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

in a way that removes the colonized organisms from skin acts as a "cleansing mechanism" that adds to the overall protective attributes. Chemical components of the antimicrobial defense include lysozymes, RNases, and antimicrobial lipids secreted via hair follicles and produced by sweat glands [55,56]. One of the key components of this natural immune defense system is a group of antimicrobial peptides such as cathelicidins and defensins produced by the epithelial cells. Evidence for the important role of antimicrobial peptides in innate immunity is well documented [57,58]. These antimicrobial proteins are gene-encoded endogenous proteins. Several *in vivo* studies have demonstrated that AMPs have a broad spectrum of activity and the capacity to protect the host against a wide range of bacteria, viruses, fungi, and parasites [59–62]. AMPs are capable of killing bacteria, fungi, viruses, and parasites at micro- and nanomolar concentrations. The minimal inhibitory concentration (MIC) of these peptides ranges from 0.1 to 100 μg/mL [63]. Production of these peptides is very limited under normal conditions; however, some of these AMPs are upregulated at sites of infection, inflammation, and tissue injury, such as human β-defensins 2 and 3 (HBD-2, HBD-3) and cathelicidin LL-37 [64–68].

Recent studies have revealed that some of these AMPs, especially cathelicidins, protect the skin through two distinct pathways: (1) by direct antimicrobial activity and (2) by inducing various immune apparatuses such as cytokine release, angiogenesis, and reepithelialization [69,70]. Most of these peptides are small 12–50 amino acids, positively charged with an amphipathic structure. Structurally, these AMPs can be divided into several categories such as peptides with an α-helix structure, peptides with a β-sheet structure stabilized by disulfide bridges, or peptides with an extended or loop structure. Beneath the epidermis of the skin are Langerhans' cells, the immature dendritic cells that phagocytose and kill microbes.

In human skin, major antimicrobial peptides include human β-defensins, cathelicidins or LL-37, dermacidin (DCD), and psoriasin [71]. In addition to these peptides, there are a number of other components (Table 5.2) such as BPI [72,73], sphingosins [74], lactic acid [75], lysozyme [63,76], RNase 7 [77] that play a role in host immune defense. These defense molecules are at work even before birth since

TABLE 5.2 Examples of Selected Defense Components of Human Skin

Defense component	Function	References
LL-37	Antimicrobial and immune modulatory activity	[14,86]
Beta-defensins	Antimicrobial, antienveloped viruses Activation of dendritic cells	[44,87–89,117]
Dermacidin	Antibacterial and antifungal	[90,127]
Psoriasin	Antibacterial and chemokine activity	[92,128]
BPI	Active against G (−) bacteria	[73]
Fatty acids	Prevent microbial growth	[53,56,75,134]
Sphingosine	Natural antibacterial agent	[74,129,130]
Lysozyme	Hydrolyzes bacterial cell wall peptidoglycan	[63,76]
RNase 7	Antimicrobial, ribonuclease activity	[77,93]

many of these molecules are present in the vernix caseosa (lipid-rich film) that attaches to fetal and neonatal skin [78].

The mode of action of most AMPs is not clearly understood; however, it is largely believed that most AMPs kill microbes by causing membrane leakage or disruption [79–81]. A number of models such as "barrel-stave" and "carpet-like" for membrane permeation by amphipathic α-helical peptides have been proposed, but these models may not properly reflect the complex processes involved in the killing of microorganism [80,82,83]. In the barrel-stave model, channel-forming peptides position in a ring, which is barrel-like, around an aqueous pore. Peptides or peptide complexes may constitute individual transmembrane spokes in the barrel, hence the term "stave" in the model. In the carpet mechanism, a high density of peptide acts upon the microbe and causes phospholipid displacement, changes in membrane fluidity, and/or reductions in membrane barrier properties, which lead to membrane disruption. The peptides initially bind to the membrane by electrostatic interactions, yet no quaternary structures are formed. Rather, the membrane integrity is lost after a sufficient concentration of peptides is reached, causing unfavorable membrane energetics. Peptides operating under this model do not form channel-like structures and may not integrate in the hydrophobic membrane core.

Cathelicidins and β-defensins are well characterized of the AMPs found in the skin. Methicillin-resistant *S. aureus* (MRSA) is an important public health problem. Skin AMPs are one of the key host factors that determine whether MRSA colonization/exposure results in infection of skin or soft tissue [84]. A better understanding of the biology of these compounds would clarify the pathophysiology of inflammatory and infectious diseases [85].

With the rise in antibiotic resistance in managing infectious diseases, these immune defense peptides may provide useful alternatives to conventional antibiotics. A brief description of key AMPs is given in the following sections.

5.5.1 Human Cathelicidin LL-37

Cathelicidins are effectors of innate immune defense in mammals. Humans and mice have only one cathelicidin gene, whereas domesticated animals such as cows, pigs, and horses have multiple cathelicidin genes. The human cathelicidin LL-37 is an α-helical, cationic antimicrobial peptide, and cathelicidin expression *in vivo* is highly resistant to proteolytic degradation. LL-37 is induced in normal keratinocytes in response to injury or inflammation or vitamin D [94,95]. However, in sebocytes, cathelicidin expression is both constitutive and inducible [96]. Cathelicidin LL-37 produced by sebocytes has been shown to kill microflora such as *S. aureus* and *P. acnes in vitro* [96]. It is now recognized that several cell types in the skin produce cathelicidin, including keratinocytes, mast cells, neutrophils, and ecrine glands [94,97–100].

Under normal conditions, LL-37 level is very minimal, but in wounded skin the LL-37 level can reach up to 2000 ng/mg of total protein [101,102]. In sweats and seminal fluids, the concentration of LL-37 can be as high as 360 ng/mL and 140 ng/mL, respectively [98]. Interestingly, some of the cathelicidins found in sweats and seminal fluids are suggested to be the novel processed cathelicidin with enhanced antimicrobial

activity [103]. LL-37 shows activity against *C. albicans in vitro*, even in physiological saline. This indicates that LL-37 can function in salty environment such as the skin surface that is salty due to the presence of sweat and its subsequent evaporation. LL-37 acts as a potent antisepsis agent by inhibiting macrophage stimulation by bacterial LPS, lipoteichoic acid, and noncapped liporabinomannan [104]. A recent study suggests that LL-37 inhibits replication of vaccinia (pox) virus [86]. It was also demonstrated that LL-37 protects mice against endotoxemia.

Advances in cathelicidin research have revealed that LL-37 is a multifunctional peptide with receptor-mediated effects on eukaryotic cells and its involvement in chemotaxis, angiogenesis, and wound healing [67,105–110]. On the other hand, cathlicidin disfunction has been linked to many skin disorders such as atopic dermatitis and psoriasis [52,86,111]. In atopic dermatitis, cathelicidin production is suppressed [52], whereas in psoriasis, cathelicidin expression is increased that has been suggested to control excessive inflammation. Downregulation of LL-37 in atopic dermatitis predisposes patients to skin infection with *S. aureus* [52].

The role of sunlight in increased expression of LL-37 has been investigated. A number of recent studies indicated that there is a link between sunlight exposure to the function of the innate immune system [112]. Individuals exposed to UVB have shown increased synthesis of vitamin D and increased expression of LL-37 in epidermis [112,113]. Vitamin D was found to induce keratinocyte LL-37 and HBD expression and antimicrobial activity against *P. aeruginosa* through consensus vitamin D response elements [112]. These observations suggest that any local deficit of immunity can be compensated by stimulating skin's antimicrobial peptide expression mediated by UVB or sunlight. Therefore, the concept that sunlight exposure to a "certain degree" may be good for the immunity is gaining some ground.

5.5.2 Human β-Defensins

Like cathelicidins, defensins are small cationic peptides (about 3–5 kDa) with a broad-spectrum antimicrobial activity against bacteria, fungi, and enveloped viruses [114–117]. Different disulfide linkage patterns separate defensin peptides into α-, β-, and θ-defensins, although humans do not produce θ-defensins [116]. Resident skin cells have not been shown to produce α-defensins. Although genes of at least 30 different human β-defensins exist, only three of them (HBD-1, -2, and -3) are expressed by keratinocytes and have been widely studied [44,118,119]. The HBDs are a group of small (4.5–5 kDa) cationic peptides with six conserved cysteine residues and three intramolecular disulfide bonds [120]. HDB-1 is constitutively produced by keratinocytes and its broad spectrum of antimicrobial activity includes Gram-negative bacteria and *C. albicans*. HBD-2 is not expressed by normal skin, but in psoriasis and in inflammatory skin conditions. It is highly expressed by keratinocytes [44,121–124]. On the other hand, HBD-3 is produced by keratinocytes only in response to infectious or inflammatory stimuli. In psoriasis, the genomic copy number of beta defensin is reported to be increased [125]. The downregulation of HBD-2 and -3 along with LL-37 is found to be relevant for the pathogenesis of atopic dermatitis [126].

