THE SKIN MICROBIOME IN PATIENTS WITH ACNE VULGARIS

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MEETING SUMMARY

Similar to some other tissues such as the gut, the skin is colonised by a dense community of commensal microorganisms. Maintaining the balance of this diverse flora may be important for healthy skin. Changes in the composition of cutaneous microbial communities have been linked to several chronic inflammatory skin diseases, including atopic dermatitis, psoriasis, and acne. Acne is a chronic inflammatory disease that affects the pilosebaceous follicle. The association between *Propionibacterium acnes* and acne vulgaris has been well established, but very few studies have investigated the total facial skin microbiota of acne-affected patients. Three-dimensional topographic analyses and microbiome profiling have, however, revealed differences in microbiome composition between healthy skin and acne lesions, as well as natural differences in microbial colonisation between the sebaceous gland and surface skin.[1] Furthermore, bacterial communities of the skin are involved in immune homeostasis and inflammatory responses important in the development of all acne lesions.[2] This improved understanding of the interactions between skin microbiota and the innate immune response in acne may provide a platform to design efficacious treatment strategies, specifically concerning the role of dermocosmetics to protect the skin microbiome.

The Cutaneous Microbiome: A Master for Healthy Skin and an Underestimated Factor in Skin Diseases

Professor Thomas Bieber

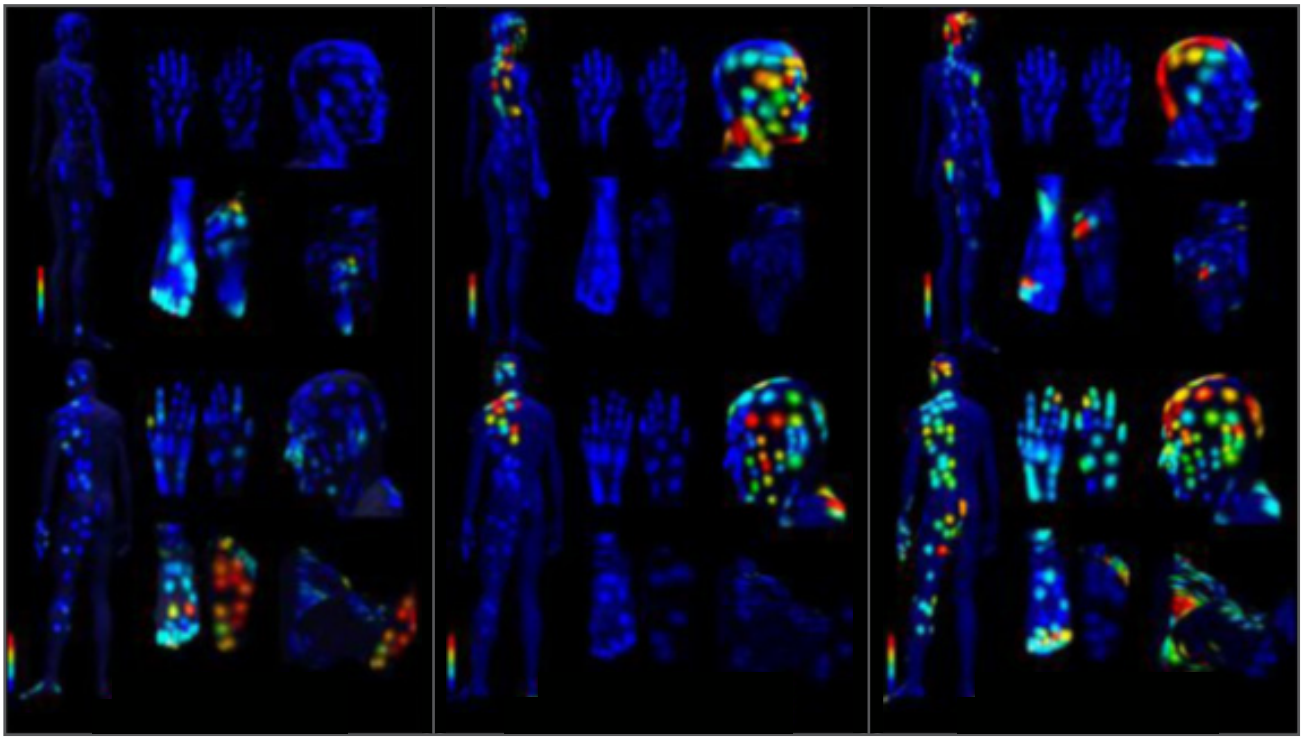
Since the discovery of microbes on the human body, much information on their composition has been gathered. However, the role of this collection of bacteria, fungi, and viruses remains a mystery. As well as the skin, most organs with an external surface are heavily colonised with different kinds of microbes, bacteria, fungi, and viruses. This 'microbiome' contains 10^{14} organisms, representing 10-times more cells than the human body itself,[2] and is often referred to as our 'second genome' owing to its fingerprint-like unique composition of diverse organisms. The microbiome can be localised to three zones of the human body: sebaceous areas and those sites that are dry or moist, each of which

