Microbial Ecology of Human Skin and Wounds

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Abstract Human skin is a complex organ that provides protection and regulates our interaction with the outside environment. The skin is composed of three layers, which include the epidermis, dermis, and hypodermis. Skin appendages include hair follicles, sebaceous glands, and sweat glands. These appendages are unevenly distributed on the skin. The stratum corneum is the outer protective layer of the epidermis and is composed of dead cells that are regularly shed from the surface. The outer layers of the epidermis are inhabited by microorganisms considered permanent skin residents as well as transient microorganisms that do not normally grow and multiply on the skin. The number and types of microorganisms inhabiting the skin are influenced by skin conditions, including the density and activity of sebaceous and sweat glands. The secretions of these glands provide nutrients and selective conditions that influence the composition of the resident microflora. This community is composed primarily of Gram-positive bacteria, including staphylococci, micrococci, and corynebacteria as well as lipophilic yeasts (*Malassezia*). This resident microflora is believed to help prevent skin colonization by pathogenic microorganisms. However, under certain conditions, skin disease can be caused by members of the resident flora. Skin infections are most often the result of injury to the skin. Cutaneous wounds enable access of microorganisms to normally sterile tissue and provide a much different niche for microbial growth than does intact skin. In the case of acute wounds, the healing process, including the immune response, is capable of stemming invasion by microbes and repairing the wound. However, in some cases wounds become chronic and fail to heal within a reasonable time frame. Most often chronic wounds afflict the ill and elderly with underlying disorders (e.g., diabetes) or weakened immune systems. Large bacterial populations in wounds have been correlated with delayed healing, and control of microbial infection is recognized as an important aspect of wound care. However, the role of specific microorganisms in preventing wound healing remains unclear. Similar types of microorganisms have been isolated from both acute and chronic wounds, although the latter tend to harbor more anaerobic bacteria. Growth of microorganisms as biofilms in wounds may also

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contribute to the delayed healing and poor response to treatment of chronic wounds. Overall, human skin and cutaneous wounds are complex ecosystems harboring diverse communities of microorganisms. A better understanding of these ecosystems may lead to improvements in human health.

1 Introduction

The skin is the largest organ of the human body and serves to regulate our interaction with the outside environment. It also provides a habitat for resident microorganisms that occupy the skin niche and help prevent colonization by pathogenic microorganisms. Regardless of the protective factors provided by skin and the resident microflora, pathogenic microorganisms can invade the skin niche and cause disease. Also, under certain conditions, members of the resident skin microflora that are usually benign can cause skin diseases. Although diseases can affect intact skin, infections are most often associated with skin trauma. Cutaneous wounds enable bacterial access to deeper tissues, which provide an environment much different than intact skin. This environment provides both nutrient-rich conditions for microbial growth and antagonistic effects of the immune system. Thus, wounds present a much different environment for microbial growth than does intact skin. Furthermore, chronic wounds involve other aspects that present a completely different niche for microbial growth than either intact skin or acute wounds. The following chapter provides an overview of skin structure and physiology, skin microbiology, wound repair, and wound microbiology.

2 Normal Human Skin

The skin acts as a barrier to microbial invasion. As well as providing protection, the skin contributes to thermoregulation, sensation, and secretion of various fluids. Although the basic structural components of skin are consistent, structural and physiological characteristics vary with body location and age. Skin characteristics also vary between individuals. Skin is inhabited by microorganisms, which include both resident microflora and transient organisms. The resident microflora is believed to be beneficial to human health when confined to the skin habitat.

2.1 Skin Structure and Physiology

The skin has been characterized into three layers, with the epidermis as the outermost layer, the dermis as the middle layer, and the hypodermis as the deepest layer (Fig. 1). The epidermis serves as a protective layer and is composed primarily of

