum, including free fatty acids, polar lipids, and glycosphingolipids accumulate in the intercellular spaces and horny layer, exhibiting antimicrobial properties, and functioning as a first line of defense. Antimicrobial peptides (AMPs) exhibit potent and targeted resistance against a wide spectrum of common pathogens [28]. When this barrier is breached, second lines of protection are provided by inflammatory cascades in the subepithelial tissue.

Approximately sixteen AMPs have been shown to be expressed in the skin (Table 1). The main antimicrobial families are known as cathelicidins and defensins [29,30]. Defensins are classified as human beta and alpha defensins, based on their size and pattern of disulfide bonding, and have been shown to be localized to the basal layer of keratinocytes, in the dendritic cells of the stratum spinosum, in the dermal glandular structures and in hair follicles [31]. Human α-defensin 1 and 3 (DEFA1 and DEFA3) are secreted by neutrophils and play a major role in phagocyte-mediated host defense against viral hemorrhagic septicemia rhabdovirus, adenoviruses, fungi, and exhibits potent antimicrobial activity against gram-negative and gram-positive bacteria, including *Burkholderia cepacia* [32,33]. The antimicrobial mechanism(s) of action of defensins comes from their ability to penetrate antigenic membranes, where they form channels or "pores," ultimately destroying the bacterium by altering membrane conductance and affecting intracellular function, with a consequent lack of antigenicity and chemical and biological resistance.

Multiple functions of AMPs have been identified by mechanisms that are not entirely understood. They are chemotactic for neutrophils, monocytes, immature dendritic cells, memory T-cells, and mast cells and play a role in recruiting them (Fig. 6). They also alter transcriptional responses in macrophages, induce degranulation of mast cells, and vascularization and epithelialization in wound healing as well as altering antibody production, thus linking innate and adaptive immunity.

5.2.2. Adaptive immunity

After an infection incurs, the adaptive immune response is regulated by Langerhans cells which are antigen presenting cells that reside in the epidermis and capture, process, and present pathogens to T lymphocytes in local lymphoid tissues (Fig. 6) [59]. Lymphocytes then migrate to the epidermis where they reside as memory T cells and upregulate inflammatory pathways [60]. During the immunostimulation phase of

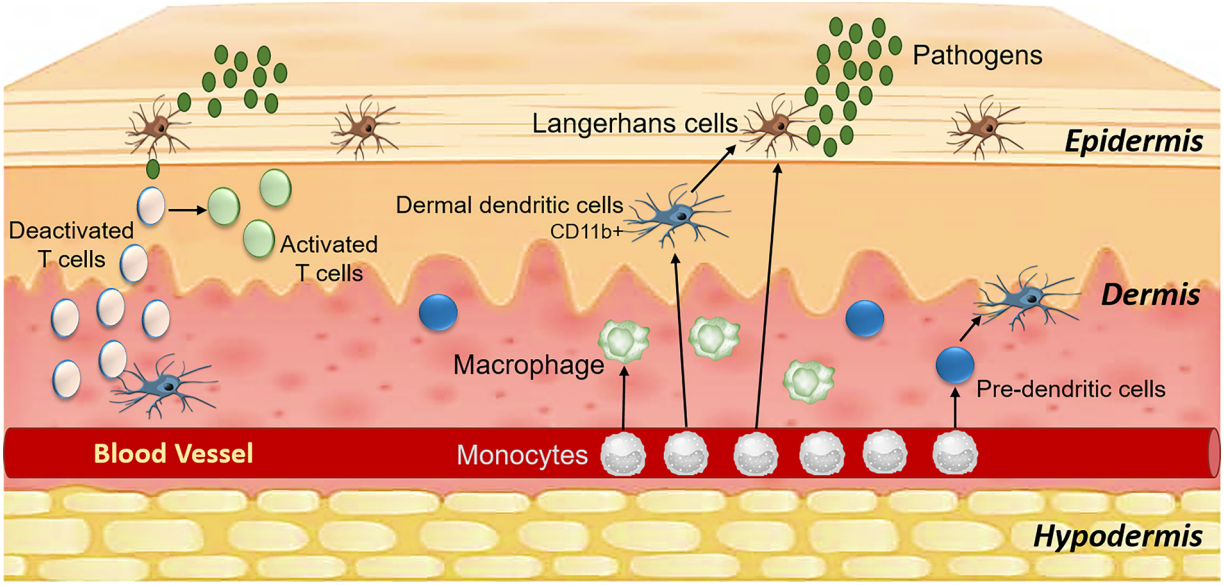

Fig. 6. Immune surveillance in the dermal compartments is controlled by a variety of skin-based dendritic cells, macrophages, and resident T cells.

the adaptive immune response, lymphocytes are converted into T helper cells.