represents differing ecological conditions and are therefore favoured by different kinds of microbes.[2]

The microbiome is strongly influenced by environmental signals and conditions. Microbiota on the skin are determined at birth and vary depending on the method of birth (natural or caesarean section) and the extent of contact with the mother shortly thereafter,[3,4] whereas the microbiota of the gut are largely dependent on early-life feeding habits.[5,6] Thus, certain events during human development are crucial in determining the basis of an individual's microbiome, which will ultimately adapt and change over the years.[7] In addition to the sex and age of a person, genetics, environmental factors (both climatic and geographic), immune response, lifestyle (e.g. occupation and level of hygiene), and underlying disease play a critical role in driving and regulating the composition of the microbiome.[8]

Microbiota have been linked to various diseases, including inflammatory bowel disease, diabetes, obesity, rheumatoid arthritis, and various allergies,[9,10] as well as neuropsychiatric diseases related to the gut–brain axis.[11] However, not all microbes are harmful. When considering the skin, microbes are present on the surface as well as deep within the epidermal compartment owing to connections with structures such as the sebaceous glands.[12] Interestingly, microbiota have been found to be in close dialogue with other cell types, including resident immunologically important cells (e.g. T cells). In addition, it has been shown that microbes in contact with Langerhans cells bridge the innate and the adaptive immune system, allowing local production of antimicrobial peptides (AMPs)[1] to counteract pathogenic microbes or even protect against atopic sensitisation and inflammation.[13] One example is *Staphylococcus epidermidis*; these bacteria themselves produce high amounts of AMPs to control the growth of pathogenic *S. aureus* strains.[14] Although diverse colonisation is key for good health, as microbes consistently deliver signals that contribute to the education of our immune system, skin is the most vulnerable organ to environmental changes, with the most unstable microbiota compared with those of the gut or other niches of the body.[15]

New technology provides 3D topography of the skin that allows visualisation of the microbiome, and the associated metabolome, for the first time (Figure 1).[1] These visualisation methods will greatly aid in developing new approaches for the design of therapeutic compounds and cosmetics.



Staphylococcus *Propionibacterium* *Corynebacterium*

Figure 1: Three-dimensional topography of the skin microbiome.[1]

How *P. acnes*, the Microbiome, and the Innate Immunity Interact in Acne

Professor Brigitte Dréno

Acne is a chronic inflammatory disease affecting the pilosebaceous follicle. Three main factors are implicated in the development of acne lesions: firstly, sebaceous glands are stimulated via activation of several receptors, including those for androgens, neuropeptides, insulin-like growth factor 1, and peroxisome proliferator-activated receptors; secondly, the combination of hormonally stimulated sebum production, abnormal keratinisation of the pilosebaceous duct, and formation of comedones; and thirdly, an inflammatory immune response to *P. acnes*, a gram-positive bacterium that results in activation of innate immunity and the development of an inflammatory reaction. Inflammation is crucial in the development of acne and could potentially contribute to the development of scars. The bacterium has been located in active lesions and also found to underpin the development of persistent post-inflammatory hyperpigmentation via the stimulation of alpha melanocyte-stimulating hormone and interleukin (IL)-1.

