Australasian Journal of Dermatology

Australasian Journal of Dermatology (2015) ••, ••–•• doi: 10.1111/ajd.12253

REVIEW ARTICLE

The skin microbiome: Associations between altered microbial communities and disease

Laura S Weyrich,[1] Shreya Dixit,[2] Andrew G Farrer,[1] Alan J Cooper[1] and Alan J Cooper[2]

[1]*Australian Centre for Ancient DNA, University of Adelaide, Adelaide, South Australia, and* [2]*Department of Dermatology, Royal North Shore Hospital, Sydney, New South Wales, Australia*

ABSTRACT

A single square centimetre of the human skin can contain up to one billion microorganisms. These diverse communities of bacteria, fungi, mites and viruses can provide protection against disease, but can also exacerbate skin lesions, promote disease and delay wound healing. This review addresses the current knowledge surrounding the healthy skin microbiome and examines how different alterations to the skin microbial communities can contribute to disease. Current methodologies are considered, changes in microbial diversity and colonisation by specific microorganisms are discussed in the context of atopic dermatitis, psoriasis, acne vulgaris and chronic wounds. The recent impact of modern Westernised lifestyles on the human skin microbiome is also examined, as well as the potential benefits and pitfalls of novel therapeutic strategies. Further analysis of the human skin microbiome, and its interactions with the host immune system and other commensal microorganisms, will undoubtedly elucidate molecular mechanisms for disease and reveal gateways for novel therapeutic treatment strategies.

Key words: acne, atopic dermatitis, bacteria, chronic wound, dermatology, psoriasis, skin microbiome.

INTRODUCTION: THE HUMAN MICROBIOME

Although the human genome is over three billion base pairs in length, it contains only about 20 000 genes, leaving numerous biological processes to the microorganisms that live on and within the human body (the microbiome).[1] An average healthy human body possesses 10^{14} microorganisms – 10-fold the number of human cells.[2] These bacterial, viral, fungal and eukaryotic microorganisms are absolutely essential to everyday life, assisting in digestion, nutrient absorption and immune development.[3,4] In contrast, a perturbation of these complex microbial communities can have deleterious effects. Although in-depth analyses of the human microbiome have just begun, the gut microbiome is by far the most extensively studied. The human gut contains nearly 10 trillion bacterial cells and over 2000 different species.[5] Diet, environment and genetic predisposition can also drastically impact on species composition, and perturbations to these microbial communities have been linked to inflammatory bowel disease, colorectal cancer and *Clostridium difficile* infections, as well as systemic disorders that include anxiety, depression, obesity, diabetes and autism.[6–8]

The bacterial communities in other parts of the body are quite distinct from those in the gut, and differ significantly depending on body site differences and are influenced by pH, temperature and oxygen content.[9] Despite the fact that the skin environment would appear to be inhospitable to microbial growth (being cool and acidic, and constantly shedding), approximately one billion bacteria have been identified inhabiting a typical square centimetre of the skin, covering the surface and extending subcutaneously.[10] These diverse microbial communities create specific ecological niches and can aid in disease prevention or contribute to disease. For example, some bacterial species limit the growth of others by hydrolysing sebum lipids into toxic fatty acids, while opportunistic skin infections (such as *Staphylococcus aureus*) are more prominent and becoming increasing difficult to control.[11,12] Similarly, large-sale alterations of skin microbial communities have been linked to several non-infectious diseases, such as atopic dermatitis (AD), psoriasis, rosacea and acne.[10] Altered bacterial community structure, in combination with epithelial

Abbreviations:

AD	atopic dermatitis
HMP	human microbiome project

Correspondence: Professor Alan Cooper, Department of Dermatology, Royal North Shore Hospital, Reserve Road, St Leonards, Sydney, NSW 2065, Australia. Email: acooper@scfa.edu.au

Laura S Weyrich, PHD. Shreya Dixit, MBBS (Hons). Andrew G Farrer, MPhil. Alan J Cooper, PHD. Alan J Cooper, FACD.

Conflict of interest: none

Submitted 15 June 2014; accepted 11 August 2014.

dysfunction, immune dysregulation or the overgrowth of pathogenic microbes, is a major cause of modern skin pathologies.[15]

This review examines what is currently known about diverse skin microbial communities and how they contribute to health and disease. After a brief overview of methodological practices, the current knowledge about 'healthy' skin microorganisms and the associations between the skin microbiome and several diseases are explored, specifically in the context of large-sale changes in bacterial diversity and colonisation by unique species. In addition, we discuss the impacts of a modern Westernised lifestyle on dermatological disease, as well as the potential to use these research findings to develop novel therapeutic strategies. Further analysis of the human skin microbiome and its associations with disease, through interactions with the host and other microorganisms, will undoubtedly reveal novel gateways for medical therapies, treatment strategies and prevention measures.