5.5.3 Dermacidin

Dermacidin (DCD) is a 47 amino acid peptide produced in ecrine sweat glands and is delivered to the skin surface via sweat. Dermacidin expression is constitutive and undergoes postsecretory proteolytic processing in sweat-yielding anionic and cationic DCD peptides. DCD has a broad spectrum of antibacterial activity against *E. coli*, *Enterococcus faecalis*, *S. aureus*, and *C. albicans* [90]. It is likely that DCD in sweat may play a role in regulating the density and composition of human skin flora. The mode of action of DCD peptides is reported to be different from that of the cathelicidin LL-37. DCD causes membrane depolarization and the provocation on the integrity of bacterial cell envelope plays a role in the antimicrobial activity of DCD [127].

5.5.4 Psoriasin

Psoriasin, also referred to as S100A7, is a small, cell-secreted, calcium binding protein that is overexpressed in psoriasis, other inflammatory skin diseases, and in cancer tissues [92]. Psoriasin is highly active against the Gram-negative *E. coli* [92]. Psoriasin acts by creating pores in microbial membrane that is pH dependent. At neutral pH, *E. coli* is killed without compromising its membrane, whereas at acidic pH *Bacillus megaterium* (Gram positive) is killed by permeabilization of its cytoplasmic membrane [128]. The antimicrobial activity of psoriasin and related protein is inhibited by zinc.

5.5.5 Antimicrobial Lipids

In human skin, sebum lipids play important role as a dynamic aspect of skin innate immunity [56,129–131]. The sources of these lipids can be varied. During epidermal differentiation, for example, sphingoid bases are synthesized locally in the epidermis and carried to the outer surface of skin where other lipids are secreted by sebaceous glands. Free sphingoid bases are known to have a broad antibacterial activity [130]. Like antimicrobial peptides, fatty acids are induced in skin upon injury or microbial stimuli through Toll-like receptor-dependent pathways [132,133]. Reduced levels of sebum fatty acids and antimicrobial peptides are found in atopic dermatitis [52,53]. Skin fatty acids prevent the production of virulence determinants and the induction of antibiotic resistance in *S. aureus* and other Gram-positive organisms. A purified human skin fatty acid was shown to be effective in treating topical and systemic infections caused by *S. aureus* [134].

5.5.6 RNase 7

Another antimicrobial protein expressed in human skin is RNase 7, a member of the RNase A superfamily. RNase 7 is characterized by homology with bovine ribonuclease A [55]. To date, 13 human RNase members (RNase 1–13) of the RNase superfamily have been identified [55]. However, the physiological role of these ribonucleases is not clear. A recent study suggests that RNase 7 may play a major

role in skin defense and contributes to the high resistance of human skin against colonization with the Gram-positive enteric bacteria *E. faecium* [93].

5.6 EFFECTS OF SKIN-CLEANSING PRODUCTS ON SKIN MICROFLORA AND SKIN IMMUNITY

Historically, the use of soap as skin cleanser dates back to some 5000 years; however, industrial production of soaps started in 19th century. It is now a universal fact that the routine use of skin cleansers has multidimensional benefits to the user in terms of reducing/eliminating the transient organisms and maintaining the resident microflora at a healthy level. Soap is still the most commonly used form of skin cleanser. Commercially available skin cleansers come mainly in two forms: (1) solid as bar soaps and (2) liquid as liquid hand and body wash and facial cleansers and lotions. The major constituents in skin cleansers are surfactants (i.e., detergents and emulsifiers/soaps), which lower the surface tension on the skin due to the emulsification of fat, thus helping to remove dirt, oil, sweat, sebum, microbes, and dead corneocytes. In addition, surfactants aid in the normal exfoliation process of the skin. Water alone removes about 65% of oil and dirt from the skin. In some cases, skin cleansers remove not only dirt but also some beneficial lipids; especially the harsh surfactants may disrupt epidermal barrier function [135] resulting in accelerated transepidermal water loss (TEWL). The use of skin-cleansing products can also have effect on the skin flora [136].

An ideal skin cleanser should effectively remove dirt and oils, including the oil from cosmetic products used, environmental pollutants, and transient microbes from the skin without damaging the barrier properties or irritating the skin. One of the most important benefits of using skin-cleansing products, especially the hand wash products, is that these products prevent the spread of infectious diseases. Laboratory and community-based studies have shown the antimicrobial and degerming effects of these products [137,138]. It is now widely recognized that proper washing of hands with soap and water is regarded as one of the most important steps in preventing the spread of infectious diseases. A recent community-based study has shown that the use of skin-cleansing soap coupled with hand washing promotion resulted in 50% decrease in pneumonia, 53% reduction in diarrhea, and 34% reduction in impetigo (skin infection) in children under 5 years of age [139].

Depending on the type, surfactants can have harmful effects on the outermost layer of the skin, that is, stratum corneum, such as postwash skin tightness, dryness, barrier damage, redness, irritation, and itching. Surfactants in the cleansers can bind to the corneocyte proteins making them overhydrated and swell. This swelling may facilitate cleanser ingredients to penetrate into the skin where they interact with nerve endings and immune system inducing inflammatory response and itching. Once the water evaporates, it leaves the skin dryer than before. Skin cleansers can also remove the natural moisturizing factor (NMF) from the skin and damage the lipid structures causing a reduction in the amounts of lipids in the skin. NMF is a collection of free amino acids, along with other physiological chemicals such as lactic acid, urea, and salts; together these elements are known as "natural moisturizing factors." The

interactions of surfactants with the skin have been extensively studied in the past [140]. In general, skin-cleansing surfactants are broadly divided into soap-based surfactants and synthetic detergent-based (syndet) surfactants. Soaps are chemically the alkali salt of fatty acids with a pH between 9 and 10. On the other hand, chemically, a synthetic detergent can be anionic, cationic, nonionic, or amphoteric depending on its chemistry and surfactant type [141]. Examples of various skin-cleansing products are listed in Table 5.3.

5.6.1 Plain Alkaline Bar Soap

The effects of skin-cleansing products on the pH, moisture, and fat content of the skin are well documented [142]. The alkaline bar soaps significantly increase the skin pH. In general, all skin cleansers remove fats and oil from skin, but alkaline bar soap has been shown to cause the highest reduction in fat content [142]. Increase in pH irritates the skin and long-term usage of these products has been shown to alter the skin surface pH and affect the skin microflora [143–145]. The use of an alkaline soap results in higher skin surface pH and significantly increases the number of *Propionibacteria* [142,146]. Compared to acidic syndet bar soap, washing of facial skin with alkaline soap in acne-prone young adults was reported to cause more inflammatory reaction [147]. On the other hand, although washing with an acidic skin cleanser (pH of 5.5) in adults was shown to increase the skin surface pH, this increase was significantly less than the increase caused by the alkaline soap [142,148,149]. On the forehead, there was a clear correlation between bacterial counts and skin pH both with *Propionibacteria* and with *Staphylococci*, but on the forearm only *Propionibacteria* count was higher with higher pH. The number of *Propionibacteria* was significantly linked to the skin pH [146]. In a crossover clinical study, Korting et al. [150] have shown that when soap was used, compared to syndet, the skin pH increased and the number of *Propionibacteria* was increased significantly, but no change in the coagulase-negative staphylococci counts was observed.

The soaps commonly recommended and used by the population at large have a pH, which is neutral to alkaline in most cases, ranging between 7 and 9. Those skin cleansers that exhibit a pH value closer to the pH of the normal skin (pH 4.5–6) are less drying and mild to skin. It is evident from the available studies that the pH of the skin changes depending on the pH of the cleanser used. This has a multifold impact on the skin in terms of the barrier damage, moisture content, the irritability, and the bacterial flora. Thus, before recommending a cleanser to a patient especially in case of atopic dermatitis, sensitive and acne-prone skin, one should carefully consider the pH of the cleanser in use.