P. acnes is a commensal bacterium of the pilosebaceous follicle microbiome that plays a physiological role by inhibiting invasion by pathogenic bacteria such as *S. aureus* and *S. pyogenes*. In addition, *P. acnes* maintains the acidic pH of the sebaceous gland and the skin by hydrolysing triglycerides, releasing free fatty acids, and secreting propionic acid.[8] This helps to maintain the physiological profile of the sebaceous microbiome in both a quantitative and qualitative manner. Recent improvements in DNA sequencing technology, together with the publication of metagenomic analyses by the Human Microbiome Project Consortium,[16,17] allowed the identification of a 16S rRNA gene, which includes hypervariable regions that provide species-specific signature sequences, permitting the identification and classification of bacteria.[18] *P. acnes* has been characterised as an anaerobic bacterium that grows particularly well in the sebaceous areas of the forehead, retroauricular crease, and back.[18,19] Furthermore, metagenomic studies have highlighted that acne is a non-infectious disease that results from shifts and imbalances in microbiota of the skin.[18,19]

P. acnes interacts with the innate immune system to promote inflammation in two different ways. The skin acts as an immunological barrier and a first-defence mechanism against infection. *P. acnes* directly modulates innate immunity by identifying pathogen recognition patterns and activating innate immune responses via Toll-like receptors,[20,21] peroxisome-activated receptors,[22,23] intracellular NOD-like receptors 1–3, retinoic acid-inducible gene-like intracellular receptors, and AMPs, thus regulating cutaneous inflammation.[22] Optimal skin health and innate immunity are maintained when the microbiome and the immune system of the skin are balanced. The second mechanism by which *P. acnes* activates cutaneous innate immunity is by quantitatively and qualitatively modifying the cutaneous microbiome. Hyperseborrhoea, starting during puberty, induces proliferation of specific *P. acnes* subtypes, *S. epidermidis*, and corynebacteria, which creates an imbalance in the microbial make-up of the skin that stimulates the activation of cutaneous innate immunity, including the secretion of IL-1β by keratinocytes and monocytes, and development of inflammatory lesions.[8,24] Additional secretion of IL-17 by monocytes in the dermis and follicular metalloproteinases are also implicated in destruction of the follicle and scarring.

Recent studies have shown that acne is not necessarily the result of the proliferation of *P. acnes*, as *P. acnes* predominates on both normal and disease-associated skin.[25] Genomic comparison of *P. acnes* strains has allowed identification of different profiles of commensal *P. acnes* subtypes between healthy skin and acne lesions, demonstrating phenotypic and functional differences of *P. acnes* as a commensal in health and as a pathogen in acne.

Skin care plays an important role in acne, as repeated cleansing of the skin results in modification of the natural protective barrier. The natural defence system of the skin can be altered by using detergents that disrupt the skin lipid barrier and induce marked loss of AMPs, allowing proliferation of *S. epidermidis* and attenuation of the innate immune response. Therefore, the main purpose of cosmetics for skin care in acne is to maintain the protective barrier function by maintaining cutaneous pH, hydration, and lipid film to protect the skin barrier and the microbiome.[26]

Research into cosmetics that support the natural defences of the skin is ongoing. A recent study demonstrated the benefits of cosmetic AMPs in the suppression of antibacterial, antiviral, and

anti-inflammatory activity, thus maintaining skin homeostasis and suppressing bacterial resistance by inhibiting the pro-inflammatory IL-1 pathway.[27] Furthermore, treatment with vitamin B12, B3, and ceramide has demonstrated benefits with regard to modulation of the transcriptome of the skin microbiome in acne pathogenesis,[28] delaying extracellular signal-regulated protein kinase activation, and reducing melanin synthesis via alpha melanocyte-stimulating hormone and inhibition of the IL-1 inflammatory pathway.[29]