MODERN TECHNIQUES FOR SKIN MICROBIAL ANALYSIS

The human microbiome project (HMP), a US National Institutes of Health initiative launched in 2008, has helped to characterise the skin microbiome of healthy volunteers and discern how it varies across different spatial niches, individuals and time.[4,9] To understand and monitor these diverse bacterial communities on and within the skin, several working groups in the HMP developed extensive methodologies to examine the skin microbiome in recent years. Prior to DNA sequencing, the experimental design should consider which methods are best suited to the aims of the project and the ability to produce data that is comparable to previously published studies and databases. Several additional procedural practices must also be considered before the study begins, including avoiding contamination by environmental DNA, storage in warm conditions, and the exposure of the samples to researchers and clinicians. Precautions must be taken to ensure a sterile technique is utilised, and that bacterial DNA sequences (not only live bacterial organisms) are not introduced into the sample from sampling equipment, lab reagents, clinicians and so on.

To obtain samples, microorganisms from the skin can be collected by swabbing, scraping or collecting biopsies using sterile techniques. While swabs or scrapings are simple and can be done quickly on large populations, only biopsies can collect the subcutaneous organisms, even though only modest differences have been detected between the different collection methodologies.[14] While it has been shown that a skin swab left exposed at room temperature for 2 weeks showed little difference in bacterial community to that of a sample from the same individual stored at –20°C, cold storage is a standard practice and all studies should comply with it to limit further microbial growth and long-term DNA degradation.[15] Freezing at –20°C, –80°C or in liquid nitrogen are the most common methods, although fixing, drying or enzyme inhibition can also be an effective means of limiting DNA degradation or exogenous microbial growth.[16] If sterile sample collection is combined with effective storage conditions, an accurate representation of the skin microbiome should be maintained prior to DNA extraction and analysis.

Once the samples are obtained and properly stored, DNA extractions can then be performed. Several different methods have been developed for the extraction of skin microbiome samples, including the REPLI-g Midi kit (Qiagen, Limberg, The Netherlands), Qiagen DNA Extraction Kit (Qiagen), and DNeasy DNA Extraction kit (Qiagen).[17–19] In an effort to obtain the most accurate representation of the microbial diversity, studies have also explored different kit and non-kit based extraction methods. For example, Yuan and colleagues suggest that methods including steps to effectively disrupt bacterial cell walls (e.g. bead beating or enzymatic lysis) will provide the most accurate diversity profile.[20] For biopsy samples that can contain significant levels of host skin cells and DNA, methods have been developed to limit the quantity of host DNA recovered by maximising the amount of microbial DNA available for sequencing.[21]

After DNA extraction, the specific target species or classes of microorganisms need to be identified to determine the most appropriate sequencing strategy. For example, bacterial communities are assessed by amplifying a variable region of the conserved 16S ribosomal RNA gene, while fungal species can be targeted by applying 18S ribosomal RNA gene or the internal transcribed spacer. Targeted sequencing approaches do not require any culturing methods and hundreds of samples can be analysed on a single sequencing run, providing an efficient and cost-effective means to examining microbial communities.[22–24] Alternatively, shotgun sequencing can be performed, which will identify a subset of random DNA sequences from the sample, although the sequencing costs and bioinformatic processing time are significantly increased.[25,26] In either approach, sequencing technologies must also be taken into account. While Roche 454 or Illumina MiSeqs can provide adequate sequencing coverage or depth for targeted amplicon sequencing, deeper coverage attainable through Illumina HiSeq or Pacific Biosciences technologies may be required for shotgun sequencing.[27,28] After sequencing, modelling the diversity of bacterial sequences at a range of given sequencing depths can provide further information to determine if an adequate depth has been achieved.