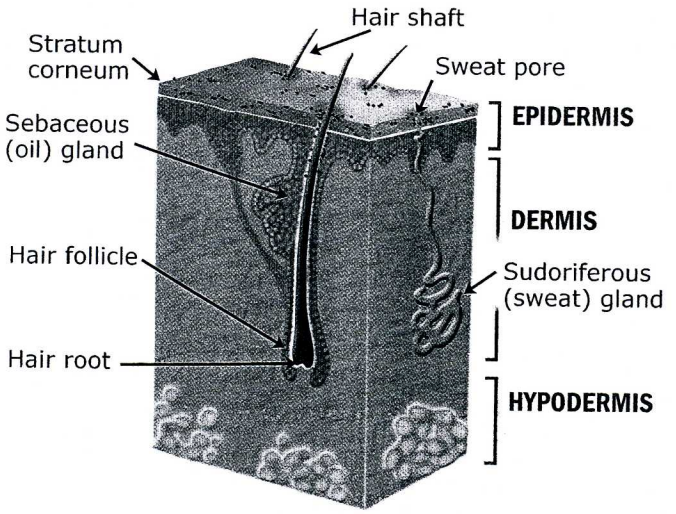


Fig. 1 Skin structure indicating sites for microflora attachment and growth. Bacteria, including cocci (*red*) and rods (*blue*), colonize the skin surface as both individual cells and small groups (microcolonies). The largest numbers of bacteria are associated with hair follicles as well as sebaceous and sudoriferous glands. Illustration by Peg Dirckx

keratinocytes. These cells produce the protein keratin, an important structural component of skin. Keratinocytes are involved in a variety of processes, including wound healing and modulating cutaneous immune responses. The outermost surface of the skin (stratum corneum) is composed of dead keratinocyte cells containing keratin and forms a tough water-repellant layer. The stratum corneum is regularly shed and varies in thickness depending on body location, thickest in certain areas such as the soles of the feet. Underlying the stratum corneum are living keratinocytes at decreasing stages of maturation with depth, as well as melanocytes, Langerhans cells, and Merkel cells. Melanocytes produce the pigment melanin, which contributes to skin color and provides protection from ultraviolet radiation. Langerhans cells are involved in the regulation of immune responses. Merkel cells are associated with nerve cells and are involved in the sensation of touch. Protruding through the epidermis are hairs and secretion ducts originating in the dermis and hypodermis. The epidermis is connected to the dermis by a basement membrane, which serves to anchor these two layers together.

The dermis is the structural layer of the skin that supports the epidermis and consists mainly of collagen and elastic fibers. A few cells such as fibroblasts, macrophages, and lymphocytes as well as blood vessels and nerves are present. The dermis contains two distinct regions, the uppermost of which is the papillary region characterized by thin collagen, elastic fibers, small blood vessels, and nerve endings. The deeper layer is the reticular region, where the collagen fibers are thick and the blood vessels and nerves are larger. Hair follicles and glands such as eccrine and sebaceous glands also reside in the reticular layer. Variations in the thickness of this

reticular region contribute to skin thickness in different areas of the body. The collagen and elastic fibers, which provide the dermis with strength and elasticity, are produced by the fibroblasts. Other cells in the dermis include immune cells such as macrophages, lymphocytes, and mast cells.

The deepest layer of skin, the subcutaneous tissue or hypodermis, attaches the skin to underlying tissues. The hypodermis is composed predominantly of adipose tissue, which provides thermal insulation, shock absorption, and energy storage. Large blood vessels in the hypodermis are essential for delivering blood to the dermis, and the main nerves that connect to the dermis are also located in the hypodermis. Some sweat glands and longer hair follicles of the scalp may extend down into the subcutaneous layer.

Appendages and glands in the skin include hair follicles, sebaceous glands, and types of sudoriferous or sweat glands. The density of these features varies with location; hair follicles and sebaceous glands are prevalent on the scalp, while eccrine glands, a type of sweat gland, are very numerous in the axillae. All of these skin features can influence the type and number of microbial flora present in certain areas of the skin. Sebaceous glands exist typically as lateral appendages of hair follicles. They secrete sebum, which is a mixture of lipids, including waxes and triglycerides, as well as protein, cholesterol, and salts. Most sebaceous glands secrete into the hair follicle, although some may secrete directly onto the surface in locations such as the lips and eyelids. They are nonexistent on the palms and soles and largest in size on the face, neck, and chest. Sebum provides nutrients for microbial growth, and bacteria on the skin are often found in microcolonies in or surrounding follicles (Fig. 1).

