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A Comprehensive Overview

Jack D. Weide

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The Skin Microenvironment and *Staphylococcus Epidermidis*: A Comprehensive Overview

By Jack D. Weide

Abstract:
The human skin microbiome is an extremely diverse and variable environment which plays a critical role in the maintenance of barrier integrity, immune regulation, and protection against disease. Among its many inhabitants, *Staphylococcus epidermidis* stands out as both a commensal and an opportunistic pathogen making it extremely relevant in skin microbiome research. This review investigates how factors such as moisture, pH, nutrient availability, and immune activity impact microbial diversity across dry, moist, and sebaceous regions. The dual role of S. epidermidis is explored through its role in immune priming, wound healing, and colonization resistance, alongside its involvement in biofilm formation and skin disorders including atopic dermatitis and rosacea. Emerging in vitro and in vivo skin models are also assessed for their flaws and strengths in replicating human skin conditions. Together, these insights highlight the balance in host-microbe interactions and the areas in current research that need to be further studied.

Introduction

The integumentary system in the human body plays a pivotal role in protecting the body from many external stressors such as temperature, the chemical environment, and bacteria.

Amongst the 19 phyla and ~1,000 different species of bacteria present on the skin, the two bacteria most common in skin microbiome research are *Staphylococcus epidermidis* and Cutibacterium acnes.[1,2] This prevalence of the two species in skin microbiome research results from their prevalence and their roles as both commensals and opportunistic pathogens in the human integumentary system. This review explores the characteristics of the skin microbiome, the impacts of these characteristics on microbial diversity and concentration, S. epidermidis as a commensal and opportunistic pathogen, and the progression of skin-relevant models for skin microbiome research.

Overview of the Skin Microenvironment

The cutaneous membrane is the largest organ in the human body and is typically divided into 3 layers: the epidermis, the dermis, and the hypodermis, each with their respective roles in skin health and impacts on the skin microenvironment and microbiota.[3] The two most important layers for this review are the epidermis and the dermis.

The outermost layer, the epidermis, is composed primarily of stratified squamous epithelial cells with a high concentration of keratinocytes, which produce lipids, an important factor in the waterproof behavior of the skin.[4]

The dermis, the secondary layer of the skin, contains nerves, hair follicles, collagen, immune cells, sudoriferous glands, and sebaceous glands.[1] The hair follicles, immune cells, sudoriferous glands, and sebaceous glands are of particular importance when discussing the skin microbiome due to their roles in nutrient availability and the physical environment.

Skin Biodiversity in Dry, Moist, and Sebaceous Conditions

The skin's environment is incredibly variable and hostile, with many factors that work against microbial growth. Despite this fact, the skin microbiota exhibits a high degree of variability, and certain regions are characterized by dense microbial colonization.[5] Factors such as nutrient availability, moisture levels, immune activity, pH, and oxygen variability or availability impact biodiversity and bacterial growth across skin regions.

In general, the skin has three different types of moisture areas which have varying oxygen saturations to accompany them. Dry areas (limbs) and moist areas (underarm and navel) have high oxygen saturation selecting for aerobic bacterial growth, and sebaceous areas (head, chest, and back) have low oxygen saturation favoring anaerobic bacterial growth, especially within the hair follicles.[6,7]

Another important factor in skin microbial biodiversity is nutrient availability in the various skin microenvironments. Dry areas have a low nutrient availability due to their low concentration of hair follicles, sudoriferous glands, and sebaceous glands. Due to the lack of nutrients in these areas, they exhibit the lowest microbial density.[8] These areas, although lacking in nutrients and microbial density, have the highest microbial diversity. This can be attributed to the lack of a dominant nutrient niche for one species to dominate.[5] Moist areas, in comparison, have a high concentration of sudoriferous glands, and some have high concentrations of hair follicles (e.g. axillary vault). These sudoriferous glands are responsible for the secretion of sweat, which is rich in water, electrolytes, urea, lactic acid, and glucose.[9] The presence of electrolytes in these areas allows for growth of halophiles such as members of the Staphylococcus genus and some members of the Corynebacterium genus, those making up the highest percentage of the microflora within these environments.[1,10] Sebaceous areas of the skin are the most nutrient-rich skin environments, fostering the highest biomass due to high nutrient concentration, but the least microbial diversity due to the specified and unique nature of the nutrient composition. The sebaceous areas of the skin contain both sudoriferous glands and sebaceous glands as well as hair follicles. The sebaceous glands produce sebum, a lipid-rich substance that is composed of triglycerides, free fatty acids, wax esters, and squalene (a chemical responsible for moisturizing the skin). The nutrients present in the sebum provide a unique energy source specific to lipid-metabolizing bacteria such as members of the Cutibacterium genus, while the nutrients from the sweat continue to select for halophilic bacteria.