Langerhans cells express an antigenic peptide with an affinity towards MHC-I molecules, which promotes cytotoxic T cells (CD8+) and effector functions with the capacity to induce the secretion of cytokines in the dermal layers [61]. Recent studies have elucidated that specific components of the microbiota selectively promote a targeted activation and proliferation of cytotoxic T cells [62]. This adaptive immune process restricts antigenic invasion and subsequently expands the innate immune barrier in an IL-17 (pro-inflammatory cytokine) dependent manner [63]. CD11b + dermal dendritic cells coordinate a precise immune defense after interacting with pathogens which stimulates the proliferation of IL-17A + CD8 + T cells by way of IL-1α production [64]. This mechanism is antigen-specific and is part of adaptive immune processes; however, it works in harmony with innate immune defenses which augments the protection against bacterial invasion and infection. The interplay between the innate and adaptive immune system is powered by the production of calprotectin (alarmins) S100A8 and S100A9 which induce microbicidal responses and act as potent chemo-attractants for neutrophils (Table 1) [61]. Also, the IL-1-controlled cascade has been observed to upregulate mRNA expression of Human β-defensins 2 and 3 in skin [65].

Most types of bacteria have been shown to increase the number of T cells in the skin [66]. Interactions between T cells and microbial antigens trigger the promotion of various immune cells to attack specific microbes in the dermal compartments (Fig. 6). Skin provides targeted protection against invasive pathogens and regulates barrier immunity via symbiotic chemo-signaling, highlighting that innate and adaptive immune responses work together as a robust first line of defense. These findings demonstrate that the skin's immune defense system is highly dynamic and can be rapidly and specifically remodeled by a full spectrum of micro-organisms.

5.3. Ultra-violet light protection

Biosynthesis of melanin involves a complex pathway that occurs in melanocytes, within membrane-bound organelles called melanosomes. Melanocytes are present in the basal and suprabasal layers of the skin and in the hair follicles and transfer melanosomes through dendritic processes where they form melanin caps that reduce the harmful effects of ultra-violet light. Each melanocyte communicates with 30–40 keratinocytes [67].

Several important steps must occur for the synthesis and distribution of melanin. Two major types of melanin are produced – pheomelanin and eumelanin, which differ in color and synthetic pathway.

The reservoir for melanocyte stem cells has been found to lie in the bulge area of hair follicles, which contain pluripotential, morphologically undifferentiated cells which develop into melanoblasts and migrate to the hair and epidermis, where they differentiate into mature melanocytes [68].

5.4. Sensory function

The skin receives, processes and transmits environmental information from multiple sensory receptors. Its information processing system and ability to release neurotransmitters and hormones that influence whole-body states and emotions, has prompted Denda to liken the organ to an auxiliary brain [69]. His analogy suggests encoding of the various sensory modalities requires keratinocyte-neuronal signaling. The resulting stimulus is translated into an action potential, which is transmitted via afferent nerve fibers to the central nervous system.

Scattered throughout the skin are specific epidermal cell receptors. Amongst the basal keratinocytes in the epidermis are Merkle cells [70]. These slowly adapting mechanoreceptor cells detect light touch, as well as spatial features such as curvature and edge. Myelinated axons (diameter about 5 μm) lose their myelin sheath, penetrate the basal lamina and branch extensively to supply up to 50 Merkel cells. Hoffman et al. have elegantly shown that Merkel cells communicate with sensory neurons through α2-adrenergic receptors at excitatory synapses [71].

Two types of sensory receptors are found in the dermis; bare nerve endings, for nociception, thermal sensation (heat and cold); and capsulated mechanoreceptors such as Meissner's corpuscles, that detect moving touch, and Pacinian corpuscles, perceiving vibration and brief touch [72].