Regardless of the sampling methods used or sequencing approaches taken, the single most time-consuming and essential portion of accurate microbiome analysis is done after the DNA sequencing occurs, during the bioinformatic processing. Quite often, large-scale computing clusters and specific bioinformatic pipelines must be established to understand and analyse these diverse bacterial communities from the millions of sequencing reads, evoking unforeseen experimental costs and hurdles. A few recently developed pipeline programs, such as QIIME, mothur, and MetaPhlAn, have been created to ease the burden of analysis, whereas considerably more visualisation packages,

such as MEGAN, Krona, MGAviewer, and MetaSee, aid in data imagining and interpretation.[29–35] Many of these programs focus on identifying bacterial species or groups (operational taxonomic units), making estimates of total species diversity within a sample (alpha diversity), and comparing differences between samples (beta diversity). For many specific and novel questions, the analysis may require additional bioinformatic scripts or the development of new analysing software or pipelines. Both pipeline analysis and ad hoc scripting can produce very different results, and the findings and conclusions of metagenomic data can be vastly different, simply because of the analysis that was applied to the data. This has encouraged the standardisation of analysis protocols and increased the need for computer programmers with biological knowledge or backgrounds.[36] Nevertheless, bioinformatics tools must adapt and change as new methods and sequencing technologies are developed, larger quantities of data are produced, and novel scientific questions are raised.

THE HEALTHY HUMAN SKIN MICROBIOME

After the development of a suitable tool to analyse the microbial diversity present on the human skin, Grice and colleagues characterised the topographical and temporal diversity of a healthy, adult skin microbiome from 20 different skin sites.[37] In all, 19 bacterial phyla were detected, but most sequences were assigned to four phyla: Actinobacteria (52%), Firmicutes (24%), Proteobacteria (17%), and Bacteroidetes (7%). Within these phyla, they found that *Propionibacterium* and *Staphylococci* species dominated sebaceous areas (glabella, alar crease, external auditory canal, occiput, manubrium and back). In moist sites, *Corynebacteria* species predominated (nare, axillary vault, antecubital fossa, interdigital web space, inguinal crease, gluteal crease, popliteal fossa, plantar heel and umbilicus), although *Staphylococci* species were also identified 'in these regions. In dry sites, such as the volar forearm, hypothenar palm and buttock, a mixed population of bacteria resided. Additional studies demonstrated that physiologically comparable sites often harbour similar bacterial communities; for example, the moist axillae and the popliteal fossae have similar microbial compositions.[9] Microbes were also robust to body site, as microbes transplanted from one habitat to another, such as from the tongue to the forehead, were not able to consistently colonise new sites or alter existing communities at a specific site.[9]

Since then, further studies have gone on to characterise the development and change of the human skin microbiome over time. Although foetal skin is sterile *in utero*, skin colonisation begins during the birthing process.[38] Vaginal microorganisms, dominated by *Lactobacillus*, *Prevotella* or *Sneathia* species, coat the skin of naturally delivered newborns immediately following birth, whereas the skin microbiomes of newborns delivered by caesarean section resemble that of adult skin, which includes various *Staphylococcus, Corynebacterium*, and *Propionibacterium* species.[39] Regardless of delivery method, the newborn skin microbiome is remarkably less complex and diverse than the adult skin microbiome, which suggests that the newborn skin microbiome is more susceptible to sweeping change and alterations. As infants come into contact with various environmental microbiota and as different areas of their skin develop distinct moisture, temperature and glandular characteristics, distinct skin microbial communities arise, becoming increasingly diverse over time.[37] These microbial niches and their inhabitants continue to transform over time with puberty, aging and environmental exposure.[15,40] Even sporting events can homogenise microbiomes from multiple groups of people over time.[41]

ASSOCIATIONS BETWEEN THE SKIN MICROBIOME AND DISEASE

Atopic dermatitis

Classic AD occurs more commonly in skin sites that harbour similar microorganisms, such as the antecubital fossa and the popliteal fossa, rather than other body sites.[9] Kong and colleagues found that more than 90% of AD patients are colonised with *S. aureus* on both lesional and non-lesional skin, compared with less than 5% of healthy individuals.[42] At the site of the lesion, there is a correlation between *S. aureus* abundance and disease severity, suggesting that an overabundance of cutaneous *S. aureus* and, therefore, an associated loss of microbiome diversity, are intimately linked to the pathogenesis of AD.[42] Other studies have also identified increases in fungal diversity and unique anaerobic bacterial species associated with AD, including *Clostridium* and *Serratia* species.[43] These studies could explain the effectiveness of common treatments for AD, such as topical or systemic antibiotics, and dilute bleach baths, which function to reduce the microbial load. In addition, the gut microbiomes of children suffering with AD were identified to be more diverse and resembled adult gut microbiomes, including a threefold decrease in Bacteriodetes and colonisation of Clostridium clusters IV and XIVa.[44] Increased diversity and bacterial load in the gut can have a significant impact on immune sensitivity, reactivity and tolerance, possibly further contributing to exacerbations of AD. Associations between AD and the human microbiome are likely to be linked to an increased microbial load at the lesion site, as well as altered immune reactivity on a systemic scale.