The pH of the skin is also a significant factor in the composition of skin microflora and the diversity of microbial communities across the various environments within the skin. The average pH of the skin is a high area of contention within dermatologic research, with studies claiming the average pH of the skin is anywhere from 4.0 to 7.0 pH.[11] This is narrowed to a range of about 4.5 - 5.7 whenever the skin is not exposed to cleansers and cosmetics for 24 hours. Although this is the generally accepted average pH of the skin mantle (the surface of the skin), it is important to note that pH, like oxygen saturation and nutrient composition, also varies between the dry, moist, and sebaceous areas of the skin. To maintain this acidic pH range, the skin releases fatty acids from sebaceous glands, as well as lactic acid and electrolytes from sudoriferous glands.[1] This suggests that sebaceous areas of the skin would be the most acidic, followed by moist areas, and then dry areas with the least acidity. Because most microflora flourish in environments with basic pH conditions, the skin provides a unique environment where only acidophiles or acid-tolerant bacteria can survive. This pH sensitivity in microflora explains, in part, why dry areas support greater biodiversity, whereas moist and sebaceous areas have less biodiversity and a more selective environment. PH-driven variation in microbial communities plays a crucial role in skin health. Many constituents of the skin microflora play a crucial role in skin barrier function; thus changes in pH of the skin mantle can cause certain bacteria to lose their functionality in the barrier or even to become opportunistic pathogens causing skin dysbiosis.[12] This leads us into our next section, exploring the skin's immune activity and its impact on microbial diversity.

Not only does the skin serve as a barrier against physical factors, but it also serves as an active component of the immune system that together works with resident microbiota to protect the body against pathogens.[8] The skin's immune function plays a central role in shaping microbial diversity across the different areas of the skin. The skin's immune system is made of both innate and adaptive components, this includes immune cells, antimicrobial peptides (AMPs), and specialized skin structures such as the skin-associated lymphoid tissue (SALT).[13] The interface between the body's immune system and the skin microbiota significantly influences the microbial composition of the skin.

The innate immune response of the skin is the first immune response involved in the skin's defense against external pathogens. It consists of physical barriers like the stratum corneum, and active components like keratinocytes' pattern recognition receptors (PRRs), which initiate the secretion of AMPs that inhibit microbial growth in the targeted microbes.[8] In areas characterized by a high concentration of skin microbiota, like the sebaceous and moist areas, this immune activity is critical for maintaining microbial balance. AMPs are thought to be involved in managing microbial density by limiting the overgrowth of beneficial microbes like C. acnes and killing pathogenic microbes like S. aureus.

The adaptive immune response is also integral in the regulation of skin microbial diversity. Specialized cells like T-cells, dendritic cells, and macrophages help detect and respond to microbial antigens.[13] Some research also suggests that resident commensals also influence the skin's immune response. For example, S. epidermidis colonization of the skin has been shown to cause increased levels of cytokine interleukin-1α(IL-1α), a cytokine associated with the inflammatory immune response of the skin.[14,15]

Imune activity in the skin also varies across the moist, dry, and sebaceous skin zones. Sebaceous areas such as the forehead and nose, rich in sebaceous glands, have a more acidic pH as previously discussed. The immune system in sebaceous areas is more specified to target lipid metabolizing bacteria such as C. acnes and S. epidermidis the two most common microflora in the sebaceous areas of the skin.[13,14] The sebaceous glands in these areas release defensins, cytokines, chemokines, and other chemicals that target lipid metabolizing bacteria to help regulate the concentration and the composition of these areas. In moist areas, a similar immune response exists where halophilic or sweat-tolerant bacteria such as Corynebacterium and Staphylococcus are targeted by AMPs released by the eccrine glands or sudoriferous glands. AMPs such as dermcidin released in eccrine sweat don't play a normal role in skin immunity like other AMPs. This AMP binds to the bacteria resulting in reduced RNA and protein synthesis. As a result, it helps control the proliferation and density of bacterial colonies in the moist areas of the skin. Recall that dry areas of the skin tend to have the highest microbial diversity in the skin due to their nutrient composition and pH. Because of this, the immune system in this area is less specified due to its exposure to a broad range of bacteria. Thus, it exhibits a more typical innate and adaptive immune response when looking at skin immune function. This allows for the microbial diversity of the dry areas to be maintained and for the skin to regulate a broad range of bacteria to prevent overgrowth of a specific type leading to an imbalance in the skin microbiome and conditions of skin dysbiosis such as psoriasis, eczema, or rosacea.[1]

