CHAPTER 5

RESIDENT MICROFLORA AND ANTIMICROBIAL PEPTIDES OF SKIN

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5.1 INTRODUCTION

Skin is the most exposed organ of human body and acts as the body's first line of defense against infection and illness. Skin is also the largest organ of the body with a surface area of about 2 m^2 in an average human adult. This vast organ is originally microbe free, but soon after birth microbes start to colonize on the stratum corneum and eventually establish a complex microbial ecosystem [1,2]. The health of skin depends upon the delicate balance between skin and millions of bacteria and other organisms such as fungi, viruses, and mites on the skin [3]. A variety of physico-chemical factors in skin such as acidic pH, moisture, and sebum content influence the growth of skin microflora.

In recent years, a comprehensive research expedition on skin microbiomes has been launched essentially to uncover and analyze all the skin microbes at the DNA and genome levels. Studies published so far have uncovered new findings [3–5]. Analysis of 16s ribosomal RNA gene sequence obtained from 20 distinct skin sites reveals the presence of a much wider array of bacteria than previously thought [3]. The study also found that body location greatly influences bacterial diversity. For example, the bacteria in the underarm are more similar among different individuals than the bacteria on the forearm.

Human skin is also equipped with a complex network of innate immune system to defend itself from microbial attacks and invasion. The skin's antimicrobial defense apparatus includes various physical and chemical factors. For example, the mechanical rigidity of the stratum corneum, increased phospholipase A_2 activity, low moisture content, lipids, lysozyme, and the continuous exfoliation process by which the outer layer of skin completely renewed every 15–28 days [6–8] are all part of the innate immune system. At cellular level, Langerhans' cells (also termed as dendritic cells) that reside in the epidermis play an important role as immune cells [9].

Innate Immune System of Skin and Oral Mucosa: Properties and Impact in Pharmaceutics, Cosmetics, and Personal Care Products, First Edition. Nava Dayan and Philip W. Wertz.

Langerhans' cells alert the immune system about in-coming threats. They produce cytokines, signal molecules that influence the immune system to take action against various external stimuli such as sun, smoke, and other environmental assaults. Langerhans' cells also modulate biochemical imbalances that lead to inflammatory skin conditions. They are involved in boosting collagen synthesis and enhance wound repair process. Recent experimental evidences are emerging that propose different role for these important cells [9,10]. The role of these cells is discussed in detail elsewhere in this book.

Functionally, skin's innate immune system is also involved in the release of various immune mediators, such as cytokines and chemokines, recruitment and activation of phagocytes, and the production of defensins or antimicrobial proteins/peptides [11–14]. Antimicrobial peptides (AMPs) act as innate chemical shield, which provides a frontline defense against microbial invasion. Apart from exhibiting a broad spectrum microbicidal properties, AMPs are also involved in activation and modulation of the immune system. Thus, improving the skin's innate immune defenses is the best way to achieve a healthier skin and may also benefit overall health of the body. The aim of this chapter is to discuss the resident microflora and how this diverse population of bacteria reacts or parts with the skin under normal health or disease conditions. We will also discuss the antimicrobial peptides of skin and highlight their respective roles in health and disease, and provide a brief overview on skin cleansers and their impact on skin health and immunity.

5.2 RESIDENT MICROFLORA OF THE SKIN

Bacterial colonization of human skin begins during birth and the process continues in next several months and years to stabilize the microflora growth on the skin. Being the most exposed organ of human body, skin constantly encounters a variety of micro-organisms. Billions of bacteria reside on the skin. Bacteria–skin relationship can be commensal (i.e., the type of relationship between two organisms in which one organism benefits but the other is unaffected), symbiotic (i.e., close, often long-term relationship between different biological species), or parasitic (i.e., the relationship in which one organism, parasite, lives off another organism or host) relative to the host's overall physical and immune status. The status of microbes on skin can be temporary or transient and permanent or resident biota. Transient bacteria are those that come in contact with the skin and live temporarily due to unfavorable skin environment. On the other hand, permanent or resident microbiota are members of the normal flora that live in or on the body permanently without causing any harm to healthy individuals under normal conditions. Permanent colonization of a bacterial species depends on the adaptability of the organism to adhere to the skin epithelium, grow in a relatively dry and acidic environment, and establish relationships that are more mutualistic than commensalistic [15–17]. Bacterial adhesion or detachment from the skin could be mediated by (a) specific interactions via lectin or sugar binding, (b) hydrophobic interactions, and (c) electrostatic interactions [18,19].