Psoriasis

Although psoriasis is commonly regarded as genetic in origin, microorganisms have been implicated in the pathogenesis of psoriasis since the 1950s. In 1955 a strong clinical association was reported between streptococcal beta-haemolytic group A throat infections and guttate psoriasis, and further evidence suggested that chronic plaque psoriasis is related to oral streptococcal infections.[45] However, anti-streptococcal treatments or the removal of infected oral streptococcal tissue were not successful in the treatment of psoriasis, leaving the link between microbes and psoriasis unknown.[46] Recently, Fahlén and colleagues

investigated the relationships between local bacterial species and psoriasis by comparing the skin microbiota of 10 patients with psoriasis to 12 healthy individuals.[47] In patients with psoriasis, Proteobacteria were present at significantly higher levels on the trunk, and higher levels of *Streptococcus* and *Propionibacterium* were identified in lesions than in healthy skin. Overall, bacterial diversity was decreased in psoriasis patients, although the difference was not significant. A further study identified two different microbial community assemblages associated with psoriasis; the skin from these patients was either dominated by Proteobacteria or Actinobacteria or Firmicutes.[48] This study was able to distinguish psoriasis patients from healthy individuals simply by the microbial diversity present on the skin, and also noted that psoriatic lesions contained less diversity than healthy skin. Although no specific aetiological microbial agent has been identified, these results demonstrate that the microbial skin habitat of psoriatic skin is less diverse, even though the fungal and viral diversity in these patients has not yet been investigated. This is in direct contrast to AD, where an increased load of microbial diversity may contributes to disease symptoms and progression. Although the mechanisms underlying these altered communities remain unknown it has been hypothesised that the combination of genetic or specific inflammatory responses elicited from unique bacterial subsets probably plays a key role in this disease.

Acne vulgaris

The aetiology and pathogenesis of acne vulgaris, an inflammatory condition of the pilosebaceous unit, remains unclear; however, the involvement of microbes is thought to be one of the main mechanisms contributing to its development. *Propionibacterium acnes* has long been implicated as an important pathogenic factor in acne, through the secretion of lipases, proteases and hyaluronidases that injure the tissue lining of the pilosebaceous unit and activate classical and alternative complement pathways, pro-inflammatory cytokines and neutrophil chemotactic factors.[49] However, as noted previously, *Propionibacterium* has also been identified as a dominant commensal skin microorganism.[9] To investigate the differences between commensal and pathogenic *P. acnes* strains, Fitz-Gibbon and colleagues recently examined *P. acnes* from both acne patients and healthy controls by sampling the pilosebaceous units on the nose.[50] Although the relative abundance of *P. acnes* did not change between the groups and the authors did not investigate inflamed follicles, the strains of *P. acnes* on patients suffering from acne were distinct from those on healthy individuals. Six different strains were dominant in the acne group, whereas only one unique strain was strongly associated with healthy skin.[51] This study also identified antibiotic resistance genes in disease-associated strains, highlighting the importance of treatment strategies other than antibiotics. In a different study, 16S ribosomal RNA sequencing studies have noted that the follicles afflicted with acne are colonised by multiple bacterial species in addition to *P. acnes*, including other commensal microorganisms, such as *Streptococcus epidermidis*, while healthy individuals are colonised by *P. acnes* alone.[52] These studies show how commensal microorganisms have the potential to be pathogenic, either through genomic evolution or through interactions with other local microbial species. In addition, these studies also highlight the potential to mitigate dermatological disease by implementing novel therapeutic solutions, such as bacterial competition or ecological exclusion.