5.6.2 Antibacterial Soap

Unlike plain soap, antibacterial soaps contain an antibacterial active that is bacteriostatic or bactericidal in nature. In addition to cleansing the skin off dirt, bacteria, body sweat, oil, and other odorous by-products, antibacterial soaps reduce/inhibit the bacterial growth on skin. The bacteriostatic or bactericidal efficacy depends on the nature of the antibacterial active and the delivery of the active on the skin surface. The

TABLE 5.3 Examples of Skin-Cleansing Products

Type of cleanser	Chemistry/additives	Usage and attributes	References
Alkaline bar soap	Saponification of fats and oils with strong alkali	Hand and body wash; drying, irritating, raises skin pH, hard water creates soap scum	[141,145,151–153]
Syndet bar	Synthetic surfactants	Hand, body, and facial wash; cleansing, high lather, mildness, skin-feel	[145,150,154]
Combar (soap + synthetic surfactants)	Combination of superfatted soap and synthetic surfactants	Hand and body wash; less irritating than soap; less mild than syndet bars	[142,147,154]
Beauty bar soap	Synthetic anionic surfactants, also contain emollients	Hand and body wash; less drying and irritating, causes follicular plugging, keeps the skin pH near neutral to alkaline	[154,156]
Antibacterial bar soap	Saponification of fats and oils with strong alkali	Hand, body, and facial wash; kills/inhibits skin bacteria; eliminates transient bacteria; long-lasting residual efficacy	[153,155,156]
Dermatologic bars/cakes	Amphoteric, anionic, and nonionic surfactants	Hand and body wash; may raise skin pH, emollients added to reduce dryness and irritation; antiacne	[150,157,158]
Cosmetic liquid cleansers	Nonionic, aninonic, amphoteric surfactants; other additives may include silicone, moisturizers, emollients, bioactive molecules	Hand, body, and facial wash; mild, skin protectant, moisturizing, and less irritating, pH around acidic similar to skin, high rinsibility factor. Antiacne	[159,160]
Antiseptic, antibacterial liquid cleansers	Amphoteric, anionic, and nonionic surfactants. Examples of antibacterial actives: triclosan, TCC, PCMX, CHG, Quats	Hand, body, and facial wash; potentially less drying and irritating than topical bactericide, may raise skin pH, adjunct to acne treatment, may help control bacteria	[161–165]

commercialization of antibacterial soap started around mid-twentieth century. During the past five decades, the use of antibacterial soap in homes, hospitals, and other institutional settings has increased significantly. A recent survey indicates that 45% of the commercially available soaps contain antibacterial agents [165]. Initially, the bar soaps with antibacterial actives were developed and marketed as deodorant soaps, but were later marketed for their antibacterial benefits as well. The most commonly used antibacterial actives in soap products includes triclosan (TCS), chlorohexidine gluconate, triclocarban (TCC), benzalkonium chloride (BKC), benzethonium chloride (BTC), and parachlorometaxylenol (PCMX).

It is widely believed that the overuse of antibiotics in human illnesses and in livestock has contributed to the emergence of antibiotic-resistant bacteria in the community. Does the regular use of antibacterial soap by general population could lead to the emergence of resistant pathogens? This issue has been widely debated in the scientific community [166–169]. Although there may be some similarities in the mechanism of action of antibacterial actives used in skin-cleansing products and antibiotics, there has been no evidence of the development of cross-resistance to antibiotics due to the use of antibacterial wash products in the community [170,171]. Multiple community-based studies have demonstrated significant reductions in infections, specifically in enteric diseases as a result of hand hygiene interventions [172–178]. A laboratory-based clinical hand wash study demonstrated significantly less transfer of *Shigella felxineri* from artificially contaminated hands to melon balls following hand washing with an antibacterial soap compared to a plain soap, suggesting potential infection reduction benefits of antibacterial soap [138].

Few laboratory-based studies [169,179,180] have reported that bacteria may develop resistance to triclosan when exposed to sublethal doses of triclosan over time. However, to date no organism originally known to be sensitive to triclosan has been found on human skin that became resistant due to the use of triclosan-containing products. A recent retrospective study (personal communication) found no increased antibiotic resistance in *Staphylococcus* isolates from groups regularly using antibacterial wash products containing TCC or triclosan, compared to the groups using wash products containing no TCC or TCS. In addition, none of the 317 study isolates was resistant to vancomycin, and the rate of MRSA detected was appreciably less than that reported in the literature for both inpatient and outpatient isolates of MRSA.

There are also some misconceptions that regular use of antibacterial soap would lead to sterile condition and increase the risk of invasion by pathogenic organisms. Interestingly, several studies have actually reported higher postwash count than prewash [181–183]. This might be due to increased shedding of the skin during the washing and increased dispersion of bacterial microcolonies. Moreover, Larson et al. found that those using the plain soap were significantly more likely to have larger proportion of higher postwash counts compared to prewash count after 1 year of use [184]. These observations may indicate that mere washing of skin with plain or antibacterial soap does not disturb or alter the bacterial population in any significant way. Antimicrobials may cause reduction in the density of the skin flora for a short duration, and the skin flora tend to regrow to the previous level within 24–48 h. While the use of antimicrobials may induce irritant and allergic contact dermatitis in some users, no evidence exists that the use of antimicrobial products may alter the ecology

of resident skin bacteria that would lead to the overgrowth of pathogenic bacteria [185]. Clearly, more work would be needed to clearly understand the effect of long-term usage of antimicrobial-containing skin cleansers on skin microflora.

Pathogenic bacteria and viruses are known to survive very well on human hands [186,187]. Pathogens are also known to be transferred from person to person via hand contact [161,188]. Proper hand washing is one of the most important intervention steps in disease transmission and control because it reduces and/or inactivates the microbial load on the hands and decreases the chance of self-inoculation or spread of microorganisms from the contaminated hands to other hands or inanimate objects and food products [138,186,189–191]. Studies conducted on human hands have shown that the use of antibacterial soaps provides greater reduction in the number of bacteria on the hands compared to the use of regular soaps [138]. Furthermore, antibacterial soap may substantially reduce the transmission of bacteria from the hands to inanimate objects and food items [138,190].

A comparative study on the effects of antibacterial deodorant soap containing triclocarban versus a plain soap on the skin flora found no significant difference in total colony counts [192]. However, more *S. epidermidis* was observed with plain soap, while washing with deodorant soap resulted in higher colonization of *Acinetobacter calcoaceticus* and *Micrococcus luteus*. A new antibacterial soap preparation containing TCC was compared in laboratory tests with commercially available hexachlorophene soap and a control, nongermicidal soap. Both antibacterial soaps showed significant reductions in the skin flora; however, there was no difference between the two preparations. More recently, an 18-month-long study conducted in a ward showed that the use of TCC soap significantly reduced the transmission of staphylococci [193]. In a clinical study involving patients with atopic dermatitis, those who bathed with soap containing 1.5% TCC had greater reductions in *S. aureus* colonization and in the severity and extent of skin lesions than those who used plain soap [194].

Chlorhexidine gluconate (CHG)-containing skin cleansers have also been shown to be effective against bacteria, fungi, and viruses [162]. CHG is an FDA-approved antimicrobial agent for topical application in products for use as surgical scrub, healthcare personnel hand wash, and patient preoperative scrub [195]. CHG-containing skin cleansers are commercially available in various liquid and foam bases at 2% and 4% concentrations. CHG-containing hand wash products have been shown to reduce skin microflora in a single wash and further reductions in subsequent multiple washes. The concentration of CHG may also impact the level of bacterial reduction; for example, in one study [196], although not significantly different, the reductions in flora among those using the 2% CHG product were less than among those using the products with 4% CHG. CHG-containing skin cleansers are mostly used in healthcare settings as surgical scrub and preoperative skin preparation and are not used as daily hand wash regimen due to its adverse effect on skin.

Alcohol or ethanol is widely used in many topical products with direct exposure to the human skin. One of the growing trends is the use of alcohol-based hand sanitizer for hand disinfection [197]. The antimicrobial effect of alcohol-based hand sanitizers is of a fast-acting nature, but due to evaporation it does not provide a lasting residual efficacy. Ethanol also dries the skin, extracts secreted lipids, and may serve as penetration enhancer that leads to irritation. Opposing arguments on the safety of

topical applications of alcohol can be found in the scientific literature and warrant further studies on this topic [198]. On the other hand, cationic chlorohexidine strongly binds with the skin resulting in persistent antimicrobial effect. Antiseptic hand wash products intended for use by healthcare workers are regulated by the US Food and Drug Administration as over-the-counter or OTC drug products. The requirements for *in vitro* and *in vivo* testing of these products as well as surgical hand scrubs are described in the FDA Tentative Final Monograph for Health-care Topical Antiseptic Drug Products issued in 1994 [199].