The main sweat glands in humans are eccrine glands, which are prevalent in areas such as the axillae, palms, and soles. The gland often extends from its opening on the epidermis down into the subcutaneous layer. The sweat secreted from these glands is an aqueous solution containing many solutes which influence growth of microorganisms. These include sodium, chloride, and potassium ions, urea, ammonia, glucose, lactic and ascorbic acids, as well as lysozyme. The pH of sweat can be neutral or slightly acidic, a factor that also influences microbial growth. Typically, increased densities of bacteria are found in areas of the skin containing a large number of eccrine glands.

Apocrine glands are sweat glands that have a thicker, milkier secretion than that of eccrine sweat glands. Located mainly on the axillae and around the genitalia, apocrine glands do not have a known purpose in humans. In other mammals, where these glands are much more developed, they serve as scent organs for sexual and territorial purposes.

As mentioned previously, composition and density of the microbial skin flora vary with body location. Factors that affect bacterial growth on the skin include, but may not be limited to, moisture, nutrients, pH, and presence of inhibitors. The moisture content of a certain area of the skin depends upon the density and activity of the eccrine glands in that area. These glands, as well as the sebaceous glands located in hair follicles, supply many different nutrients for microorganisms, and eccrine glands also help regulate pH for optimized growth conditions of some

species. Therefore, body locations that contain high numbers of these appendages are likely to house larger bacterial populations that can be quite diverse based on the variety of available nutrients.

In addition to variations in skin structure and physiology between individuals and among various body locations, skin also undergoes considerable changes with age, which influences the skin microflora (Somerville 1969). Although active at birth, because of the influence of maternal androgens, sebaceous glands are inactive in children and then become active again at puberty. Apocrine glands also become active at puberty. In general, adult glandular secretions (sebaceous, eccrine, and apocrine) decrease with age. The skin of adults also tends to have a higher water content than that of infants, children, or the elderly.

2.2 *Skin Microflora*

Research into the microbiology of human skin has been reviewed since 1965 in books authored or edited by Marples (1965), Sommerville and Noble (1973), and Noble (1981, 1993). The following section is covered in more detail by the most recent edition of *The Skin Microflora and Microbial Disease* edited by Noble (2004).

A huge variety of microorganisms have been isolated from human skin, many of which are transient from exposure of the skin to environmental sources such as soil and water and do not grow or multiply on the skin. Certain microorganisms are considered permanent inhabitants of human skin, while others may establish populations on the skin, temporarily, under certain conditions. Nobel (1981) established the terms *transient* for bacteria that do not multiply on the skin, *temporary residents* for those multiplying and persisting for a short period of time, and *residents* for those organisms believed to be permanent skin inhabitants.

As discussed earlier, the number of microorganisms on skin varies with body location. Areas such as the scalp, axillae, and groin, with numerous hair follicles, sebaceous glands, and eccrine glands, harbor the largest and most diverse populations of bacteria. There are also variations between individuals and individuals living in different environments with respect to the types of microorganisms present on the skin.

The resident microflora consists primarily of Gram-positive bacteria, including the genera *Staphylococcus*, *Micrococcus*, *Propionibacterium*, *Corynebacterium*, and others. In addition, yeasts of the genus *Malassezia* are also considered permanent residents of human skin. These microorganisms colonize the hair follicles and superficial layers of the epidermis and recolonize newly formed tissue as the stratum corneum is shed, utilizing secretions of the sebaceous, eccrine, and apocrine glands as nutrients (Fig. 1). The number and diversity of microorganisms are dependant on skin location, with the largest populations and most diverse communities in areas such as the axillae and groin, which have the most numerous glands. Although, all humans have a resident skin flora, the numbers and microbial species present vary widely between individuals. The resident microflora likely evolved

with humans and is well adapted to the skin environment. It is accepted that these microorganisms generally provide protection by occupying the skin niche and preventing colonization by pathogens, with minor consequences to the host (e.g., body odor). Resident microbes may also more directly inhibit invading microorganisms by the production of inhibitory compounds such as antibiotics, bacteriocins, and lytic enzymes (Allaker and Noble 2004).