Chronic wounds

Chronic wounds, that is, wounds that persist for longer than 3 months, occur most commonly in diabetic, elderly and immunocompromised individuals. While a bacterial infection may not have caused the initial wound, microorganisms can contribute to the lack of healing and persistent inflammation that is associated with these lesions.[49] Several studies have examined microbial communities associated with chronic wounds from venous leg and diabetic ulcers.[53–55] In addition to decreased bacterial diversity, several different anaerobic bacteria, including *Staphylococcus*, *Serratia* and *Clostridium* species, were all identified in chronic wounds, indicating that a drastic micro-environmental change allows for the opportunistic colonisation of specifically adapted microbes. In addition, a diverse mix of fungi, protozoa and viruses were also identified in chronic wounds, suggesting that these communities are more complex than initially identified.[55] Although bacterial wound communities were responsive to antibiotic treatment, the fungal and viral diversity suggests that multiple treatment strategies should be applied for successful treatment.[55] Given the significant morbidity and economic burden caused by slow-healing wounds, a individualised approach to dealing with chronic wounds, such as by examining the microbes present in a specific wound, could provide the best individualised treatment possible, resulting in a better way forward for healing management and patient prognosis.[56]

FUTURE RESEARCH AVENUES

Each of these dermatological diseases has an identified microbial component, although how alterations to the microbiome cause or exacerbate these diseases remains largely unknown. In contrast, the recent increased prevalence of each of these diseases has been closely tracked and recorded. The prevalence of AD in industrialised countries has increased over the past 30 years, now affecting up to 20% of children in industrialised countries.[57] The incidence of acne is up to 80% in most Westernised countries, even though it remains surprisingly rare in communities with hunter–gather diets.[58,59] Furthermore, the incidence of diabetes, a major contributor to chronic wounds, has more than doubled in the last 10 years in the USA.[60] The recorded increase of each of these diseases suggests that a common element may underlie each of these epidemiological observations.

Several recent studies have suggested that the human microbiome has been concurrently affected in recent history as we have changed our diet, hygiene and medical practices. For example, a large-scale ancient DNA study revealed dramatic shifts in the human oral microbiome over time, when farming was introduced in ancient Europe and during the Industrial Revolution, suggesting that dietary, environmental and cultural changes have significantly impacted on the microbial communities that inhabit the human body.[61] More recently, several studies have examined different microbiomes in modern rural hunter–gatherer communities and compared them with those from the USA or Europe.[62,65] Modern hunter–gatherers have distinctly different microbiomes and contain numerous beneficial species that are now absent from industrialised countries.[62,64] Furthermore, the relatively modern use of antibiotics has also been shown to significantly and permanently affect the murine and human gut microbiomes.[65] Taken together, these studies suggest that the modern microbiome is significantly different from what it was a century ago. Similarly, it is likely that the modern skin microbiome has also undergone significant changes in recent history. We can speculate that relatively recent shifts in diet, hygiene and medicine have affected the diversity and colonisation of specific, and potentially protective, microbial species on the human skin. Further alterations in gut microbial communities, which can have significant impacts on immune development and inflammatory responses to microbes elsewhere in the body, may have also had an impact on skin diseases. Further research should aim to understand how these recent changes within the human microbiome affect immune priming and tolerance, and how these changes impact on the environmental triggers of specific genetic skin diseases.

Moving forward, these investigations into the human skin microbiome and its association with disease can also be utilised and focused to develop novel treatment strategies. The use of prebiotics and probiotics on the skin is an area of research with great promise, as several interspecies competition mechanisms have already been explored. For example, *P. acnes* has been utilised to outcompete methicillin-resistant *S. aureus* using fermentation, and *S. epidermidis* has been shown to limit growth of *Micrococcus*, *Corynebacterium*, and *Streptococcus* species *in vitro*.[66,67] The transplantation of 'protective' microbes can provide valuable antimicrobial therapy, if the correct conditions, such as pH, moisture and nutrients that support transplantation can also be co-administered.[15] In addition, therapies to augment the increased growth of commensal microorganisms or maintain the limited growth of potential pathogens would also be beneficial. Metagenomic analyses of skin microbial communities can also aid in understanding the mechanisms behind effective treatments, such as by monitoring skin microbial communities before and after topical steroidal treatments. In any case, the introduction of microorganisms and novel treatments that alter skin microbial communities can also have significant risks that will need to be investigated to ensure that modified microbial communities do not result in additional or alternative complications or alterations.

CONCLUSIONS

This article reviews the current knowledge of the healthy human skin microbiome and documents how alterations to these communities can contribute to disease. The increased density of bacteria (AD), reduced bacterial diversity (psoriasis), augmentation of commensal organisms to cause disease and co-infections (acne), and alterations to microenvironments and colonisation of unique species (chronic wounds) can each contribute differently to dermatological disease. Future studies will need to explore how changing lifestyles, environments, and even medical practices impact on the microbial communities on the human body. Further research in this field will also provide key insights into how alterations to these communities contribute to disease progression and symptoms, as well as how these microbial communities can be manipulated for novel therapeutic strategies.

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