The skin, however, has the capacity to detect a pattern of mechanical stimuli on a smaller scale than would be expected merely by the location of the nerve terminals [73]. This is due to the keratinocytes themselves containing sensors that can recognize and respond to a wide range of physical and chemical environmental factors. Members of the transient receptor potential family (TRP) are expressed with the ability to sense a range of environmental stimuli. For example, TRPV1 is strongly expressed near the surface of the skin which is consistent with its role in detecting temperature changes [74]. TRPV3 and 4 are activated by mechanical stress and changes in osmotic pressure,

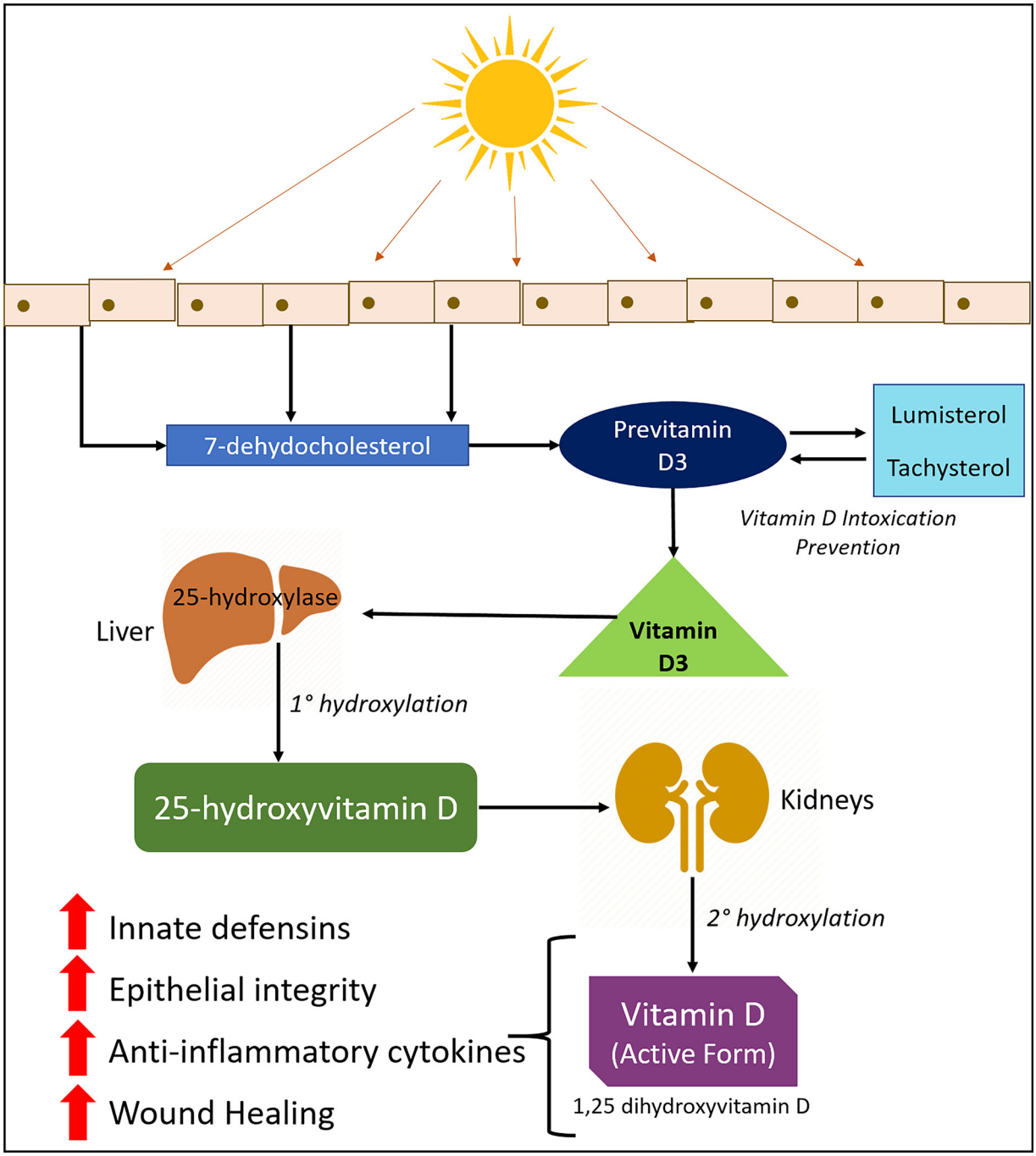

Fig. 7. Vitamin D synthesis promotes biological activity, initially facilitated by the skin.

respectively [75,76]. In addition, a variety of environmental factors, including visible light and sound can influence epidermal permeability barrier homeostasis. Indeed, keratinocytes express photo-receptor like proteins present in the retina [77].

