Cindy Wang

An Introduction to the Skin Microbiome and Acne

INTRODUCTION

The largest organ in the human body, the skin, consists of not only host skin cells but also whole communities of microorganisms. With advances in high-throughput sequencing and computational analysis methods, scientists have begun making strides towards uncovering the complex interactions between host cells and the skin microbiota, which could have significant implications for better understanding disease pathology and overall human physiology. In this paper, we explore the composition and functions of the skin microbiome, the contributions of the skin microbiome to acne vulgaris, the effects of modulating host-metabolite levels on the skin microbiome and skin disease progression, and future directions in the field.

WHAT IS THE MICROBIOME?

Our skin consists not only of our various keratinocytes, melanocytes, and other skin cells, but also millions of commensal microorganisms. These communities of microorganisms, including bacteria, archaea, fungi, viruses, and protists are referred to as microbiota. And within these communities, the collection of genes available and expressed compose the microbiome. [1]

To study the composition of the microbiota and microbiome, researchers use sequencing techniques such as amplicon sequencing, where small (~300 bp) subsections of conserved marker genes like the 16s rRNA gene for bacteria are amplified and compared to reference databases for taxonomic identification. Recently, next-generation sequencing techniques such as shotgun metagenomics have allowed scientists to capture and analyze all genetic material in a sample. This allows for greater resolution to clarify classifications among strains of the same species of microorganism and to infer the relative abundances of varying species in the microbiota.[1]

The skin can generally be split into three different categories of microenvironments: sebaceous (chest, face, back), dry (palm and forearm), and moist (inner elbow joint, groin, back of knee). [1] Within each region, the composition of the microbiota varies, and the variation continues in each of the categories as we travel deeper into the layers of the skin. [1-3] These categories are also largely determined by the relative abundance of sweat glands, sebaceous glands, and hair follicles. As the name suggests, sebaceous glands are denser in sebaceous regions of the skin, and these sites are dominated by the *Propionibacterium* species, which are lipophilic, appropriate for the lipid-rich environment caused by the secretion of sebum by the sebaceous glands. Bacterial species such as *Staphylococcus* and *Corynebacterium* are found in higher abundances in moist regions as they thrive in humid environments. Unlike bacteria, fungi tend to have similar composition throughout the various skin microenvironments, with the exception of the foot sites, which have shown greater diversity in species. [1]

Compared to the gut microbiome, the microbiome of the skin has much lower biomass due to how inhospitable the skin can be. The skin consists largely of only the most basic lipids and proteins and is thus quite nutrient poor. Hence, the microbiota that are commonly found on the skin have special adaptations to repurpose the materials present in the skin from the skin appendages. Due to the necessity of these adaptations to survive in the skin, it is difficult for new microorganisms to colonize the skin. Thus, the composition of the skin microbiome remains relatively stable for individuals. [1]

The initial colonization of the skin microbiome occurs during birth and is heavily dependent on the method of delivery. While the composition of the microbiota stays largely constant throughout adulthood, it is restructured in adolescence during puberty. Since the specific microorganisms residing on our bodies have specific adaptations to reflect the relative abundances of resources and skin appendages, the additional sebum produced by changing levels of hormones during puberty alters the microbiota. Prior to puberty, children have a more diverse fungal community, and larger relative amounts of Bacteroidetes and Proteobacteria, and

Firmicutes. In contrast, postpubescent individuals have greater abundances of lipophilic microorganisms including fungal *Malassezia* spp., and bacterial *Corynebacterium* spp. and *Propionibacterium* spp. The exact mechanisms by which these changes occur is still unclear, but an interesting area of future exploration as many skin diseases are associated with certain ages.[1]

The microbiota are not only passive, commensal microorganisms residing on our skin, but active participants in protecting our bodies from pathogenic attack. In what is referred to as colonization resistance, it has been shown that our skin microbiota can aid our immune system by competing with harmful microorganisms and preventing them from breaking the skin barrier. For example, some human skin bacteria such as *Staphylococcus epidermidis* have adapted antimicrobial mechanisms to prevent biofilm formation of *S. aureus* bacteria.[1, 4] Further investigation is warranted to better understand the intricate relationships between distinct species and strains within our skin microbiota and pathogenic microorganisms from the environment.