The coagulase-negative staphylococci inhabiting human skin include *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and others (Noble 2004). These bacteria are not considered skin pathogens, although they can cause disease in other niches of the human body, having been implicated in endocarditis and many device-related infections.

In contrast to the coagulase-negative staphylococci, the coagulase-positive species, *Staphylococcus aureus*, is considered a true pathogen (Lowy 1998). The primary human habitat of *S. aureus* is the nostrils, although they also are commonly members of the resident skin flora, particularly in the axillae, groin, and toe webs (Noble 2004).

Early studies of human skin flora used the term *diptheroids* to describe Gram-positive nonsporing rods; this group of bacteria was later referred to as *coryneform* bacteria because these pleomorphic bacteria often assumed club-shaped forms. The coryneform group is composed of numerous genera, including both aerobic and facultative species (Leyden and McGinley 2004). Many of the coryneform bacteria either depend on lipids for growth or show enhanced growth in the presence of lipids. Aerobic coryneforms considered residents of human skin include *Corynebacterium* species, such as *C. minutissimum*, *C. xerosis*, and *C. jeikeium* as well as *Brevibacterium epidermis*. Aerobic coryneforms have been linked to axillary odor (Leyden et al. 1981; Taylor et al. 2003). Anaerobic coryneforms constituting the resident flora are members of the genus *Propionibacterium*, with species including *P. acnes*, *P. granulosum*, and *P. avidum*. Of the propionibacteria, *P. acnes* is the most commonly isolated species of propionibacterium from human skin.

A variety of species of *Micrococcus* are skin residents, including *M. luteus* and *M. varians*. *Micrococcus* species are not considered skin pathogens and rarely cause infections in humans. However, they have been implicated in axillary malodor (Taylor et al. 2003).

Gram-negative bacteria are not generally considered part of the resident flora, with the exception of *Acinetobacter* species (Noble 2004). Species of this genus are found in locations such as the axillae, groin, and toe webs of some individuals. The toe webs may also be inhabited by other Gram-negative genera, including *Pseudomonas* and *Proteus*, although these genera are not considered resident flora (Noble 2004).

Yeasts of the genus *Malassezia* (*Pityrosporum*) are also considered part of the resident flora of human skin (Hay 2004). These yeasts are lipophilic and inhabit superficial layers of the stratum corneum near sebaceous glands, much like the lipophilic cutaneous bacteria.

Recent studies of skin microflora have utilized culture-independent techniques (PCR and cloning) to determine the presence of difficult-to-culture bacterial

species. These studies have served to further confirm the presence of species determined from the numerous culture-based studies described earlier. In addition, these studies also discovered bacteria not previously associated with human skin. These include such genera as *Acidovorax*, *Dietzia*, and *Methylophilus* found on human foreheads (Dekio et al. 2005). A study of human forearms revealed the presence of additional phyla not normally associated with human skin, including *Thermomicrobia*, *Cyanobacteria*, and *Deinococcus-Thermus* (Gao et al. 2007). In most instances, these new skin genera appear to be associated with a limited number of the volunteers sampled rather than on every individual. These studies serve to distinguish skin microflora on a subject-to-subject basis and provide further insight into the species diversity resident on normal human skin.

2.3 Microbial Skin Diseases

A vast number of microbially related skin diseases have been documented. As with the previous section on skin microflora, more detailed information on these diseases can be found in *The Skin Microflora and Microbial Disease* edited by Noble (2004). Some of these diseases are caused by invading microorganisms and viruses that are not considered part of the normal human skin flora. Such diseases include cellulitis and necrotizing fasciitis, caused by streptococcal species (Bisno and Stevens 1996). Other diseases can be caused by microorganisms considered normal resident skin flora that usually are benign. Such diseases include erythrasma, caused by *Corynebacterium minutissimum*, and pityriasis versicolor, caused by *Malassezia* yeasts. A common disease associated with a normally benign skin resident is acne vulgaris. In this case, increased sebum production and abnormal desquamation of follicular epithelium lead to proliferation of *Propionibacterium acnes* (Leyden and McGinley 2004). In most cases microbial skin diseases are associated with skin damage such as minor abrasions, burns, or other trauma. Infection of cutaneous wounds is discussed in more detail later. Diabetics and immunocompromised individuals are also predisposed to microbial skin infections caused by both members of the normal skin flora and invading microorganisms.