The colonization of specific bacterial species and their population density on various location of the skin depend on physicochemical characteristics of the skin and

environmental factors of that particular niche, for example, the anatomical location, amount of sebum and sweat production, pH, moisture content, temperature, and light exposure [20,21]. Skin's microbial composition and density are also influenced by the age, personal habits, and immune and hormonal status of the host [22,23]. Not all bacteria are able to reside on skin; only those bacteria that protect the host from pathogenic bacteria both directly and indirectly are allowed to take the resident status on skin. These resident bacteria provide protection to the host by producing antibiotics (e.g., bacteriocin) and toxic metabolites, inducing a low reduction–oxidation potential, depleting essential nutrients, preventing attachment of competing bacteria, inhibiting translocation by degrading toxins, and so on [15,22]. Minor racial and gender differences as factors in skin microflora have been suggested [4,24]. Interestingly, in a recent study, in which the taxonomic diversity, evenness, and richness of each sites' microbiome were examined using the ecological diversity statistics [3], revealed that the richest site with diverse microbiome was the volar forearm. The most even site was the politeal fossa; the least even sites were back, retroauricular crease, and toe web space. In general, the sebaceous sites were less diverse, less even, and less rich than moist and dry sites [3].

Common resident bacterial species on normal skin belong to Gram-positive bacteria that include *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Brevibacteria*, *Propionibacteria*, and *Acinetobacter* [20,22,25,26]. These bacterial species have specific affinity for different skin sites, some are more populated in underarm and groin area and others are more prominent in drier areas (Table 5.1).

The Gram-negative bacteria make up the minor constituents of the normal skin flora. Acinetobacter is one of the few Gram-negative bacteria commonly found on skin. Generally, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia*

TABLE 5.1 Resident Skin Bacteria and Their Dominant Sites

Bacteria	Skin site
S. epidermidis	Upper trunk
S. hominis	Glabrous skin
S. aureus	Nostrils, sebaceous sites
S. capitis	Head
S. saccharoliticus	Forehead/antecubital
S. saprophyticus	Perineum
M. luteus	Forearm
Corynebacterium xerosis	Perineum, axilla, conjuctiva
C. minutissimum	Toe webs, axilla, intertriginous area
C. jeikeium	Intertriginous (e.g., axilla) area
P. acnes	Sebaceous gland, forehead
P. granulosum	Sebaceous gland, forehead, axilla
P. avidum	Axilla
Brevibacterium spp.	Axilla, toe webs
Dermabacter spp.	Forearm
Acinetobacter spp.	Forearm, forehead, toe webs

coli, and *Pseudomonas aeruginosa* can be isolated from skin as transient colonizers [25,27]. The presence of *E. coli* on the skin surface is indicative of fecal contamination. Yeasts and fungi are uncommon on the skin surface, but the lipophilic yeast *Pityrosporum ovalis* is occasionally found on the scalp.

In recent years, the advances in sophisticated molecular techniques have led to new discoveries in the microbiome of the human body including skin [28–33]. Using molecular tools some unknown species that had never before been identified on skin or described in the literature were identified [4,34]. A total of 182 species of bacteria were identified on human forearm skin, of which 8% were unknown species [35]. Roughly, half the bacteria identified in the samples were normal resident bacteria such as *Propionibacteria*, *Corynebacteria*, *Staphylococcus*, and *Streptococcus*. Interestingly, the study also noticed subtle individual and gender differences in the type of bacterial species isolated.

A recent study in which the palmar surfaces of the dominant and nondominant hands of 51 healthy young adults were examined using a novel pyrosequencing-based method for bacterial diversity and variability within and between individuals [36]. The study found highly diverse bacterial communities, >150 unique species-level bacterial phylotypes, and identified a total of 4742 unique phylotypes across all the hands examined [36]. The study also found a higher bacterial diversity in women than men, and bacterial community composition was greatly affected by handedness, time since the last hand washing, and gender.