The antimicrobial wash products do not exhibit moisturizing properties to skin. Lotions have been developed to provide antimicrobial activity to inactivate/destroy microorganisms on the surface of the skin while also providing a moisturizing benefit. Moisturizing lotions usually contain emollients such as glycerin that has been shown to improve the hydration of the horny layer of skin [200,201]. Skin cleansing particularly in institutional settings such as hospital, nurseries, day care centers, and food establishments requires potent antimicrobial agents to kill and/or eliminate germs from hands of care-givers and also provide, in conjunction with antimicrobial properties, skin moisturizing benefit. Excessive hand washing with soaps is not totally harmless. In particular, harsh soaps can alter the acidic mantle of the skin that can hinder some of the protective fatty acid secretions. Excessive washing can also remove protective microflora leaving the newly exposed skin susceptible to colonization by other potentially pathogenic microorganisms. People working in healthcare settings who scrub their hands frequently are prone to sustain barrier damage and skin infections. Dry and barrier-damaged skin also suffers from irritation and inflammation.

Although a number of studies have been published on the role of surfactants in affecting the skin pH, depletion of proteins, and lipids, there has been very little work done to understand the effects of skin cleansers on skin's innate immunity. In recent years, there has been a growing interest in developing skin care products that boost skin immunity and its defense mechanisms. Enhancing the skin's natural antimicrobial defenses would help to prevent infection and maintain skin health and can possibly improve the over-all health. Patients with atopic dermatitis are susceptible to bacterial skin infections because patients with atopic dermatitis fail to produce effective amounts of the antimicrobial peptides LL-37 and β-defensin 2 and 3. These findings suggest that boosting the synthesis of antimicrobial peptides may ameliorate the skin conditions. Skin's immunity provides skin with power to repair and regenerate and maintain a healthy balance between the host and microbe and the environment. As skin gets older, its natural immune system starts to slow down. As a result, skin loses its power to effectively protect, prevent, and repair damages due to biological, chemical, or physical factors.

5.7 CONCLUDING REMARKS

Skin is a vital organ of the human body responsible for protecting the body from various physical, chemical, biological, and environmental stressors. In that role, skin possesses a network of defensive apparatuses. The resident microflora, antimicrobial peptides,

antimicrobial lipids, and other immune components play respective roles in defending the skin. The resident microflora protects the skin from invasion by pathogenic microbes by producing antibiotics and enzymes, making the local environment hostile for the invading microbes, and preventing unwanted inflammation. The acidic pH of the skin also plays a role in controlling the microbial colonization of skin. Under normal conditions, skin microflora is in a mutualistic relationship with the host; however, in altered circumstances, the same resident bacteria can get involved in skin conditions such as atopic dermatitis, rosacea, psoriasis, and acne. Recent advances in human microbiome project have uncovered interesting variations within and between individuals in microbial ecology. Such observations in a way will help in defining what constitutes a healthy bacterial community of the skin.

Among the various immune components that constitute skin's innate immunity, antimicrobial peptides, especially cathelicidin LL-37 and human β-defensins 1 and 2, are the key players involved in the protective mechanisms. Normally, production of these peptides is minimal; however, their synthesis is upregulated at the site of infection, inflammation, and tissue injury. These antimicrobial peptides are very important in maintaining a healthy skin as evident from some of the instances where downregulation of HBD-2 and -3 and LL-37 has been linked to the pathogenesis of atopic dermatitis. Recent advances in this area indicate that some of these antimicrobial peptides not only kill microorganisms but are also involved in immune regulation and other functions. Antibacterial lipids present in the epidermis are also a factor in skin's protective mechanism. Further work is required to fully understand how antibacterial lipids are synthesized and what is their mode of action. Based on *in vitro* and *in vivo* studies [202,203] on the antimicrobial activities of lipids, the possibilities of using such lipids as active ingredients in skin-cleansing products should be considered. A better understanding of skin microflora and its innate immunity will advance the treatment of skin diseases, prevent skin infection, and improve the skin health.

The use of skin-cleansing products both removes dirt, sweat, and oil and reduces the microbial load on the skin. Skin-cleansing products with exfoliating agents also help skin cell differentiation and exfoliation. Skin cleansers with antibacterial actives further help to prevent the growth of skin flora including the odor-causing bacteria. Antibacterial hand wash products are known to be effective in eliminating transient and potentially pathogenic organisms from hands. In hospital and other institutional settings, the use of antibacterial wash products has led to the reduction in the outbreaks of infectious diseases. A clear evidence for infection reduction in homes due to the use of antibacterial wash products is yet to be demonstrated.

Skin-cleansing products, especially those formulated with harsh surfactants, may cause skin dryness due to loss of natural moisturizing factors and beneficial lipids. Resident bacteria otherwise nonpathogenic in normal skin condition can become pathogenic when skin becomes damaged [23]. Investigations in this area would lead to novel skin cleansers that would enhance local immunity and at the same time maintain healthy microflora. Generally, surfactant-based skin-cleansing products help to maintain skin's normal physiology; however, surfactant-induced barrier disruption triggers immune response resulting in irritation and inflammation. In order to minimize skin barrier damage, skin care products supplemented with emollients,

humectants, and lipid analogues may protect skin from drying and irritation. The selection of a skin cleanser is very important to maintain a healthy skin. An ideal skin cleanser should remove unwanted waste materials and microbes from skin, leaving beneficial components behind to continue to nourish and protect the skin. Delivery of bioactive materials such as vitamins and antioxidants on skin via skin cleansers would also help the skin to neutralize environmental stresses.

REFERENCES

1. Aly R. Cutaneous microbiology. In: Orkin M, Maibach HI, Dahl MV, editors. *Dermatology*. Los Altos: Appleton & Lange, 1991. pp. 22–25.
2. Leyden J, McGinley K, Hoelzle E, Labows JN, Kligman AM. The microbiology of the human axilla and its relationship to axillary odor. *J. Invest. Dermatol.* 1981;77:413–416.
3. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, et al. Topographical and temporal diversity of the human skin microbiome. *Science*, 2009;324:1190–1192.
4. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. *Proc. Natl. Acad. Sci. USA* 2007;104:2927–2932.
5. Gao Z, Tseng C-H, Strober BE, Pie Z, Blaser MJ. Substantial alternations of the cutaneous bacterial biota in psoriatic lesions. *PLoS ONE* 2008;3(7):e2719.
6. Ansari SA. Skin pH and skin flora. In: Barel AO, Paye M, Maibach HI, editors. *Handbook of Cosmetic Science and Technology*, 3rd ed. New York: Informa Healthcare USA Inc., 2008. pp. 221–233.
7. Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J. Invest. Dermatol.* 2001;117:44–51.
8. Öhman H, Vahlquist A. The pH gradient over the stratum corneum differs in X-linked recessive and autosomal dominant ichthyosis: a clue to the molecular origin of the "acid skin mantle". *J. Invest. Dermatol.* 1998;111:674–677.
9. Mathers AR, Larregina AT. Professional antigen presenting cells of the skin. *Immunol. Res.* 2006;36:127–136.
10. Kaplan DH, Jenison MC, Saeland S, Shlomchik WD, Shlomchil MJ. Epidermal Langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* 2005;23:611–620.
11. Lai Y, Gallo R. AMPed up immunity: how antimicrobial peptides have multiple role in immune defense. *Trends Immunol.* 2009;30:131–141.
12. Kai-Larsen Y, Agerberth B. The role of multi-functional peptide LL-37 in host defense. *Front. Biosci.* 2008;13:3760–3767.
13. Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I. Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. *Eur. J. Immunol.* 2001;31:1066–1075.
14. Yang D, Chertov O, Oppenheim JJ. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). *J. Leukoc. Biol.* 2001;69:691–697.
15. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. *Sci. Am.* 1978;238:86–95.
16. Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int. J. Cosmet. Sci.* 2006;28:359–370.
17. Rippke F, Schriener V, Schwantiz H-J. The acidic milieu of the horny layer: new findings on the physiology and pathophysiology of skin. *Am. J. Clin. Dermatol.* 2002;3:261–272.
18. Ansari SA, Scala D, Kaplan S, Jones K, Ghaim J, Polefka T. A novel skin cleansing technology that reduces bacterial attachment to the skin. Poster Abstract, 102nd General Meeting of the American Society for Microbiology, Salt Lake City, Utah, USA, 2002.
19. Ofek I, Beachy EH. General concepts and principles of bacteria adherence in animals and man. In: Beachy EH,editor. *Bacterial Adherence*. London: Chapman and Hall, 1980. pp. 3–29.