Other modes of communication exist between the epidermis and the central nervous system. Keratinocytes release neurotransmitters, neuropeptides, hormones and cytokines which transmit and process sensory information in the CNS and can affect the physiology of multiple systems [78].

5.5. Vitamin D synthesis

The skin is a ready source of vitamin D following sun exposure. It is a fat-soluble prohormone steroid primarily acknowledged for its endocrine role in calcium homeostasis maintaining levels of serum calcium through control of calcium and phosphate absorption from the intestine, and resorption of bone [79]. However, it also has other biological effects playing a lesser role in the regulation of skin differentiation, immune function, hair follicle cycling, photo-protection, upregulation of innate immune defenses and wound healing [80–82].

Vitamin D exists in the plasma membranes of basal and suprabasal keratinocytes and dermal fibroblasts in its inactive form 7-dehydrocholesterol, and is first converted to previtamin D3 prior to hydroxylation in the liver and in the kidneys to its active form 1,25-dihydroxyvitamin D. Overproduction of vitamin D is prevented by a feedback loop whereby previtamin D3 is converted to the inactive photoproducts lumistrol and tachysterol (Fig. 7).

5.6. Temperature control

The skin controls body temperature. The underlying adipose tissue insulates against conductive heat loss, whereas loss of heat is facilitated actively by evaporation of sweat from the skin surface and by increased blood flow through the rich vascular network within the dermis.

From the thermoregulation perspective, our skin is heterogeneous. There is non-hairy glabrous skin that covers the most distal body parts and hairy non-glaborous skin, covering the rest of the body.

The main thermosensory-related role of non-hairy glabrous skin is to assess local temperatures and tactile signals. It is characterized by the absence of hair, dense vascularization, the presence of arteriovenous anastomoses and a large surface-to-volume ratio. It can mount two opposite responses. The body's thermoregulatory response to overheating is achieved by vasodilatation with increasing blood flow and rapid release of heat into the environment. Remarkably, in the human finger, the flow can increase by 500 % [83]. Secondly, cutaneous vasoconstriction and closing of the anastomoses cause an abrupt cessation of heat loss.

The body is covered, however, mostly by hairy (non-glabrous) skin, which is typically insulated from the environment (with clothes in humans and with fur in non-human mammals). Thermal signals from hairy skin represent a temperature of the insulated superficial layer of the body and provide feedback to the thermoregulation system [84]. The hairy skin is characterized by the lack of arteriovenous anastomoses and by the presence of hair follicles. Both features make the skin better suited to serve as a thermal insulator.

6. Discussion

Skin is an intricate, self-renewing organ that is our primary defense barrier against a hostile environment. It protects against harmful antigens and chemicals, dehydration and overhydration, and ultraviolet radiation. It provides structural integrity and resilience, allows selective absorption, antioxidant storage, controls thermoregulation through fluctuations in cutaneous blood supply and perspiration and can stimulate epidermal regeneration when injured.

The clinical significance of skin is illustrated by the morbidity associated with burns and cutaneous defects. Current models of biological research have not yet surpassed the skin graft as a standard of care for the resurfacing of full-thickness wounds. We highlight the importance of attaining induced organ regeneration to replicate all essential processes of skin.

Author contributions

JA and SM composed the original draft, JA designed and created 4 of the figures and designed and created Table 1. JA also contributed to multiple revisions and drafts of this manuscript. NA and SM determined the scope and aims of this review article. NA ensured scientific accuracy of the paper, consulted on the design and flow of the figures as well as contributed to multiple revisions and drafts of the manuscript. SM contributed multiple revisions and drafts of this manuscript, designed 3 of the figures and ensured clinical accuracy of the article.

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Ethical statement

This work does not involve the use of human subjects and did not utilize animal-model experimentation. This paper is a comprehensive review of recent literature; therefore, informed consent, and adherence to various regulatory protocols were not incorporated in this work.

Declaration of competing interest

This work was supported by the Department of Research and Development at PolarityTE™, Inc. Salt Lake City, UT, USA. Joseph Abdo, Nikolai Sopko and Stephen Milner are employees of PolarityTE. PolarityTE had no role in the design, information collection and analysis, decision to publish, or preparation of the manuscript. The authors have no other conflicts of interest regarding this work.

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