Staphylococcus epidermidis

Staphylococcus epidermidis, a gram-positive coagulase-negative bacteria, is one of the most prominent species of bacteria present in the varying skin microbiomes.[1] Often referred to as a major "skin sentinel," S. epidermidis plays a dual role in the skin as both a commensal organism and an opportunistic pathogen.[16] In homeostatic skin conditions, it actively primes the cutaneous immune response, aids in maintaining homeostasis, and prevents other opportunistic pathogens from causing disease through colonization resistance. Due to its prevalence in the skin compared to other microflora, along with its functional importance in skin health and its dual role in the skin, S. epidermidis has been selected as a critical model organism for understanding the dynamics and conditions of the skin microbiome. As such, in this section we will discuss its priming of the cutaneous immune response, how it aids in the maintenance of homeostasis, its colonization resistance, and its role in two of the most common forms of skin dysbiosis, atopic dermatitis and rosacea.

S. epidermidis as a commensal organism plays a functional role in skin health. Whether it's immune priming, responding to barrier injury, or contributing to colonization resistance, this bacterium is a major player in the prevention of infection and maintenance of skin homeostasis. In this section, we will discuss how S. epidermidis fills these roles and why it is so important.

One of the ways that S. epidermidis impacts the skin microbiome and aids in the immune function of the skin is through priming host cells for immune response. S. epidermidis has been shown to do this in many ways. One way it does so is by early colonization of the host skin microenvironment. It has been shown that this early colonization, specifically within hair follicles, induces the chemokine CCL20 and the migration of Treg Cells to the follicle following induction of said chemokine.[16]

Response to barrier injury is also a major factor when considering S. epidermidis's role as a commensal. Studies done on mice to determine S. epidermidis's role in skin barrier function have shown that S. epidermidis converts aromatic amino acids into trace amines which were shown to increase the rate of wound healing.[17] This increased wound healing rate helps prevent the colonization of wound sites by pathogenic bacteria which may cause inflammation and infection of the skin. The S. epidermidis also produced a molecule called 6-N-Hydroxyaminopurine (6-HAP) which selectively inhibited the proliferation of tumor cell lines in the mouse model and significantly suppressed ultraviolet tumor growth.[16] This is important because it shows how S. epidermidis not only strengthens our bacterial immunity but also suppresses and even prevents neoplasms of the skin.

The last major factor to consider when looking at S. epidermidis's role as a commensal is colonization resistance. S. epidermidis plays a crucial role in colonization resistance as a commensal organism, preventing the overgrowth of pathogenic microbes on the skin through various means. One of these means is via competitive exclusion. Being one of the most prevalent microbes on the skin, S. epidermidis is a dominant player in nearly every microenvironment within the skin.[5] This can be seen in the sheer volume of S. epidermidis present in the skin when compared to other skin microbes as stated previously in our section on nutrient availability impacts on microbial biodiversity. Additionally, S. epidermidis prevents pathogenic colonization of the skin through various other direct and indirect mechanisms. One such mechanism is the activation of the distinct innate immune response and the amplification of the keratinocyte antimicrobial immune response through the secretion of a certain unidentified factor or factors by S. epidermidis.[16] Another mechanism is the secretion of an unidentified small molecule (<2 kDa) by S. epidermidis, and subsequent activation of the keratinocyte aryl hydrocarbon receptor (AHR) and downstream IL-1α and human β-defensin 3 (hBD3) expression to promote an innate immune defense response.[13,16]

While S. epidermidis plays a crucial role as a commensal in the skin microenvironment during homeostatic conditions, under abnormal skin conditions it becomes an opportunistic pathogen commonly associated with both skin dysbiosis and nosocomial infection. In this section, we will primarily be covering the implications of S. epidermidis as an opportunistic pathogen in skin dysbiosis, though it is pertinent to consider that this research also has implications in the prevention and treatment of nosocomial infection.

The first thing that needs to be discussed when considering the pathogenic state of S. epidermidis is its proliferation into a biofilm and the lack of research done on same. The exact mechanism of biofilm formation by S. epidermidis is currently unknown, but the process itself has been broken down into four major steps: adherence, accumulation, maturation, and detachment.[18] A fully matured S. epidermidis biofilm contains an amalgamation of adhesive molecules, among which are polysaccharide intercellular adhesin (PIA), proteinaceous factors (Bhp, Aap, and Embp), teichoic acids, and extracellular (e) DNA. It has been identified that not all isolates of S. epidermidis present on the skin encode the factors that are believed to cause this biofilm formation. A study done by Bowden et al. stated that only 13 percent of colonizing skin flora isolates encoded Bhp, a protein that has been associated with the formation of strong biofilms by both S. epidermidis and S. aureus.[19] Given the role of S. epidermidis biofilms in conditions of skin dysbiosis and nosocomial infection, along with the lack of understanding of the mechanisms responsible for the formation of a mature S. epidermidis biofilm, future research needs to be focused on identifying these mechanisms and building an understanding of these prevalent and relevant biofilms.

