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Consumer Safety Considerations of Skin and Oral Microbiome Perturbation

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Summary

Microbiomes associated with human skin and the oral cavity are uniquely exposed to personal care regimes. Changes in the composition and activities of the microbial communities in these environments can be utilised to promote consumer health benefits; for example by reducing the numbers, composition or activities of microbes implicated in conditions such as acne, axillary odour, dandruff and oral diseases. It is however important to ensure that innovative approaches for microbiome manipulation do not unsafely disrupt the microbiome or compromise health, and where major changes in the composition or activities of the microbiome may occur, these require evaluation to ensure that critical biological functions are unaffected. This article is based on a two-day workshop held at SEAC Unilever, Bedford, United Kingdom, involving 31 specialists in microbial risk assessment, skin and oral microbiome research, microbial ecology, bioinformatics, mathematical modelling and immunology. The first day focused on understanding the potential implications of skin and oral microbiome perturbation, while approaches to characterise those perturbations were discussed during the second day. This article discusses the factors that the panel recommend are considered for personal care products that target the microbiomes of the skin and the oral cavity.

INTRODUCTION

The human microbiome

The last two decades have seen the effective application of culture-independent methods to study the human microbiota (the microbial cells) or microbiome (the associated DNA) (1). This has led to a deeper and more comprehensive analysis of the diverse range of organisms that inhabit the body, where a substantial proportion are not readily amenable to culture (2). In the process some but certainly not all knowledge gaps have been addressed. High-throughput sequencing is currently performed using a range of platforms including Illumina and Ion Torrent, which can rapidly sequence millions of fragments of DNA in parallel (3). Hypervariable regions of the bacterial 16S rRNA genes, or whole genome DNA is targeted to analyse complex microbial communities. For 16S amplicon sequencing in particular, bioinformatic analyses have been applied to cluster the generated sequences according to their similarity to define different operational taxonomic units (OTU), which are then compared to databases to reveal community composition. However, tools such as DADA2 are being increasingly used to obtain exact sequence variants (4) giving greater resolution (5). The often short sequencing reads and the large data volumes generated through NGS presents challenges and taxonomic classification and relative abundances can vary depending on the

bioinformatic pipeline used (3). Microbiome research has nevertheless identified considerably greater microbial diversity than had been previously characterised, overcoming some of the limitations of culture including issues of non-culturability. Whilst microbiome research in humans has focussed primarily on the gut, studies of the oral cavity (6-9) and skin (10-14) have facilitated the deeper understanding of these sites, which are of particular relevance to personal care. The use of personal care products can result in changes in microbiome that may be intentional or otherwise. It is however important to note that "oral microbiome" and "skin microbiome" are simplified terms referring to biogeography-dependent sets of communities where microbial composition and activities can vary markedly depending on site.

The challenge of establishing causality

The human microbiome provides protection against pathogenic organisms (14) and can stimulate the immune system (15, 16) and participate in the maintenance of different ecological niches present in the body (17). Fluctuations in micobiome composition may therefore perturb beneficial microbial functions with potential health implications for the host. The following section will consider some notable diseases of the skin and the oral cavity where differentiating between cause and association for microbiome composition has been challenging.

Atopic dermatitis (AD) is a chronic, relapsing inflammatory condition characterised by pruritis (itchiness), wheels and flares, and in severe cases, broken, bleeding skin. A high *Staphylococcus aureus* load has been reported to correlate with AD flares and *vice versa* in clinical studies involving AD patients, where coagulase negative staphylococci (CoNS) were more abundant in healthy controls (18). Colonisation with commensal staphylococci early in life appears to be protective against the development of AD (19), and AD is also strongly associated with mutations in the barrier protein, filaggrin (20). It has therefore been hypothesised that an abnormal epidermal

environment caused by a leaky skin barrier predisposes the skin to infection by exposing environmental niches that would normally be inaccessible to *S. aureus.*