While colonization resistance plays a critical role in our body's ability to defend itself from infection, sometimes commensal and even mutualistic members of our skin microbiota can become pathogenic. Several skin disorders have been found to be correlated to or have contributions from dysbiosis, changes in the skin microbiota.[1]

Acne vulgaris is a common skin condition that affects more than 80% of adolescents and young adults.[3, 5] Associated with a primary bacterial causative agent, *P. acnes*, acne has been correlated with differential expression of genes in *P. acnes* in people with the condition compared to those without. For example, *P. acnes* populations in people with acne have been shown to produce greater concentrations of porphyrins, chemicals that lead to hyper-secretion of sebum and inflammation.[3] In atopic dermatitis, commonly referred to as eczema, the abundances of *S. aureus* and *S. epidermidis* are positively correlated with the severity of flare-ups and increase greatly during flares compared to pre- and post-flare periods.[1]

In those with primary immunodeficiency (PID), there is often less stability in the composition of the microbiota and greater permissibility of atypical microorganisms. However, the species that are generally associated with the skin still appear at levels similar to their regular abundances, further indicating that there exist specific adaptations microorganisms need to survive the harsh environment of the skin. This relative instability of the microbiota has also been observed in individuals with chronic wounds. However, a study on diabetic foot ulcers and their associated microbiota showed the favorability of such instability. More frequent changes in the microbiota reflect the difficulty harmful microorganisms face when attempting to colonize and enter the body. Thus, the more unstable the skin microbiota, the less likely pathogenic microorganisms can enter the wound and cause internal infection. [1]

The skin microbiota and microbiome play major roles in protecting our bodies from the surrounding environment and understanding the interactions between the various members of the microbiota and with our own cells appears to be critical to elucidating the dynamics of various disease states and the basic functioning of our skin and immune system.

ACNE AND THE SKIN MICROBIOME

Propionibacterium acnes, *P. acnes*, is one of the most common species of bacteria found in the skin microbiota. There are three major subspecies of *P. acnes* classified based off of their susceptibility to phages, serum lectin response, and cell wall sugar content. And among the three subspecies, many smaller classifications of *P. acnes* strains can be made, with some classifications occurring in varying abundances correlating with disease states.[3]

In healthy individuals, *P. acnes* plays an important role in the skin microbiota's colonization resistance efforts against pathogenic strains of *S. aureus* and *Streptococcus*. They also help to maintain a lower skin pH by inducing the synthesis of acetic and propionic acid, which enable them to break down triglycerides and release free fatty acids. [2] At the same time, however, *P. acnes* is the main species of bacteria associated with acne vulgaris.

The pilosebaceous unit, which is comprised of the sebaceous gland and hair follicle, is where acne appears and is also a skin site dominated by *P. acnes*, with a density of up to $(10^5 - 10^6 cm^2)$ in the scalp and facial regions.[1] While the exact involvement of *P. acnes* in the pathophysiology of acne remains unclear, researchers have proposed a few main mechanisms of action.