20. Feingold DS. Bacterial adherence, colonization, and pathogenicity. *Arch. Dermatol.* 1986;122:161–163.
21. Jacobi U, Gautier J, Sterry W, Lademann J. Gender-related differences in the physiology of the stratum corneum. *Dermatology* 2005;211:312–317.
22. Chiller K, Slekin BA, Murakawa GJ. Skin microflora and bacterial infections of the skin. *J. Invest. Dermatol. Symp. Proc.* 2001;6:170–174.
23. Roth RR, James WD. Microbial ecology of the skin. *Annu. Rev. Microbiol.* 1988;42:441–464.
24. Ogawa T, Katsuoka K, Kawano K, Nishiyama S. Comparative study of the staphylococcal flora on the skin surface of atopic dermatitis patients and healthy subjects. *J. Dermatol.* 1994;21: 453–460.
25. Akiama H, Morizane S, Yamazaki O, Oono T, Iwatsuki K. Assessment of *Streptococcus pyogenes* microcolony formation in infected skin by confocal laser scanning microscopy. *J. Dermatol. Sci.* 2003;32:193–199.
26. Noble WC. Observations on the surface flora of the skin and on skin pH. *Brit. J. Dermatol.* 1968;80:279–281.
27. Wesley NO, Maibach HI. Racial (ethnic) differences in skin properties: the objective data. *Am. J. Clin. Dermatol.* 2003;4:843–860.
28. Bik EM, Eckburg PB, Gill SR, Nelson KE, Purdom EA, et al. Molecular analysis of the bacterial microbiota in the human stomach. *Proc. Natl. Acad. Sci. USA* 2006;103:732–737.
29. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* 2007;449:811–818.
30. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L. Diversity of the human intestinal microbial flora. *Science* 2005;308:1635–1638.
31. Fredricks DN. Microbial ecology of human skin in health and disease. *J. Invest. Dermatol. Symp. Proc.* 2001;6:167–169.
32. Hayashi H, Takahashi R, Nishi T, Sakamoto M, Benno Y. Molecular analysis of jejunal, ileal, caecal and reco-sigmoidal human colonic microbiota using 16S rRNA gene libraries and terminal restriction fragment length polymorphisim. *J. Med. Microbiol.* 2003;54:1093–1101.
33. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, et al. The human microbiome project. *Nature* 2007;449:804–810.
34. Dekio I, Hayashi H, Sakamoto M, Kitahara M, Nishikawa T, et al. Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J. Med. Microbiol.* 2005;54:1231–1238.
35. Behne MJ, Barry NP, Hanson KM, Aronchik I, Clegg RW, et al. Neonatal development of the stratum corneum pH gradient: localization and mechanisms leading to emergence of optimal barrier function. *J. Invest. Dermat.* 2003;120:998–1006.
36. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of surface bacteria. *Proc. Natl. Acad. Sci.* 2008;105:17994–17999.
37. Forfar JO, Gould JC, MacCabe AF. Effect of hexachlorophene on incidence of staphylococcal and Gram-negative infection in the newborn. *Lancet*, 1968;ii:177–180.
38. Puhvel SM, Reisner RM, Amirian DA. Quantification of bacteria in isolated pilosebaceous follicles in normal skin. *J. Invest. Dermatol.* 1975;65:525–531.
39. Bibel DJ, Aly R, Shinefield HR, Maibach HI. The *Staphylococcus aureus* receptor for fibronectin. *J. Invest. Dermatol.* 1983;80:494–496.
40. Peterson PK, Verhoef J, Sabath LD, Quie PG. Extracellular and bacterial factors influencing staphylococcal phagocytosis and killing by human polymorphonuclear leukocytes. *Infect. Immun.* 1976;14:496–501.
41. Hentges DJ. The anaerobic microflora of the human body. *Clin. Infect. Dis.* 1993;16:175–180.
42. Chung WO, Dale BA. Innate immune response of oral and foreskin keratinocytes: utilization of different signaling pathways by various bacterial species. *Infect. Immun.* 2004;72:352–358.
43. Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv. Lipid Res.* 1991;24:1–26.
44. Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin. *Nature* 1997;387:861.

45. Van der Hoeven E, Hinton, NA. An assessment of the prolonged effect of antiseptic scrubs on the bacterial flora of the hands. *Can. Med. Assoc. J.* 1968;99:402–407.
46. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? *Brit. J. Dermatol.* 2008;158:442–455.
47. Hackett SP, Stevens DL. Super-antigens associated with staphylococcal and streptococcal toxic shock syndrome are potent inducers of tumor necrosis factor-beta synthesis. *J. Infect. Dis.* 1993;168:232–235.
48. Baker BS. The role of microorganisms in atopic dermatitis. *Clin. Exp. Immunol.* 2006;144:1–9.
49. Paulino LC, Tseng CH, Strober BE, Blaser MJ. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *J. Clin. Microbiol.* 2006;44:2933–2941.
50. Holland KT, Cunliffe WJ, Roberts CD. Acne vulgaris: an investigation into the number of anaerobic diphtheroids and members of the Micrococcaceae in normal and acne skin. *Brit. J. Dermatol.* 1977;96:623–626.
51. Gilani SJK, Gonzalez M, Hussain I, Finlay AY, Patel GK. Staphylococcus re-colonization in atopic dermatitis: beyond the skin. *Clin. Exp. Dermatol.* 2005;30:10–13.
52. Ong PY, Ohtake T, Brandt C, Strickland L, Boguniewics M, Ganz T, Gallo RL, Leung DY. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.* 2002;347:1151–1160.
53. Takigawa H, Nakagawa H, Kuzukawa M, Mori H, Imokawa G. Deficient production of hexadecanoic acid in the skin is associated in part with vulnerability of atopic dermatitis patients to colonization by *Staphylococcus aureus*. *Dermatology* 2005;211:240–248.
54. Dahl MV, Ross AJ, and Schlievert PM. Temperature regulated bacterial protein production: possible role in rosacea. *J. Am. Acad. Dermatol.* 2004;50:266–272.
55. Dyer KD, Rosenberg HF. The RNase a superfamily: generation of diversity and innate host defense. *Mol. Divers.* 2006;10:585–597.
56. Wertz PW. The nature of the epidermal barrier: biochemical aspects. *Adv. Drug Deliv. Rev.* 1996;18:283–294.
57. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell* 1996;86:973–980.
58. Wilson CL, Ouellette AJ, Satchell DP, Ayabe T, Lopez-Boado YS, et al. Regulation of intestinal beta-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999;286:113–119.
59. Andreu D, Rivas L. Animal antimicrobial peptides: an overview. *Biopolymers* 1998;47:415–420.
60. Chromek M, Slamova Z, Bergman P, Kovacs L, Podracka L, et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. *Nat. Med.* 2006;12:636–641.
61. Hancock REW, Scott MG. The role of antimicrobial peptides in animal defenses. *Proc. Natl. Acad. Sci. USA* 2000;97:8856–8861.
62. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, et al. Innate antimicrobial peptides protect the skin from invasive bacterial infection. *Nature* 2001;414:454–457.
63. Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respir. Res.* 2000;1:141–150.
64. DeSmet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol. Lett.* 2005;27:1337–1347.
65. Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, et al. Cutaneous injury induces release of cathelicidin antimicrobial peptides active against group A *Streptococcus*. *J. Invest. Dermatol.* 2001;117:91–97.
66. Schröder JM, Harder J. Antimicrobial skin peptides and proteins. *Cell Mol. Life Sci.* 2006;63:469–486.
67. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. *J. Leukoc. Biol.* 2004;75:39–48.
68. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* 2002;415:389–395.
69. Braff MH, Bardan A, Nizet V, Gallo RL. Cutaneous defense mechanisms by antimicrobial peptides. *J. Invest. Dermatol.* 2005;125:9–13.