5.3 PROTECTIVE ROLE OF RESIDENT MICROFLORA

The permanent residency of the skin flora is the direct result of a mutually beneficial relationship between the bacteria and the host. One of the attributes of skin's innate immune system is that it allows commensal bacteria to establish residency so that it prevents invasion by potential pathogenic organisms. Normal flora thrives on the host-generated nutrients and in turn the microflora acts as protective barrier and directly or indirectly prevents invasion and growth of pathogenic bacteria [37,38]. A healthy population of the resident bacteria effectively denies the colonization by transient bacteria including *E. coli*, coagulase positive *S. aureus*, group A *Streptococci* (GAS), *Pseudomonas* spp., and *Candida albicans*. Some resident bacteria produce antibiotics, for example, bacteriocins, toxic metabolites, create low reduction–oxidation potential, deplete essential nutrients, prevent attachment, and degrade toxins produced by the invading microbes. For example, *S. epidermidis* binds to keratinocyte receptors and prevents attachment of pathogenic *S. aureus* [39,40]. *S. aureus* strain 502A releases bacteriocin that inhibits other virulent staphylococci [40]. *Propionibacterium acnes* releases fatty acids from sebum breakdown, thus acidifying the milieu and inhibiting the growth of *S. pyogenes* [41].

It has also been suggested that skin pathogenic and commensal bacteria such as group A *Streptococci* and *S. epidermidis*, respectively, upregulate keratinocyte human β-defensin 2 (HBD2) utilizing different signaling pathways [42]. The signal transduction leads to the expression of antimicrobial peptides, proinflammatory cytokines, chemokines, and inducible enzymes. Indirectly, resident bacteria can

induce host immune system; for example, they stimulate phagocytosis and augment interferon and other cytokines' production. This protective role of normal flora suggests that an excessive use of antimicrobial skin cleansers because of not exhibiting a selective mode of action may make the skin vulnerable to infection by more hostile Gram-negative bacteria rather than protecting it [43–45].

5.4 ROLE OF SKIN MICROFLORA IN SKIN DISEASES

One of the important benefits of the normal resident flora is preventing transient pathogenic organisms from colonizing the skin. Therefore, cutaneous invasion and infection depends on the health status of the skin, its local microenvironment, immune status, and pathogenic potential of invading microbes. Resident microbes can also cause skin infections and in some cases can lead to life-threatening conditions in the host, particularly immunocompromised people [46]. Among the transient Gram-negative organisms, *Pseudomonas aeruginosa*, *Pasteurella multocida*, *Klebsiella rhinoscleromatis*, and *Vibrio* spp. are known to cause skin infection. Pathogenicity of bacteria depends on their ability to attach to, grow on, and invade the host tissues [20]. Bacteria possess virulence genes that facilitate the invasion and subsequent disease process. For example, *S. aureus* and *S. pyogenes* produce toxins that may elicit superantigen response triggering a massive release of cytokines. These superantigens cause Staphylococcal scalded skin syndrome, toxic shock syndrome, and scarlet fever [47]. The normal flora also carries the opportunistic character. Resident organisms have been shown to play a role in noninfectious skin diseases such as atopic dermatitis [48], rosacea, psoriasis [49], and acne [50]. Bacteria otherwise nonpathogenic in normal skin condition can become pathogenic when the skin is compromised [23].

There is a selective shift in microbial community linked to some skin diseases. In atopic dermatitis, recurrent bacterial infection and heavy recolonization of *S. aureus* was linked to the antibiotic resistance, nasal carriage, and treatment contamination [51–53]. In psoriasis, the microbial population was higher than for other normal skin samples [5] and a substantial alteration in microbial flora was observed. Bacteria isolated from patients with rosacea have shown to function very differently from the same type of bacteria isolated from normal rosacea-free skin. Bacteria isolated from patients with rosacea also produced different types of proteins in different amounts at 37°C versus at 30°C temperature. These observations indicate that increase in facial skin temperature in rosacea patients is likely to play a role in altered bacterial protein synthesis [54]. A great deal of research is required to understand the shift in specific microbiota under disease condition, which would lead to novel approaches to control numbers of overrepresented organisms and help repopulate normal resident flora that diminish in disease.

5.5 INNATE ANTIMICROBIAL SKIN DEFENSES

The physical properties of skin, for example, dryness, acidic pH, and temperature lower than 37°C, act as defensive barriers are mostly unfavorable conditions for bacterial growth. The dead keratinized cells that are constantly being sloughed off

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