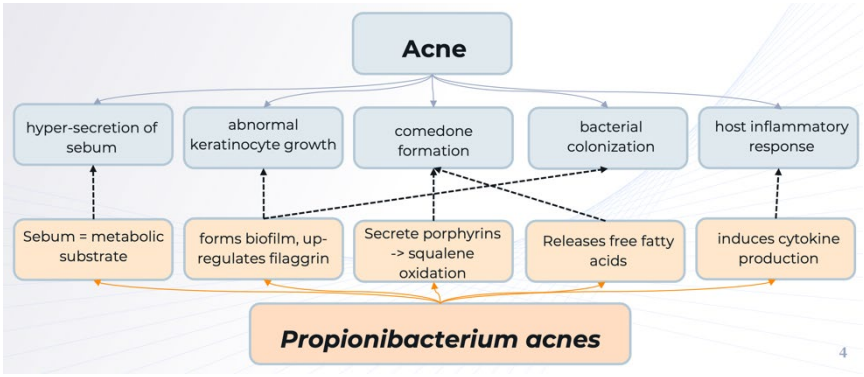

Figure 1. The mechanisms of *P. acnes*-driven acne pathogenesis. Acne is caused by five main mechanisms, represented in blue. *P. acnes*, a main component of the bacterial colonization factor, also impacts the other driving mechanisms of acne development as represented by the orange boxes and black and orange arrows.

One key driver of acne pathogenesis is the overproduction of sebum, a mixture of lipids (such as triglycerides and squalene) secreted by the sebaceous glands that can cause blockages in the pores.[6] *P. acnes* positively regulate the synthesis and secretion of sebum as they can upregulate the activity of diacylglycerol acyltransferase, an enzyme involved in triglyceride synthesis,[7] while relying on the metabolization of sebum for growth.[3]

The formation of comedones, closed or open skin lesions that develop around pores, is also a main contributor to acne development. Comedogenesis can be attributed to the combination of free fatty acids and oxidized squalene[6] as well as the retention of hyper-proliferating keratinocytes in the hair follicle duct. Studies have shown that *P. acnes* will both break down the triglyceride components of sebum and secrete porphyrins, catalytic factors that enable the oxidation of squalene. Furthermore, *P. acnes* can form biofilms that help keratinocytes adhere to the follicular duct and regulate keratinocyte proliferation and differentiation by activating the insulin-like growth-factor 1 (IGF-1) signaling pathway.[3]

Finally, *P. acnes* can worsen existing and trigger new inflammation by binding to Toll-like receptor 2 (TLR-2) and TLR-4 on the surface of keratinocytes. This binding induces host cells, such as monocytes, to produce various interleukins and cytokines to further inflammatory responses. Interleukins and other chemical factors secreted by *P. acnes* also promote the maturation of naïve T cells to Th17 cells, and enzymes produced by *P. acnes*, such as lipases and metalloproteins cause damage to sebaceous glands, hair follicles, and the dermal extracellular matrix, further aggravating inflammation.[3, 4]

While several other microorganisms, including *S. epidermidis*, *S. hominis*, *Malassezia*, have been shown to play roles in acne development, *P. acnes* is recognized as the main bacterium involved with the disease's pathogenesis.[4] Due to this relative simplicity and its widescale impact on the general population, acne serves as a promising model for studying how the skin microbiota and host interact as the disease progresses.

Although *P. acnes* has been proposed to act in many ways to promote acne pathogenesis, *P. acnes*, along with the other major bacterial phyla of the skin, is also found in similarly abundant proportions in the microbiota of individuals without acne.[5] Why does the presence of *P. acnes* have such varying effects?

HOW VITAMIN B12 SUPPLEMENTATION CAN CAUSE ACNE

Since *P. acnes* is found in both people with and without acne, researchers investigated what differences there may be between the *P. acnes* populations on people with acne compared to those without. A study performed by researchers at the University of California, Los Angeles, published in 2015 examines the differences in gene expression in *P. acnes* in people with and without acne, investigating the role of the vitamin B12 biosynthesis pathway in particular.