70. Schauber J, Gallo RL. Expanding the roles of antimicrobial peptides in skin: alarming and arming keratinocytes. *J. Invest. Dermatol.* 2007;127:510–512.
71. Lehrere RI. In defense of skin. *J. Invest. Dermatol.* 2005;125:108–115.
72. Elsbach P, Weiss J. Bactericidal/permeability-increasing protein (BPI), a potent element in host-defense against Gram-negative bacteria and lipopolysaccharide. *Immunology* 1993;187:417–429.
73. Takahashi M, Horiuchi Y, Tezuka T. Presence of bactericidal/permeability-increasing protein in human and rat skin. *Exp. Dermatol.* 2004;13:55–60.
74. Arikawa J, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, et al. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by *Staphylococcus aureus*. *J. Invest. Dermatol.* 2002;119:433–439.
75. Bibel DJ, Miller SJ, Brown BE, Pandey BB, Elias PM, et al. Antimicrobial activity of stratum corneum lipids from normal and essential fatty acid-deficient mice. *J. Invest. Dermatol.* 1989;92:632–638.
76. Niyonsaba F, Ogawa H. Protective roles of the skin against infection: implication of naturally occurring human antimicrobial agents β-defensins, cathelicidin LL-37 and lysozyme. *J. Dermatol. Sci.* 2005;40:157–168.
77. Harder J, Schröder JM. RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J. Biol. Chem.* 2002;277:46779–46784.
78. Marchini G, Lindow S, Brismar H, Stabi B, Berggren V, et al. The newborn infant is protected by an innate antimicrobial barrier: peptide antibiotics are present in the skin and vernix caseosa. *Brit. J. Dermatol.* 2002;147:1127–1134.
79. Brogden KA, Ackermann M, McCray PB, Jr., Tack BF. Antimicrobial peptides in animals and their role in host defenses. *Int. J. Antimicrob. Agents* 2003;22:465–478.
80. Henzler Wildman KA, Lee DK, Ramamoorthy A. Mechanism of lipid bilayer disruption by the human antimicrobial peptide, *LL-37*. *Biochemistry* 2003;42:6545–6558.
81. Shai Y. Mode of action of membrane active antimicrobial peptides. *Biopolymers* 2002;66: 236–248.
82. Gutsmann T, Hagge SO, Larrick JW, Seydel U, Wiese A. Interaction of CAP18-derived peptides with membranes made from endotoxins or phospholipids. *Biophys. J.* 2001;80:2935–2945.
83. Yamasaki K, Gallo RL. Antimicrobial peptides in human skin disease. *Eur. J. Dermatol.* 2008;18:11–21.
84. Harrison CJ. Innate immunity as a key element in host defense against methicillin resistant *Staphylococcus aureus*. *Minerva Pediatr.* 2009;61:503–514.
85. Doss M, White MR, Tecle T, Hartshorn KL. Human defensins and LL-37 in mucosal immunity. *J. Leukoc. Biol.* 2010;87:79–92.
86. Howell MD, Jones JF, Kisich KO, Streib JE, Gallo RL, Leung DY. Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum. *J. Immunol.* 2004;172:1763–1767.
87. Liu L, Wang L, Jia HP, Zhao C, Heng HH, Schutte BC, et al. Structure and mapping of the human beta-defensin HBD-2 gene and its expression at sites of inflammation. *Gene* 1998;222:237–244.
88. Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova, E, Shakhov A, et al. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 2002;298:1025–1029.
89. Ding J, Chou YY, Chang TL. Defensins in viral infections. *J. Innate Immun.* 2009;1:413–420.
90. Schittek B, Hipfel R, Sauer B, Bauer J, Kalbacher H, Stevanovic S, et al. Dermacidin: a novel human antibiotic peptide secreted by sweat glands. *Nat. Immunol.* 2001;12:1133–1137.
91. Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, et al. Cathelicidin antimicrobial peptide expression in sweat, an innate defense system for the skin. *J. Invest. Dermatol.* 2002;119:1090–1095.
92. Glaser R, Harder J, Lange H, Bartels J, Christophers E, et al. Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat. Immunol.* 2005;6(1):57–64.
93. Köten B, Simanski M, Gläser R, Podschun R, Schröder J-M, et al. RNase 7 contributes to the cutaneous defense against *Enterococcus faecium*. *PLoS One* 2009;4:e6424.
94. Frohem M, Agerberth B, Ahangari G, Stahle-Backdahl M, Linden S, et al. The expression of the gene coding for the antimicrobial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J. Biol. Chem.* 1997;272:15258–15263.

95. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsojo A, et al. Vitamin D induces the antimicrobial protein hCAP18 in human skin. *J. Invest. Dermatol.* 2005;124:1080–1082.
96. Lee DY, Yamasaki K, Rudsil J, Zouboulis CC, Park GT, et al. Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill *Propionibacterium acnes*. *J. Invest. Dermatol.* 2008;128:1863–1866.
97. Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J. Immunol.* 2003;170:2274–2278.
98. Murakami M, Dorschner RA, Stren LJ, Lin KH, Gallo RL. Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. *Pediatr. Res.* 2005;57:10–15.
99. Sorensen OE, Cowland JB, Theilgaard-Monch K, Liu I, et al. Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. *J. Immunol.* 2003;170:5583–5589.
100. Turner I, Cho Y, Dinh NN, Waring AI, Lehrer RI. Activities of LL-37, a cathelicidin-associated antimicrobial peptide of human neutrophils. *Antimicrob. Agents Chemother.* 1998;42:2206–2214.
101. Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, et al. Cutaneous injury induces the release of cathelicidin antimicrobial peptides active against group A *Streptococcus*. *J. Invest. Dermatol.* 2001;117:91–97.
102. Zaiou M, Gallo RL. Cathelicidins, essential gene-encoded mammalian antibiotics. *J. Mol. Med.* 2002;80:549–561.
103. Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL. Post-secretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. *J. Immunol.* 2004;172:3070–3077.
104. Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. *J. Immunol.* 2002;169:3883–3891.
105. Bals R, Wilson JM. Cathelicidin: a family of multifunctional antimicrobial peptides. *Cell Mol. Life Sci.* 2003;60:711–720.
106. Barlow PG, Li Y, Wilkinson TS, Bowdish DME, Lau E, et al. The human cationic host defense peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary cells of the innate immune system. *J. Leukoc. Biol.* 2006;80:509–520.
107. Gudmundsson GH, Agerbert B. Neutrophil antibacterial peptides, multifunctional effector molecule in the mammalian immune system. *J. Immunol. Methods* 1999;232:45–54.
108. Kamysz W, Okroj M. Novel properties of antimicrobial peptides. *Acta Biochim. (Polish)* 2003;50:461–469.
109. Risso A. Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. *J. Leukoc. Biol.* 2000;68:785–792.
110. Zaiou M, Nizet V, Gallo RL. Antimicrobial and protease inhibitory functions of the human cathelicidins (hCAP18/LL-37) prosequence. *J. Invest. Dermatol.* 2003;120:810–816.
111. Leung DY, Bieber T. Atopic dermatitis. *Lancet* 2003;361(9395):151–160.
112. Wang TT, Nestel FP, Bourdeau V, Nagai Y, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J. Immunol.* 2004;173:2909–2912.
113. Mallbris L, Edstrom DW, Sunblad L, Granath F, Stahle M. UVB up-regulates the antimicrobial protein hCAP18 mRNA in human skin. *J. Invest. Dermatol.* 2005;125:1072–1074.
114. Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, et al. Defensins: natural peptide antibiotics of human neutrophils. *J. Invest. Dermatol.* 1985;76:1427–1435.
115. Hoover DM, Wu Z, Tucker K, Lu W, Lubkowski J. Antimicrobial characterization of human beta-defensin 3 derivatives. *Antimicrob. Agents Chemother.* 2003;47:280–289.
116. Lehrer RT, Ganz T. Antimicrobial peptides in mammalian and insect host defense. *Curr. Opin. Immunol.* 1999;11:23–27.
117. Lehrer RT, Daher K, Ganz T, Selsted ME. Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes. *J. Virol.* 1985;54:467–472.
118. Duits LA, Ravensbergen B, Rademaker M, Hemstra PS, Nibbering PH. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. *Immunology* 2002;106:517–525.