The next thing to be considered is the role of S. eipidermidis in the various conditions of skin dysbiosis with which it's associated. The first major skin condition we will discuss is atopic dermatitis. Atopic dermatitis is defined as a chronic relapsing, pruritic inflammatory skin condition that is non-contagious, affecting approximately 15 percent of children in the United States at a given time.[20] It used to be associated with the colonization of S. aureus in the skin, but more recent research has shown that disease flares have been associated with an overall decrease in the diversity of the skin microenvironment in areas where the lesions form.[21] It also showed that both S. aureus and S. epidermidis were responsible for an increase in lesions and inflammation. Although this new research shows that both organisms are responsible for the flare-ups of this disease, it doesn't address the relationship between the two bacteria in this disease.

The other major skin condition we will cover is rosacea. Like atopic dermatitis, there are a plethora of holes in the research correlating S. epidermidis to the disease. Despite this, it seems to be commonly accepted that S. epidermidis is one of the primary bacteria responsible for the disease.[22] This idea was also supported in a study by Woo et al. that showed, via 16S rRNA PCR, that S. epidermidis was the most prevalent bacteria in pustules and skin sites of rosacea, making up 28 percent of the microbial population.[23] Despite this shared acceptance that it is one of the bacteria responsible for the skin condition, it is glaringly clear that further research into the disease and its correlation to S. epidermidis is necessary.

Skin Relevant Research Models

As stated previously, the complex and dynamic microbial community present in the skin microbiome plays a pivotal role in skin health, immunity, and disease prevention. As research on these microbial communities becomes more relevant, it is increasingly important that research models that accurately represent the skin are available.

In the past, much of the research done on human skin microflora has been done on in vivo models where mouse skin was used to represent the skin. The mouse model for skin microbiome research especially in the research of bacterial infections of the skin and immune response to them has proven to be a reliable model.[24] Despite its capabilities as a research model, the mouse model also has a plethora of shortcomings including inherent immunological and physiological differences between humans and mice. It has been suggested, however, that human skin xenografts on mice could aid in the mitigation of some of these physiological and immunological differences, though that remains to be proven.

Previous in vitro skin-relevant models for understanding the growth and metabolism of bacteria such as S. epidermidis and C. acnes have used high concentrations of nutrients and oxygen which despite giving an accurate representation of the optimal metabolism of said bacteria fails to represent the actual environment on the skin for which they're being studied.[1] Recent research has altered that though, using modified M9 salt media with nutrient, amino acid, and oxygen concentrations similar to what would be found in the different environments on the skin. However, this research falls short in its ability to accurately represent the moisture levels, temperature, and physical characteristics of the skin.

Conclusions

There has been immense progress in research on the characteristics of the skin microbiome, the impacts of these characteristics on microbial diversity and concentration, S. epidermidis as a commensal and opportunistic pathogen, and the progression of skin-relevant models of skin microbiome research. Despite this, there is still much research to be done on topics such as S. epidermidis's role in colonization resistance, specifically on the unknown small molecules that are secreted by it, as well as its role in various conditions of skin dysbiosis such as atopic dermatitis and rosacea. Additionally, the recent progress seen in skin-relevant research models is incredible, but there is still progress to be had in accurately representing temperature variance in different skin areas and understanding its impact on those areas. Overall, despite the incredible progress on skin microbiome and microflora research that has occurred over the last decade and a half, there are significant holes in our understanding of the roles of various bacteria in disease and how we can work to prevent them. Further discussion in future reviews should address the implications of bacteria such as S. epidermidis in nosocomial infection, as well as covering other prevalent skin microflora such as C. acnes.



About the Author

Jack Weide is a freshman at Montana State University majoring in Chemistry with a concentration in Biochemistry and a minor in Mathematics. Jack has worked in the Fields Lab of the Center for Biofilm Engineering since the beginning of his senior year of high school and has since pivoted to work in Computational Chemistry with the Mosquera group in the epartment of Chemistry and Biochemistry. Jack has lived in Bozeman nearly his entire life and enjoys skiing, hiking, and running. In the future Jack hopes to go to graduate school and to conduct research in Medicinal Chemistry.

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