3 Skin Wounds

Skin wounds include superficial scrapes and cuts, bite wounds, traumatic wounds, surgical wounds, and pressure ulcers. As well as damaging the integrity of the skin, wounds also result in the introduction of cutaneous microflora and other microorganisms into the underlying tissues. In the case of acute wounds, the normal immune response and wound repair process are adequate to heal the wound in a relatively short time frame (days to weeks). This process can be augmented through the use of skin cleansers as well as topical and systemic antimicrobial agents.

In contrast to acute wounds, chronic wounds fail to heal within a reasonable time frame (months to years) and often remain in an inflammatory state. The microbiology of acute and chronic wounds is different, with the latter tending to harbor more diverse microbial communities that include anaerobic species. However, the role of microorganisms in preventing the healing of chronic wounds remains unclear.

3.1 Normal Wound Repair

Following injury to the skin, the inflammation phase begins, initiating the process of wound repair. Blood coagulation as well as platelet adhesion and aggregation form a blood clot, which serves to reestablish hemostasis and provide a provisional matrix for cell migration. This provisional matrix is rich in fibrin and also contains fibronectin. The platelets release chemotactic factors for blood leukocytes and growth factors. Blood clotting also leads to the generation of vasoactive agents and activation of the classical and alternative complement cascades, which further attract leukocytes to the wound. Concurrently with inflammation, epithelial cells migrate across the wound under the scab and proliferate, reestablishing the epithelial barrier.

Neutrophils are the first leukocytes to arrive at the wound site, drawn and activated by a variety of chemoattractants. The neutrophils engulf invading bacteria by phagocytosis and subsequently destroy them using enzymes and oxidative compounds. The length of the neutrophil infiltration phase depends on the amount of contamination in the wound. If a large number of bacteria and other foreign objects are present, neutrophil infiltration will persist along with further activation of the alternative complement cascade.

Following the initial neutrophil invasion of the wound, monocytes arrive on the scene, drawn by various chemoattractants, and are activated to become macrophages. The macrophages migrate along the provisional matrix phagocytosing bacteria, tissue debris, and exhausted neutrophils. Activated macrophages also produce chemoattractants and activators, recruiting additional inflammatory cells as well as fibroblasts. Cytokines produced by macrophages stimulate the formation of provisional extracellular matrix by fibroblasts as well as the formation of new blood vessels by endothelial cells.

The invasion of macrophages, fibroblasts, and endothelial cells into the wound bed, along with the formation of new blood vessels, gives the newly formed tissue a granular appearance, resulting in the term *granulation tissue*. Although fibroblasts initially produce primarily fibrin, synthesis of collagen eventually becomes predominant.

The final phase of wound healing is maturation, which involves matrix formation and remodeling. Fibroblasts form myofibroblasts, which contract the wound. The matrix matures, with the fibrin and hyaluronic acid of the provisional matrix replaced by collagen bundles of increasing thickness. As maturation progresses, collagen fibers become more organized, blood vessels are restored to normal, the scab is shed, and the epidermis is restored to normal thickness.

The wound healing process is guided by a large number of growth factors and enzymes. These compounds are released by various cells and regulate the proliferation, migration, and function of cells involved in the wound healing. Both interleukin-1 and tumor necrosis factor alpha are produced by keratinocytes in response to tissue damage. Release of these cytokines contributes to macrophage activation and induction of an inflammatory response. Macrophages also produce these cytokines along with interferon gamma. Important enzymes in the wound repair process include matrix metalloproteinases (MMPs) which facilitate cellular migration and tissue remodeling by specific cleavage of extracellular matrix protein. These enzymes are regulated by tissue inhibitors of metalloproteases (TIMPs), which bind and inactivate MMPs.

3.2 Chronic Wounds

Wounds that persist for more than about 2 months are considered chronic. These wounds often remain in an inflammatory state. Patients with chronic wounds are commonly also suffering from systemic disease, such as diabetes mellitus or peripheral vascular disease. A variety of factors may provide a barrier to wound healing. These factors include infection, poor blood perfusion, low oxygen pressure, malnutrition, and systemic disease.