The Skin Biome: A New Player in Acne Management

Doctor Sophie Seité

In collaboration with L'Oréal Research and Innovation and the University of Boulder (Boulder, Colorado, USA), La Roche-Posay has initiated studies to analyse the microbiomes of various face skin surface areas of acne patients compared with healthy individuals. Superficial intrapersonal sampling of inflammatory lesions and non-inflammatory lesions in close, non-affected areas, coupled with amplification of the 16S rRNA specific to each bacterium in the skin samples allows identification of the bacterial landscape at the surface of the skin of acne patients and assessment of the quantity and the diversity of the superficial microbiome.[30] Three outcomes were considered to be of the greatest importance: differences between the microbiomes of superficial acneic and normal healthy skin in two individuals, differences in the microbiomes of an inflammatory lesion and adjacent unaffected skin of the same individual, and variability between the microbiome of a non-inflammatory lesion and adjacent unaffected skin of the same individual.

Bacterial biodiversity seems lower on the skin surface of the cheeks of healthy individuals compared to patients with acne possessing the same skin type, age, and gender, particularly following treatment.[30] The main difference in the phyla present on both skin types is the quantity of actinobacteria (including the *Propionibacterium* genus), which are observed at higher levels in healthy skin of healthy individuals, together with firmicutes and proteobacteria. The lower levels of actinobacteria present on acneic patients translate into increased levels of firmicutes and proteobacteria, even on unaffected skin (Figure 2).[30]

The comparison of unaffected skin with non-inflammatory and inflammatory lesions sampled from acne patients revealed similar profiles of phyla. Whereas the level of actinobacteria was similar across the three sampled sites, the abundance of proteobacteria was lower for both lesions compared with unaffected skin. Conversely, firmicutes were observed at a higher level on non-inflammatory lesions in comparison with unaffected skin. This increased quantity of firmicutes was mainly due to a significant rise in staphylococci in both lesions compared with unaffected areas (Table 1). When comparing healthy individuals with those affected by acne, propionibacteria made up 38% and less than 2% of the skin phyla, respectively.[31]

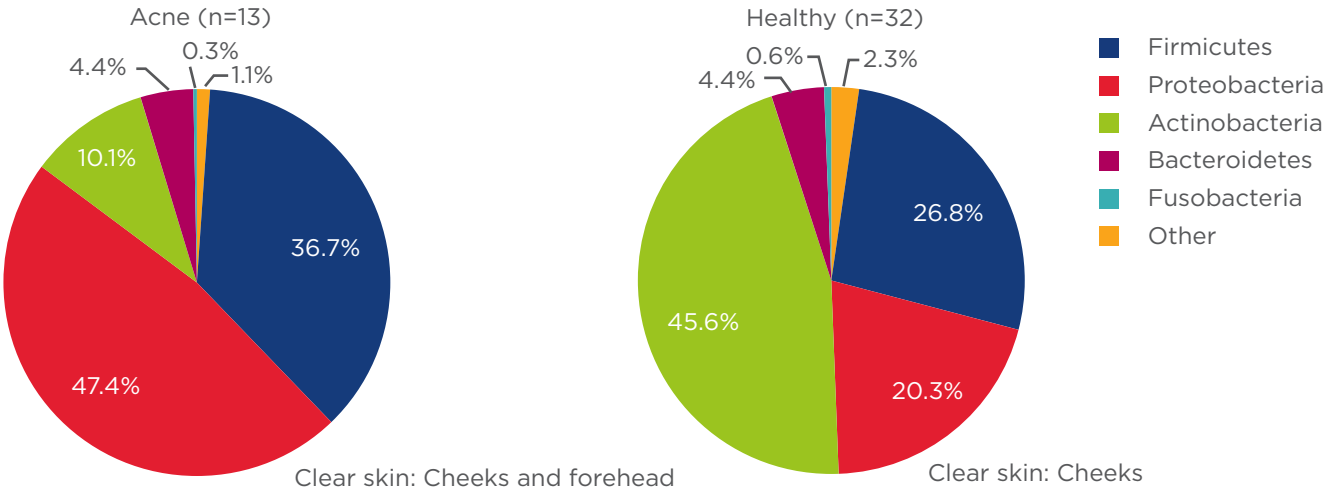


Figure 2: Phylum-level comparison of the microbiomes of healthy skin (cheeks) and acne-affected skin (skin without any lesion - cheeks and forehead).