Unravelling the role of the microbiome in dermal diseases is confounded by the physiological changes in host tissues that characterise the pathology. Acne vulgaris, for example, has been associated with overgrowth of *Cutibacterium* (formerly *Proprionibacterium) acnes*, but this association is not necessarily causal. In addition, Acne vulgaris has been potentially linked to changes in the dermal environment proposed to be driven by factors including a Western style diet, which may influence signaling in the hair follicle resulting in overproduction of sebum (21). The photodermatosis, polymorphic light eruption (PLE) that is characterised by a rash on exposure to UV light has been associated with the abnormal expression of antimicrobial peptides in the skin, (22) distinct from that seen in psoriasis or AD, suggesting a microbiota involvement. PLE is however also associated with other changes in the immune system of the skin (23) (24). The common inflammatory skin condition psoriasis has been associated with changes in the skin microbiota (25) (26) but this association is not necessarily causal because the massive systemic inflammatory response that is a feature of psoriasis may also profoundly influence the composition of the skin microbiota (as reviewed by (27)).

Whilst the relationship between the oral microbiome and oral disease is arguably better understood, knowledge gaps remain. Common conditions such as dental caries, gingivitis and periodontitis are closely associated with potentially harmful changes in the composition and activities of the oral microbiota (sometimes referred to as dysbiosis) (28) (29) that have environmental triggers. The development of caries for example, is related to high intake of sugary foods and the consequent production of lactic acid by caries-associated bacteria within the oral microbiome. This in turn favours the growth of acid-tolerant, acidogenic organisms such as *Streptococcus mutans* which, along with other oral bacteria, forms biofilms on the tooth surface (30). Acid produced by these organisms can alter the balance of enamel demineralisation/remineralisation of the tooth, leading to

loss of mineral, and caries formation. In periodontitis, the persistent presence of subgingival biofilms associated with poor oral hygiene can lead to inflammation and bone loss (31). The pathology of periodontitis is largely caused by the host response and the primary risk factor is host susceptibility (as reviewed by Wade (32)). However, certain species of bacteria favour inflamed sites including *Porphyromonas gingivalis*, which can subvert the host response leading to a "dysbiotic" microbiota, which further exacerbates lesions (33). Whilst the role of the host response in periodontitis is well established, the roles of host response and microbiome for gingivitis merits further research. Additionally, some reports suggest that oral bacteria can translocate from the mouth into the systemic circulation and whilst causality has not been confirmed, periodontitis for example, has been associated with other conditions such as coronary artery disease (34), rheumatoid arthritis (35) and respiratory disease (36) (37).

Targeting specific microbes with personal care products

As well as investigating the role of the microorganisms present in health and disease, microbiome research is increasingly being applied to investigate the fundamental biology of various skin conditions (38), oral hygiene (39), dandruff (40), dental caries (41), acne (42) and periodontitis (28, 29) (Table 1). Recent advances in this field include improved knowledge of the bacterial and fungal composition of the scalp in individuals with and without dandruff (43), and the identification of bacteria involved in axillary (44) and oral (45) malodour. In addition, the importance of bacterial strain variability in acne is also now appreciated; although the overall relative abundance of *C. acnes* is comparable between acne and healthy individuals, significant differences at the strain level have been observed (42). Manipulation of the compositional structure or function of skin and oral microbiomes can potentially counteract certain undesirable health conditions where use of probiotics, prebiotics and targeted antimicrobials may provide opportunities to restore the healthy microbial composition of the skin (46) and oral cavity (47) (48). Manipulating innate immunity of the skin and oral cavity is a another route through which this could be achieved (39) (49)

Aims and Objectives

Whilst differentiating between association and causality remains a key issue in microbiome research, the fact that in some cases interactions between the microbiome and the host play a role in health and disease has been established (as previously reviewed (50)). It is therefore important that the effect of personal care regimes on the microbiome receives adequate consideration. Understanding of the factors that cause fluctuations in the microbiome is likely to contribute to the development of novel approaches to understand potential links to undesirable health conditions, and to the identification of microbiome-based biomarkers. It is in this context that the U.S. National Academy of Sciences have discussed the need to incorporate interactions between the microbiome and chemicals in assessing human health risks associated with environmental chemical exposure (51). As understanding of the functional significance of the human microbiome progresses, and the exploration of host-microbial interactions advances, understanding the effects of intentional manipulation of the human microbiome in the context of human safety should be addressed.