Chronic wounds provide a much different environment than acute wounds. One of the key differences is the highly proteolytic condition (Yager and Nwomeh 1999). Typically, chronic wounds display a much higher ratio of MMPs to TIMPs than acute wounds do. This is at least partially due to a higher number of neutrophils present in chronic wounds (Yager and Nwomeh 1999). The proteolytic conditions in chronic wounds are also believed to be responsible for the degradation of growth factors and extracellular matrix proteins. However, the concentrations of some growth factors are elevated in chronic wounds. These include proinflammatory cytokines, such as interleukin-1, tumor necrosing factor alpha, and interferon gamma (Trengove et al. 1996a,b, 2000).

3.3 Wound Microbiology

Cutaneous wounds provide a much different environment for microbial growth than does intact skin. Depending on wound depth, tissues may be exposed, which provide a rich milieu for microbial growth. However, wounds also present a hostile environment for microorganisms because of the activity of the immune system. The microbial flora of wounds reflects these environmental differences, being quite different than the resident skin microflora. Although microbial infection of wounds is recognized as a barrier to healing, the role of microorganisms in chronic wounds remains unclear.

A myriad of microbial species have been isolated from wounds, including both aerobic and anaerobic bacteria. The predominant aerobic microorganisms isolated from clinical wound samples included *S. aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella* species, *Streptococcus* species, *Enterococcus* species, and *Proteus* (Howell-Jones et al. 2005; Bowler et al. 2001). *S. aureus* is the most commonly isolated species from wounds (Bowler 1998) and is a recognized pathogen with a suite of virulence factors. However, it is often also isolated from chronic wounds showing no clinical signs of infection. In a comparative study of chronic and acute wounds, *S. aureus* predominated in infected acute wounds, while infected chronic wounds were primarily colonized by anaerobic bacteria (Bowler and Davies 1999a,b).

Genera of anaerobes cultured from wound samples include *Peptostreptococcus*, *Clostridium*, *Propioinacterium*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Veillonella*, and *Bacteroides* (Howell-Jones et al. 2005;Wall et al. 2002; Bowler et al. 2001). Bowler and colleagues have stressed the potential role of anaerobic bacteria as a barrier to healing in chronic wounds (Bowler 1998; Bowler et al. 2001). *Peptostreptococcus* species, in particular, have been implicated in this regard (Wall et al. 2002). These authors suggested production of short-chain fatty acids and hydrolytic enzymes by *Peptostreptococcus* as potential factors in delaying wound healing. Anaerobes also appear to be responsible for malodor in wounds. Wounds that exhibit no odor were rarely colonized with anaerobic bacteria (Bowler et al. 1999). The lack of recognition of the importance of anaerobes in wound infections has been blamed on the difficulties of isolating and culturing anaerobes (Bowler 1998; Bowler et al. 2001). There appear to be instances of aerotolerance in some anaerobic species. The presence of *Clostridium tertium*, for example, was determined using 16S rDNA sequencing but has been mistaken as *Lactobacillus* or *Bacillus* using routine culture methodologies (Fujitani et al. 2007). Indeed, molecular-based studies have revealed that the microbial community of wounds is more diverse than revealed by culture (Davies et. al. 2001, 2004).

In polymicrobial communities, the growth of specific bacterial species may be aided by synergistic effects from growing in the presence of other bacterial species. For example, in a study of leg ulcers, *S. aureus* appeared to increase growth rates of Gram-negative anaerobes (Bowler and Davies 1999). Changes in virulence have been shown to occur in noncapsulate *Bacteroides fragilis*, which becomes capsulate (virulent) after passage with either *E. coli* or *S. pyogenes* (Brook 1988).

While the presence of bacteria does not necessarily indicate a wound infection, the concept of bacterial load appears to be an important determinant (Howell-Jones et al. 2005). As discussed by Bowler et al. (2001), quantitative culture of tissue biopsies or wound swab samples has been correlated with delayed wound healing. Generally, bacterial loads greater than 10^5 organisms are considered to indicate an infected wound (Robson et al. 1999). However, this guideline has been questioned (Bowler 2003). The use of rigorous and long incubation culture techniques has shown a positive correlation of increased bacterial diversity with nonhealing status of venous leg ulcers, instead of bacterial numbers alone (Davies et al. 2007).