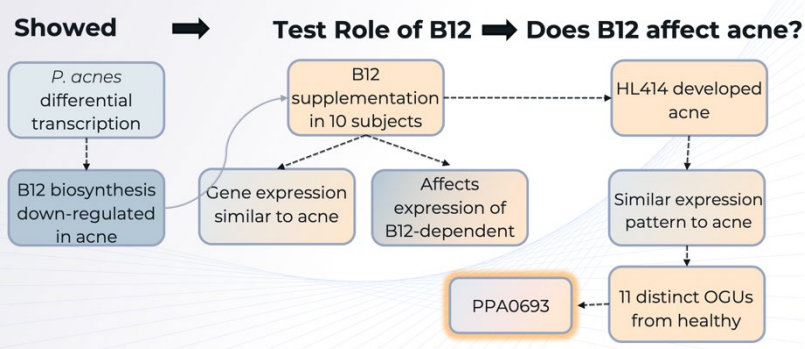

Figure 2: Investigating the role of Vitamin B12 synthesis in acne pathogenesis. The light blue represents the initial experiment conducted comparing 5 healthy and 4 acne individuals' transcriptomes; the darker blue represents the investigation into B12 specific gene regulation in acne patients; the orange represents the *in vivo* B12 supplementation study. The findings indicated by a gradient of two of the three colors represent findings resulting from both studies.

By applying RNA-seq to nose skin samples obtained from a group of five healthy individuals and four acne patients, they observed that there was indeed distinct transcriptional activity. They clustered all of the genes found specific to *P. acnes* and kept as operational gene units (OGUs) the genes that were found conserved in all strains of *P. acnes*. Out of a master set of 3725 OGUs, 136 had differential gene expression in acne patients compared to healthy people. Interestingly, the differentially expressed OGUs were largely associated with metabolism and protein transport, and a few were linked to previously predicted virulence factors.

Several metabolic pathways, including the vitamin B12 biosynthesis pathway, porphyrin metabolism, and the glutamine amino acid family synthesis, were affected. Specifically, when the researchers looked at three genes critical to vitamin B12 biosynthesis, cysG+cbiX, cbiL, and btuR, they found cysG+cbiX and cbiL to be significantly downregulated in patients with acne. In years past, vitamin B12 supplementation has been clinically noted to cause acne in a subset of patients, the mechanism unclear.

It is important to note that vitamin B12 acts as a negative regulator of its own synthesis pathway. Thus, to further clarify the role of vitamin B12 biosynthesis in acne progression and how it can be modulated, the study investigated how vitamin B12 supplementation would affect the skin microbiome of ten healthy, non-acne individuals. Upon sequencing nostril-skin samples collected from the subjects two weeks after intramuscular vitamin B12 supplementation, they

verified that supplementing vitamin B12 does indeed decrease the expression of vitamin B12 biosynthesis genes. When comparing to the skin microbiomes of patients with acne from the initial experimentation, they found that healthy patients supplemented with vitamin B12 had similar changes in gene expression in their *P. acnes* populations.

In fact, one out of the ten patients developed acne after the first week of vitamin B12 supplementation. Upon examining the differences between that patient and the other healthy supplemented patients, they identified 11 distinct OGUs, one of which was PPA0693, a gene also found to be significantly downregulated in individuals with acne from the initial experiment.

Building off of this discovery, the study proposed a mechanism through which B12 supplementation contributes to acne pathogenesis. Supplementing B12 decreases the overall expression of genes associated with B12 biosynthesis, which could impact the expression of genes dependent on the B12 biosynthesis pathway as well. PPA0693 encodes the E2 component of the 2-oxoglutarate dehydrogenase complex, which ordinarily converts 2-oxoglutarate to succinyl co-A in the tricarboxylic acid cycle. However, when B12 is supplemented and in people with acne, PPA0693 is downregulated, thus leaving an excess of 2-oxoglutarate. It turns out that 2-oxoglutarate is a precursor for either vitamin B12 biosynthesis or porphyrin biosynthesis. Previous studies have shown that when vitamin B12 biosynthesis decreases, the concentration of porphyrins increases. Thus, since supplementation of B12 would greatly stunt B12 biosynthesis, the excess 2-oxoglutarate would largely be consumed in the synthesis of porphyrins.[4] Porphyrin, as described earlier, is a chemical factor that can lead to the oxidation of squalene, a main component of sebum[6], and stimulate inflammatory mediators in keratinocytes, thus promoting acne development.

Metabolites like vitamin B12 can greatly affect the expression of genes in the skin microbiome. These findings strongly suggest that host metabolite levels can be modulated, such as via supplementation of vitamin B12, to possibly control the expression of genes in the skin microbiota and affect disease states.