In October 2016, a workshop was organised at Colworth Science Park in the UK including 31 specialists in the areas of microbial risk assessment, skin and the oral microbiome, microbial ecology, bioinformatics, bacterial modelling and immunology. This manuscript emerged from exploration of the areas discussed during the workshop. It considers factors that the panel agreed require consideration when evaluating the safety of personal care products that aim to benefit the consumer by affecting the composition or activities of the skin and oral microbiomes.

PROTECTION OF THE ORAL AND SKIN MICROBIOME FUNCTIONS TO PROMOTE HEALTH

The human microbiome in health and wellbeing

Microbiotas associated with the oral mucosa and the skin help programme the human immune system to recognise pathogens (52, 53), reduce the risk of invasion by undesired organisms (54), produce vitamins and other metabolites such as short-chain fatty acids (55). In skin, Phenol Soluble

Modulins (PSMs) and bacteriocins (56) contribute to the ecological and structural maintenance of the niche (54). Commensal skin organisms such as *S. epidermidis* and *C. acnes* use distinct mechanisms to inhibit pathogens and maintain a healthy skin barrier. *S. epidermidis* produces antimicrobial peptides which can reportedly control the growth of *S. aureus* (16) as well as serine proteases to inhibit biofilm formation (16), fermentation products such as succinic acid that may inhibit the overgrowth of the opportunistic pathogen *C. acnes* (46), and a unique form of lipoteichoic acid that can inhibit skin inflammation during skin injury (57). *C. acnes* has also a protective role as a commensal by converting sebum to free fatty acids, which in consequence inhibit colonisation of opportunistic pathogens and contribute to the maintenance of an acidic skin pH (46).

In the oral cavity, some streptococci generate hydrogen peroxide that can inhibit the caries-associated bacterium *S. mutans* (58). The oral microbiome also has non-antimicrobial functions of importance to health and disease where nitrate-reducing oral bacteria can convert dietary nitrate into nitrites, which can influence cardiovascular health and blood pressure (59). Nutritional functions of the oral microbiota are delivered by complex communities via cross feeding and syntrophy. For example, streptococci have both glycosidic and endopeptidase activity, whilst species of *Prevotella* and *Porphyromonas* species have endopeptidase activity and *Fusobacterium* and *Peptostreptococcus* have aminopeptidase activity (60). Bearing in mind the roles of the skin and oral microbiome that are currently understood and the fact that other activities remain unknown, the maintenance and protection of the healthy functionality of the microbiome is an important consideration when assessing the effect of personal care products.

Microbiome Composition *versus* function

Initiatives such as the Human Microbiome Project (HMP) (13) (53) (55) and other studies (14, 52, 61) have enhanced our understanding of baseline skin and oral microbial composition but the search for attributes that define a healthy human microbiome continues. As part of the HMP, where 200

healthy individuals were examined, the “core” microbiome of different body sites, including saliva, plaque, tongue and other oral tissues, ranged from zero to eight operational taxonomic units (OTUs) when analysed for percentage prevalence of 100% compared to a higher range of 19-75 OTUs when the percentage was lowered to 50% (62). Interpretation of the core microbiome to measure the similarity of samples depends on the taxonomic resolution employed since samples may decrease in apparent similarity when analysed to genus or OTUs compared to phylum level (61) (63) (64). Whilst a specific group of microorganisms may be shared between individuals, inter-individual variation may still be considerable at the species-level, and for the presence of rare microorganisms (8, 14, 61). Care is therefore required when classifying microbiome composition as healthy or otherwise, especially in the absence of species-level classification. This is of particular importance in the oral cavity where different species within the same genera can have contrasting associations between health and disease.