3.4 *Biofilms in Chronic Wounds*

It has been speculated for several years that bacteria colonizing chronic wounds exist as biofilm communities (Serralta et al. 2001; Mertz 2003; Percival and Bowler 2004). Chronic wound infections share two important attributes with other biofilm diseases – persistent infection that is not cleared by the host immune system and resistance to systemic and topical antimicrobial agents (Costerton et al. 1999). Frequent debridement is one of the most clinically effective treatments to help heal chronic wounds (Steed et al. 1996). This may be an effective treatment because it removes the biofilm from the wound. This is similar to resolving infections from biofilm-colonized catheters; where antibiotic therapy is ineffective, the most effective approach is to remove the colonized catheter (Raad et al. 2002). However, direct evidence of biofilm involvement in chronic wound infections is scarce. One of the first published studies of biofilm formation in wounds was conducted using a porcine model (Serralta et al. 2001). The pigs were wounded, inoculated with *P. aeruginosa*, and a polyurethane dressing was applied. When the wounds were sampled, loosely adhered cells were removed with a saline flush and enumerated. Following flushing, the wounds were scrubbed to remove adhered bacteria and these too were enumerated. The scrub technique resulted in much higher bacterial counts than did the rinse technique, suggesting that the bacteria were tightly adhered to the wound as a biofilm. In this study plastic cover slips were also placed in some wounds for subsequent microscopic imaging. Clusters of bacterial cells surrounded by an extracellular polymer matrix were observed on the cover slips, which also indicated that biofilm formed in the wounds. These researchers also demonstrated that a *P. aeruginosa* strain isolated from a burn wound rapidly formed biofilms in vitro (Harrison-Balestra et al. 2003). Overall, preliminary evidence indicates that polymicrobial biofilm forms on chronic wounds, and clinical aspects of chronic wound infections resemble those of other biofilm infections. Recent research indicates that biofilms may be present in chronic wounds (James et al. 2008; Bjarnsholt et al. 2008). Further research is necessary to evaluate the role of biofilms in the prevention of wound healing.

4 Conclusion

Human skin is a complex organ that provides protection from the outside environment. The outermost layers of the skin are inhabited by microorganisms that are termed the resident microflora. In addition, a variety of other microorganisms that do not normally grow and multiply on the skin may be present on the skin. The resident microflora consists primarily of Gram-positive bacteria and yeasts of the genus *Malassezia*. Resident bacteria include staphylococci and micrococci as well as both aerobic and anaerobic coryneforms. These microorganisms grow and multiply on the skin using secretions from sebaceous glands as well as eccrine and apocrine sweat glands. The density of these glands and the associated microbial populations vary

with body location. The resident skin microflora is believed to serve a protective function by preventing colonization of the skin by pathogenic microorganisms, although in some cases microorganisms that are considered normal microflora can cause disease. Injuries to the skin result in the initiation of an immune response and cutaneous repair processes. Wound repair is complex and involves numerous cell types, growth factors, and enzymes. Infection with microorganisms can impair the healing process. Acute wounds heal within a few days or weeks, while chronic wounds persist for months or years. Chronic wounds usually have an underlying cause, such as systemic disease (e.g., diabetes) or immune dysfunction. A wide variety of bacteria have been isolated from wounds. The most common species of bacteria isolated from wounds is *S. aureus*. The role of specific bacteria in delayed healing of chronic wounds is unclear because many of the same bacterial species have been isolated from both acute and chronic wounds. Some studies have shown that anaerobic bacteria are more prevalent in chronic wounds than in acute wounds and have implicated them in delayed healing. Many studies may have overlooked anaerobic bacteria because they are difficult to grow in culture. It has been speculated that biofilms may play a role in the delayed healing of chronic wounds, but further research is necessary to evaluate this hypothesis. Overall, human skin and wounds are ecosystems often supporting complex microbial communities that can have both beneficial and detrimental influences on human health.

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