Table 1: Phyla profiles of non-inflammatory lesions (NIL), inflammatory lesions (IL), and unaffected skin (UAF) taken from acne patients.

PHYLUM	GENUS	NIL (%)	IL (%)	UAF (%)
Actinobacteria		13.61	14.15	13.75
	Propionibacterium	1.04	1.20	1.36
	Corynebacterium	7.93	8.54	7.71
Firmicutes		52.01*	49.27	47.01
	Staphylococcus	33.87*	34.00*	26.85
Proteobacteria		28.90*	31.30*	34.10

*p<0.05 vs UAF, n=26.

The differing severity of acne was reflected in the proportion of staphylococci, without apparent differences for propionibacteria or *P. acnes*. Therefore, while the bacterial microbiota of follicles from acne-affected patients are dominated by *P. acnes*,[32] current data indicate that the microbiota of the skin surface are dominated by staphylococci relating to the severity of acne.

When assessing the effect of topical treatment with 4% erythromycin or a dermocosmetic on the skin surface microbiota of acne-affected patients, both agents significantly decreased the number of non-inflammatory and inflammatory lesions after short-term use. These benefits may be the result of a significant reduction in actinobacteria, particularly corynebacteria and propionibacteria, after antibiotic treatment and a smaller effect on staphylococci and propionibacteria following treatment with the dermocosmetic used in this study. Therefore, dermocosmetics targeting staphylococci in monotherapy or in combination with additional treatments provide an effective treatment strategy to manage more widely the microbiotic imbalance observed on the skin surface of patients affected by acne.

Q&A session

The effect of method of birth and influences immediately afterwards on the microbiome are well understood. To what extent does the microbiome change throughout a person's lifetime?

Prof Bieber: It is important to note that past studies were based on swab sampling, whereas in current studies cutaneous swabs or scratching can be performed to analyse samples, allowing us to assess more carefully and distinguish between the skin surface microbiome and the follicular microbiome. It has been suggested that standardised microbiomes could be used to correct the composition of the skin biome following birth by caesarean section. However, the composition of the microbiome is largely dependent on genetic and epigenetic pressures. Manipulation of the microbiome is likely to be transient and would require long-term and consistent application of agents designed to change the microbiome of the skin.

What was the make-up of the agent used as dermocosmetic?

Dr Seité: The study presented was based on a cosmetic (Effaclar Duo[+], La Roche-Posay) containing β-lipohydroxy acid, salicylic acid, linoleic acid, niacinamide, and piroctone olamine, and which has been shown to be very effective in the treatment of acne, mainly via rebalancing the skin surface microbiome without eradicating *P. acnes* and with no risk of inducing *P. acnes*-resistant strains.

Does each individual harbour different phenotypes of *P. acnes* or is a single phenotype involved in the development of acne?

Prof Dréno: Different phenotypes of *P. acnes* are involved in the development of the skin disorder and this is very individual to each patient. The severity of acne most likely depends on the ratio of the various phenotypes, as well as the predominant phenotype, as the effect on innate immunity activation can vary.

Can different skin diseases, such as acne and psoriasis, be explained by differences in microbiota?

Prof Bieber: The development of cartographic analysis of the microbiotic composition of various

body regions will aid greatly in establishing 'concrete' profiles that will aid the treatment of specific skin disorders. However, the question remains whether changes in microbiomic composition are a cause or the result of skin disorders. The unexpected finding of staphylococci on the acneic skin surface, as presented during this symposium, may be interpreted as a secondary phenomenon owing to a particular kind of inflammation that favours the growth of bacterium in unexpected regions of the skin. Furthermore, topical antibiotic treatment used in acne-affected patients may lose its activity due to bacteria becoming resistant. However, many topical antibacterials appear to have an additional benefit as anti-inflammatory compounds.

Dr Seité: One limitation of studies assessing the microbiome is the inability to evaluate antibiotic resistance of *P. acnes* or staphylococci. Therefore, antibiotics can induce the development of bacterial resistance but are also, as demonstrated in this study, unable to manage the microbiotic imbalance observed on the skin surface of patients affected by acne.

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