The functions provided by compositionally different microbiomes can be relatively similar between individuals (55). Exploring which of these general functions are associated with health represents an alternative to the concept of “healthy composition” (65). A proposed functionality-based definition of a “healthy microbiome” involves three functions: those associated with health-related housekeeping functions, human functions, and specialised functions (53). Housekeeping functions involve energy production and the generation of metabolites and other requirements to maintain the microbial community itself; human-associated functions comprise interactions with the host such as developing and influencing the activity of the immune system and specialised functions include regulation of the pH in a specific body site. A functional core has been described for metabolic pathways detected in more than 75% of individuals (55). Pathway cores were identified for either multiple or single body sites, reflecting the fact that some core functions are broadly distributed and general to the human host whilst others are an adaptation to a specific body site. It should be noted that core functions are not necessarily beneficial to the host. Among site-enriched pathways, nitrate

reduction has been identified as important in the oral cavity (55). These core pathways are generally associated with microbial consortia. Such functional observations may provide further insights when studied across populations and during longer temporal studies with a controlled microbial change. If functional characterisation of the human microbiome can be achieved, measuring or predicting the loss of a beneficial function or the introduction of an undesired function could be used as a functional index during consumer safety assurance.

FACTORS THAT CONTRIBUTE TO PERTURBATION OF THE SKIN AND ORAL MICROBIOME

Microbiome stability as an indicator of health

The stability of the microbiome over time in healthy individuals has been assessed (66). Temporal stability has been explained as a state of equilibrium for a community regardless of the fact that some microbes may at the same time be changing as response to disturbances (67). The ability of the microbiome to remain balanced when exposed to a perturbation and to recover to a healthy functional profile afterwards has also been proposed as a key feature of a healthy microbiome (53). Despite their importance for understanding microbial community dynamics and responses to perturbations, long-term longitudinal studies are still rare. However, based on the available evidence, the composition of the human microbiome is relatively stable over time, with the main variation within an individual being between body sites (13) and considerable temporal stability has also been reported for the microbiome in healthy skin. Oh and colleagues (68) generated metagenomic sequence data from longitudinal samples collected over 2 years and reported that bacterial, fungal, and viral communities were largely stable over that time despite exposure to the external environment. This stability was observed to be site-specific, with body sites harbouring high microbiome diversity being more variable than low diversity (sebaceous) body sites. Observations of temporal stability in the skin microbiome have been interpreted as evidence for colonization resistance and used as the basis for clinical studies exploring skin microbiome in

disease states, where compositional changes in the microbiome have been reported. Costello *et al.* (10) assessed the resilience of the skin microbiota by disinfecting plots on the forehead and left volar *(i.e.* underside of) forearms of volunteers and then inoculating them with "foreign" microbiotas *(i.e.* taken from the tongue and skin of other individuals). The microbiotas of forearm plots (n= 16) that had been inoculated with tongue scrapings were more similar to tongue communities than to those normally associated with the forearm in relative abundance between 2 and 8 h after inoculation. However, communities more similar to those normally associated with the forehead, developed on forehead plots that had been similarly inoculated with tongue material. It can be inferred therefore, that for some reason (potentially the presence of sebaceous lipids), the forehead environment exerted a stronger selection pressure than the forearm. Furthermore, following interpersonal and inter-gender reciprocal swaps of forehead and forearm microbiotas, developing communities resembled the recipient rather than the donor, demonstrating the importance of the environment and possibly, the action of endogenous mechanisms for individualisation and microbiota perpetuation. The authors hypothesised that the stronger selection at forehead sites was due to sebaceous secretions which, in contrast to dry sites like the volar forearm, may have i) been more strongly selective and/or ii) could have supported the more rapid recolonisation from appendageal structures, which is in agreement with the hypothesis outlined above.

The oral microbiota may also remain stable over time in healthy individuals (6) although it is also sufficiently malleable to be beneficially manipulated through hygienic intervention (39). It is however important to consider what stability means when referring to a host-associated microbiota. Belstrøm and colleagues collected saliva from five volunteers without oral disease every 4 h for 24 h, repeated this seven days later (69) and profiled the salivary microbiome. Whilst caution is necessary given the small sample size, the author's tentative conclusion was that "little or no variation" within salivary microbiomes was observed over time. The oral cavity is a complex

environment with various distinct areas, and saliva, often purported to contain microorganisms originating from multiple sites on the mouth may vary less in terms of microbiome composition than for example, a tooth surface where in individuals following the recommended oral health regime of twice daily brushing microbial abundance will be very low immediately after cleaning, but can exceed 10^7 bacteria per cm^2 following regrowth.