119. Fang XM, Shu Q, Chen QX, Book M, Sahl G, et al. Differential expression of alpha- and beta-defensins in human peripheral blood. *Eur. J. Clin. Invest.* 2003;33:82–87.
120. Lehrer RI. Primate defensins. *Nat. Rev. Microbiol.* 2004;2:727–738.
121. Ali RS, Falconer A, Ikram M, Bissett CE, Cerio R, Quinn AG. Expression of the peptides antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. *J. Invest. Dermatol.* 2001;117:106–111.
122. Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M,et al Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. *J. Clin. Invest.* 1998;102:874–880.
123. Chronnell CM, Ghali LR, Ali RS, Quinn AG, Holland DB, et al. Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions. *J. Invest. Dermatol.* 2001;117:1120–1125.
124. Liu L, Roberts AA, Ganz T. By IL-1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide. *J. Immunol.* 2003;170:575–580.
125. Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, et al. Psoriasis is associated with increased beta-defensin genomic copy number. *Nat. Genet.* 2008;40:23–25.
126. Rupec R, Boneberger S, Ruzicka T. What is really in control of skin immunity: lymphocytes, dentritic cells, or keratinocytes? Facts and controversies. *Clin. Dermatol.* 2010;28:62–66.
127. Senyurek I, Paulmann M, Sinnberg T, Kalbacher H. et al. Dermicidin-derived peptides show a different mode of action than the cathelicidin LL-37 against *Staphylococcus aureus. Antimicrob. Agents Chemother.*, 2009;53:2499–2509.
128. Michalek M, Gelhaus C, Hecht O, Podschun R, Schröder JM, Leippe M, Grötzinger J. The human antimicrobial protein psoriasin acts by permeabilizing of bacterial membranes. *Dev. Comp. Immunol.* 2009;33:740–746.
129. Bibel DJ, Aly R, Shah S, Shinefield HR. Sphingosines: antimicrobial barriers of the skin. *Acta Derm. Venereol.* 1993;73:407–411.
130. Drake DR, Brogden KA, Dawson DV, Wertzi PW. Antimicrobial lipids at the skin surface. *J. Lipid Res.* 2008;49:4–11.
131. Thormar H, Hilmarsson H. The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chem. Phys. Lipids* 2007;150:1–11.
132. Georgel P, Crozat K, Lauth X, Makrantonaki E, Seltmann H. A toll-like receptor 2-responsive lipid effector pathway protects mammals against skin infections with Gram-positive bacteria. *Infect. Immun.* 2005;73:4512–4521.
133. Schauber J, Dorschner RA, Coda AB, Buchau AS, Liu PT, et al. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J. Clin. Invest.* 2007;117:803–811.
134. Clarke SR, Mohamed R, Bian Li, Routh AF, Kokai-Kun JF, Mond JJ, Tarkowski A, Foster SJ. The *Staphylococcus aureus* surface protein IsdA mediates resistance to innate defenses of human skin. *Cell Host Microbe*, 2007;1:199–212.
135. Okuda M, Yoshiike T, Ogawa H. Detergent-induced epidermal barrier dysfunction and its prevention. *J. Dermatol. Sci.* 2002;30:173–179.
136. Holland KT, Bojar RA. Cosmetics: what is their influence on the skin microflora? *Am. J. Clin. Dermatol.* 2002;3:445–449.
137. Ansari SA, Sattar SA, Springthrope VS, Wells GA, Tostowaryk W. *In vivo* protocol for testing efficacy of hand-washing agents against viruses and bacteria: experiments with rotavirus and *Escherichia coli. Appl. Environ. Microbiol.* 1989;55:3113–3118.
138. Fischler GE, Fuls JL, Dail EW, Duran MH, Rodgers ND, et al. Effect of hand wash agents on controlling the transmission of pathogenic bacteria from hands to food. *J. Food Prot.* 2007;70:2873–2877.
139. Luby SP, Abogatwalla M, Felkin DR, Painter J, Billhimer W, et al. Effect of handwashing on child health: a randomized controlled trial. *Lancet* 2005;366:225–233.
140. Rhein L. *In vitro* interactions: biochemical and biophysical effects of surfactants on skin. In: Rieger MM, Rhein LD, editors. *Surfactants in Cosmetics*, 2nd ed., vol. 68 New York: Marcel Dekker, Inc., 1997. pp. 397–426.
141. Friedman M, Wolf R. Chemistry of soaps and detergents: various types of commercial products and their ingredients. *Clin. Dermatol.* 1996;14:7–13.

142. Gfatter R, Hackl P, Braun F. Effects of soap and detergents on skin surface pH, stratum corneum hydration and fat content in infants. *Dermatology* 1997;195:258–262.
143. de Almeida e Borges LF, Silva BL, Gontijo Filho P.P. Hand washing: changes in the skin flora. *Am. J. Infect. Control* 2007;35:417–420.
144. Korting HC, Megele M, Mehringer L, Vieluf D, Zienicke H, et al. Influence of skin cleansing preparation acidity on skin surface properties. *Int. J. Cosmet. Sci.* 1991;13:91–102.
145. Thune P, Nilsen T, Hansatad IK, Gustavsen T, Lovig Dahl H. The water barrier function of the skin in relation to the water content of stratum corneum, pH and skin lipids. The effect of alkaline soap and syndet on dry skin in elderly, non-atopic patients. *Acta Derm. Venereol.* 1988;68:277–283.
146. Aly R, Maibach HI, Rahman R, Shinefield HR, Mandel AD. Correlation of human *in vivo* and *in vitro* cutaneous antimicrobial factors. *J. Infect. Dis.* 1975;131:579–583.
147. Korting HC, Bruan-Falco O. The effect of detergents on skin pH and its consequences. *Clin. Dermatol.* 1996;14:23–27.
148. McGinley KJ, Labows JN, Zechman JM, Nordstrom KM, Webster GF, et al. Analysis of cellular components, biochemical reactions, and habitat of human cutaneous lipophilic diphtheroids. *J. Invest. Dermatol.* 1985;85:374–377.
149. Visscher M, Hoath SB, Conroy E, Wickett R. Effect of semipermeable membranes on skin barrier repair following tape stripping. *Arch. Dermatol. Res.* 2001;293:491–499.
150. Korting HC, Ponce-Poschi E, Klovekorn W, Schmotzer G, Arens-Corell M, et al. The influence of the regular use of a soap or an acidic syndet bar on pre-acne. *Infection* 1995;23:89–93.
151. Barel AO, Lambrecht R, Clarys P, Morrison BM, Jr., Paye M. A comparative study of the effects on the skin of a classical bar soap and a syndet cleansing bar in normal use conditions and in soap chamber test. *Skin Res. Technol.* 2001;7:98–104.
152. Corraza M, Lauriola MM, Zappaterra M, Bianchi A, Virgili A. Surfactants, skin cleansing protagonists. *J. Eur. Acad. Dermatol. Venereol.* 2010;24:1–6.
153. Lakshmi C, Srinivas CR, Anand CV, Mathew AC. Irritancy ranking of 31 cleansers in the Indian market in a 24-h patch test. *Int. J. Cosmet. Sci.* 2008;30:277–283.
154. Ananthapadmanabhan KP, Moore DJ, Subramanyan K, Misra M, Meyer, F. Cleansing without compromise: the impact of cleansers on the skin barrier and the technology of mild cleansing. *Dermatol. Ther.* 2004;17 S1:16–25.
155. Billhimer WL, Berge CA, Englehart JS, Rains GY, Keswick BH. A modified cup scrub method for assessing the antibacterial substantivity of personal cleansing products. *J. Cosmet. Sci.* 2001;52:369–375.
156. Ertel K. Modern skin cleansers. *Dermatol. Clin.* 2000;18:561–575.
157. Draelos ZD, Ertel K, Hartwig P, Rains G. The effect of two skin cleansing systems on moderate xerotic eczema. *J. Am. Acad. Dermatol.* 2004;50:883–888.
158. Draelos ZD. Concepts in skin care maintenance. *Cutis* 2005;76:19–25.
159. Draelos ZD. The effect of a daily facial cleanser for normal to oily skin on the skin barrier of subjects with acne. *Cutis* 2006;78(Suppl. 1):34–40.
160. Kuehl BL, Fyfe KS, Shear NH. Cutaneous cleansers. *Skin Therapy Lett.* 2003;8:1–4.
161. Ansari SA, Sattar SA, Springthorpe VS, Wells GA, Tostowaryk W. *In vivo* protocol for testing efficacy of hand-washing agents against viruses and bacteria: experiment with rotavirus and *Escherichia coli*. *Appl. Environ. Microbiol.* 1989;55:3113–3118.
162. Leyden JJ, McGinley KJ, Kaminer MS, et al. Computerized image analysis of full-hand touch plate: a modified method for quantification of surface bacteria on hands and the effect of antimicrobial agents. *J. Hosp. Infect.* 1991;18(Suppl. B):13–22.
163. Morrison BM, Jr., Scala D, Fischler GE. Topical antibacterial wash products. In: Rieger M, Rhein LD, editors. *Surfactants in Cosmetics*, 2nd ed. New York: Marcel Dekker, Inc., 1997. pp. 331–356.
164. Oughton MT, Loo VG, Dendukuri N, Fenn S, Libman MD. Hand hygiene with soap and water is superior to alcohol rub and antiseptic wipes for removal of *Clostridium defficile*. *Infect. Control Hosp. Epidemiol.* 2009;30:939–944.
165. Perencevich EN, Wong MT, Harris AD. National and regional assessment of the antibacterial soap market: a step toward determining the impact of prevalent antibacterial soaps. *Am. J. Infect. Control* 2001;29:281–283.