FUTURE AREAS OF INTEREST REGARDING THE SKIN MICROBIOME

In areas of research such as antibiotic resistance and automated diagnosis, scientists have been working to apply deep neural networks to better understand and approach various medical challenges. For example, researchers at MIT, such as James Collins, have created deep learning models that turn millions of chemical compounds into continuous vectors whose similarities, connections and capacity for use as an antibiotic can be explored *in silica* much more efficaciously than traditionally. [8] For the human microbiome specifically, researchers have created deep learning methods that utilize the idea of convolutional neural nets to represent the phylogenetic relationships between microorganisms in our microbiota and predict various characteristics like age and gender of individuals from gut microbiome data. [9] Since much microbiome data is high-dimensional, other researchers such as those who created DeepMicro, have created deep learning models that can reduce the dimensionality of human microbiome data to better represent the microbiota and the interactions between the microorganisms in the microbiota and host cells for specific investigative purposes. [10]

Similarly, building off of discoveries like that of the vitamin B12 study, the skin microbiome could be analyzed *in silica*. Due to vast improvements in genome sequencing technology in the last few years, despite having lower density than the gut microbiome, much of the skin microbiome has been sequenced including skin microbiomes of individuals with various diseases. Using deep learning techniques and accurate dimension-reduction, researchers could transform the existing sequenced data on the various genes of the skin microbiome and the microorganisms that reside in the skin microbiota into continuous vectors to be further analyzed by a deep learning model. A training dataset could be created for such investigation of how the various skin microorganisms affect each other and the relationship between disease and dysbiosis, from experiments like those conducted in the vitamin B12 study. From this training set of known interactions of various metabolite changes and disease-state impacts, our deep

learning model could further uncover connections between the host and the skin microbiome. Through this model, we may be able to better understand how the skin microbiome affects our overall health. We may also uncover strategies to manipulate the microbiome, by topical probiotics or targeted antibiotic treatments, to improve treatment of certain disease-states and aid accuracy of differential diagnosis of various skin conditions.

Overall, better understanding the connection between the skin microbiome and the host is critical to identifying novel solutions to various skin conditions and human physiology as a whole. Applying existing high-throughput sequencing and convolutional neural net technology to study the skin microbiome could open pathways that may transform the field of dermatology and medicine for all.

REFERENCES

1. Byrd, A.L., Y. Belkaid, and J.A. Segre, *The human skin microbiome.* Nature Reviews Microbiology, 2018. **16**(3): p. 143-155.
2. Kanwar, I.L., et al., *Models for acne: A comprehensive study.* Drug Discoveries & Therapeutics, 2018. **12**(6): p. 329-340.
3. Xu, H. and H. Li, *Acne, the Skin Microbiome, and Antibiotic Treatment.* American Journal of Clinical Dermatology, 2019. **20**(3): p. 335-344.
4. Kang, D., et al., *Vitamin B12modulates the transcriptome of the skin microbiota in acne pathogenesis.* Science Translational Medicine, 2015. **7**(293): p. 293ra103-293ra1.
5. Li, C.X., et al., *Skin microbiome differences relate to the grade of acne vulgaris.* The Journal of Dermatology, 2019. **46**(9): p. 787-790.
6. Pappas, A., et al., *Sebum analysis of individuals with and without acne.* Dermato-Endocrinology, 2009. **1**(3): p. 157-161.
7. Roe, N.D., et al., *The Role of Diacylglycerol Acyltransferase (DGAT) 1 and 2 in Cardiac Metabolism and Function.* Scientific Reports, 2018. **8**(1).
8. Stokes, J.M., et al., *A Deep Learning Approach to Antibiotic Discovery.* Cell, 2020. **180**(4): p. 688-702.e13.
9. Wang, Y., et al., *A novel deep learning method for predictive modeling of microbiome data.* Briefings in Bioinformatics, 2020.
10. Oh, M. and L. Zhang, *DeepMicro: deep representation learning for disease prediction based on microbiome data.* Scientific Reports, 2020. **10**(1).