166. Aiello AE, Larson EL, Levy SB. Consumer antibacterial soaps: effective or just risky? *Clin. Infect. Dis.* 2007;45:S137–S147.
167. Gerba CP. Benefits of antibacterial products well documented. *Am. J. Infect. Control* 2002;30:257–258.
168. Russell AD. Antibiotic and biocide resistance in bacteria: comments and conclusions. *J. Appl. Microbiol.* 2002;92:171S–173S.
169. Russell AD. "Whither triclosan?" *J. Antimicrob. Agents Chemother.* 2004;53:693–695.
170. Cole EC, Addison RM, Rubino JR, Leese KE, Dulaney PD, et al. Investigation of antibiotic and antibacterial agents cross-resistance in target bacteria from homes of antibacterial product users and nonusers. *J. Appl. Microbiol.* 2003;95:664–676.
171. Aiello AE, Marshall B, Levy SB, et al. Relationship between triclosan and susceptibilities of bacteria isolated from hands in the community. *Antimicrob. Agents Chemother.* 2004;48: 2973–2979.
172. Aiello AE, Coulborn RM, Perez V, Larson EL. Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am. J. Public Health* 2008;98:1372–1381.
173. Black RE, Dykes AC, Anderson KE, Wells JG, Sinclair SP, et al. Handwashing to prevent diarrhea in day-care centers. *Am. J. Epidemiol.* 1981;113:445–451.
174. Carabin H, Gyorkos TW, Soto JC, Joseph L, Payment P, et al. Effectiveness of a training program in reducing infections in toddlers attending day care centers. *Epidemiology* 1999;10:219–227.
175. Falsey AR, Criddle MM, Kolassa JE, McCann RM, Brower CA, et al. Evaluation of a handwashing intervention to reduce respiratory illness rates in senior day-care centers. *Infect. Control Hosp. Epidemiol.* 1999;20:200–202.
176. Luby SP, Agboatwalla M, Painter J, Altaf A, Billhimer W, et al. Combining drinking water treatment and hand washing prevention, a cluster randomized controlled trial. *Trop. Med. Int. Health* 2006;11:479–489.
177. Peterson AF, Rosenberg A, Alatary SD. Comparative evaluation of surgical scrub preparations. *Surg. Gynecol. Obstet.* 1978;146:63–65.
178. Shahid NS, Greenough WB, 3rd, Samadi AR, Huq MI, Rahman N. Hand washing with soap reduces diarrhoea and spread of bacterial pathogens in a Bangladesh village. *J. Diarrhoeal Dis. Res.* 1996;14:85–89.
179. Heath RJ, Rubin JR, Holland DR, Zhang E, Snow ME, et al. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *J. Biol. Chem.* 1999;274:11110–11114.
180. McMurry LM, Oethinger M, Levy SB. Triclosan targets lipid synthesis. *Nature* 1998;394:531–532.
181. Larson EL, Hughes CA, Pyrek JD, Sparks SM, Cagatay EU, et al. Changes in bacterial flora associated with skin damage on hands of health care personnel. *Am. J. Infect. Control* 1998;26:513–521.
182. Larson EL, Aiello AE, Gomez-Duarte C, Lin SX, Lee L, et al. Bioluminescence ATP monitoring as a surrogate marker for microbial load on hands and surfaces in the home. *Food Microbiol.* 2003;20:735–739.
183. Meers PD, Yeo GA. Shedding of bacteria and skin squames after hand washing. *J. Hygiene (London)* 1978;81:99–105.
184. Larson EL, Aiello A, Lee LV, Della-Latta P, Gomez-Duarte C, Lin S. Short- and long-term effects of handwashing with antimicrobial or plain soap in the community. *J. Community Health* 2003;28:139–150.
185. Elsner P. Antimicrobials and skin physiological and pathological flora. *Curr. Probl. Dermatol.* 2006;33:3–41.
186. Ansari SA, Sattar SA, Springthorpe VS, Wells GA, Tostowaryk W. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J. Clin. Microbiol.* 1988;26:1513–1518.
187. Ansari SA, Springthorpe VS, Sattar SA. Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Rev. Infect. Dis.* 1991;13:448–461.
188. Rusin P, Maxwell S, Gerba CP. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of Gram-positive bacteria, Gram-negative bacteria, and phage. *J. Appl. Microbiol.* 2002;93:585–592.

189. Bloomfield S, Exner M, Fara GM, Scott EA. Prevention of the spread of infection: the need for a family-centered approach to hygiene promotion. *Euro. Surveill.* 2008;3:18889.
190. Fuls JL, Rodgers ND, Fischler GE, Howard JM, Patel M, et al. Alternative hand contamination technique to compare the activities of antimicrobial and non-antimicrobial soaps under different test conditions. *Appl. Environ. Microbiol.* 2008;74:3739–3744.
191. Scott E, Duty S, Callahan M. A pilot study to isolate *Staphylococcus aureus* and methicillin-resistant *S. aureus* from environmental surfaces in home. *Am. J. Infect. Control* 2008;36:458–460.
192. Bibel DJ. Ecological effects of a deodorant and a plain soap upon human skin bacteria. *J. Hyg. (London)* 1977;78:1–10.
193. Wilson PE. A comparison of methods for assessing the value of antibacterial soaps. *J. Appl. Microbiol.* 2008;33:574–581.
194. Breneman DL, Hanifin JM, Berge CA, Keswick BH, Neuman PB. The effect of antibacterial soap with 1.5% triclocarban on *Staphylococcus aureus* in patients with atopic dermatitis. *Cutis* 2003;66:296–300.
195. Bruch, M. Newer germicides: what they offer. In: Maibach H, Aly R.editors. *Skin Microbiology: Relevance to Clinical Infection.* New York: Springer-Verlag, 1981. pp. 103–112.
196. Larson EL, Laughon BA. Comparison of four antiseptic products containing chlorhexidine gluconate. *Antimicrob. Agents Chemother.* 1987;31:1572–1574.
197. Bloomfield SF, Aiello AE, Cookson B, O'Boyle C, Larson EL. The effectiveness of hand hygiene procedures in reducing the risks of infection in home and in community settings including hand washing and alcohol-based hand sanitizers. *Am. J. Infect. Control* 2007;35:S27–S64.
198. Lachenmeier DW. Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *J. Occup. Med. Toxicol.* 2008;3:26–31.
199. Food and Drug Administration. Tentative final monograph for healthcare antiseptic drug products: proposed rule. *Fed. Regist.* 1994;59(116):31402–31452.
200. Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. *Brit. J. Dermatol.* 2008;159:23–34.
201. Gloor M, Bettinger J, Gehring W. Modification of stratum corneum quality by glycerin-containing external ointments. *Hauarzt* 1998;49:6–9.
202. Bergsson, G, Arnfinnsson J, Steingrimsson O, Thormar, H. Killing of Gram-positive cocci by fatty acids and monoglycerides. *APMIS* 2001;09:670–678.
203. Thormar H, Hilmarsson H, Bergsson G. Stable concentrated emulsions of 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the foodborne bacteria *Campylobacter jejuni, Salmonella* spp. and *Escherichia coli. Appl. Environ. Microbiol.* 2006;72:522–526.