Maintaining microbiome stability in healthy individuals will ensure that the beneficial microbial functions are maintained (70) so the measurement of microbiome stability and its recovery following disturbance are important in understanding potential risks. Whilst the human microbiome is relatively stable, its composition can be altered both by pathologies such as gingivitis and dandruff, or by treatment.

Consumer products can alter microbiome composition or function

The hypothesis that the skin microbiota, once established, is perpetuated by continuous endogenous inoculation is supported by an investigation by Grice *et al.* (12) in which skin microbiota was sampled using swabs and biopsies and profiled by high-throughput sequencing. An attractive explanation is that secretions from sweat glands and the outward migration of differentiating skin cells could transport bacteria cells from within appendageal structures continuously onto the skin surface (as proposed by Kong et al. (71)). Daily hygiene regimens may however affect the microbiome and some routines tooth brushing and hand washing do this intentionally to respectively control reduce the risk of oral disease and to reduce the transmission of pathogens, (54, 72). Exposure to antimicrobials through the use of household and personal care products has shown minimal long-term effects on the microbiome. In this respect, two human studies monitored how the use of toothpaste, liquid and bar soap, and dishwashing liquid, with and without triclosan perturbed the microbiome. The first study; a crossover control study involving healthy individuals, showed no significant impact on human oral or gut microbiome composition during 4 months exposure to the antibacterial compound triclosan (73). A longitudinal survey of the gut microbiota in infants and

mothers during the first year following birth also did not show major compositional changes or loss of microbial diversity (74). It is highly likely that environmental modulation of the skin microbiota has been occurring since the ancient origins of the microbiome for the skin through UV irradiation, friction and washing, and for the oral cavity through diet, friction and cleaning. In personal care, antiperspirants are used by approximately 50% of the global population and have been shown to reduce bacterial load in the axilla. Individuals that do not use antiperspirants have been observed to harbour greater axillary microbiome diversity than individuals that use antiperspirants do (75). For antiperspirant and deodorant users who ceased use of product, an increase in Staphylococcaceae was observed, in comparison to *Corynebacterium* species dominating in non-users. Perhaps surprisingly, microbiome diversity was reported to be greater in antiperspirant users compared to deodorant or non-users. In a separate study of nine cohorts, axillary diversity was similarly found to be greater in antiperspirant (and deodorant) users compared to non-users (76). A recent study on effect of cosmetic products on the microbiome of facial skin of high and low hydration groups indicated that baseline bacterial diversity was greater in the low than that of high hydration group, and that the use of cosmetic products decreased the differences between the two groups (38).

Microbiome individualisation

Evidence suggests that both environment and host genetics play important roles in determining the composition of individual microbiomes. Salivary microbiome studies in twins indicate that overall microbial abundance and some aspects of the microbial population structure are influenced by heritability (77). With respect to the skin microbiome, Blekhman and colleagues (78) analysed shotgun metagenomic data from the HMP, collecting data on host genetic variation for 93 individuals. They reported significant associations between host genetics and microbiome composition for ten of the fifteen sites they assessed, including the oral cavity and the skin. Thus, as well as extrinsic environmental factors, host genetics appears to play a role in the composition of the oral and skin microbiotas, probably through immunological and other mechanisms. These

examples partly explain the variability between individuals observed in microbiome research (8) and highlight the need to separate a significant change from individual variation when assessing specific perturbations.

Extrinsic factors also influence the stability of the microbiome since activities such as smoking tobacco have been shown to influence the composition of oral biofilms (79), suggesting that smoking promotes the acquisition and colonisation of pathogenic bacteria. The development of gingivitis and its progression from gingivitis to periodontitis and the promotion of dental plaque biofilm colonisation partly depends on the host immune response (80). Gomez and colleagues (81) illustrated the impact of host genetics through a human volunteer study involving a large cohort of monozygotic and dizygotic twin children with and without active caries, with the aim of elucidating the contributions of host genotype and shared environment on the oral microbiomes (supragingival plaque) of children. They observed that similarity in oral microbiomes was higher between monozygotic twins regardless of caries state, with certain taxa being identified as highly heritable but that most of the variation was determined by the specific growth microenvironment. The caries state however, was not associated with the more highly heritable bacteria suggesting that lifestyle, diet and oral hygiene practices might outweigh parental heritability in establishment of a caries associated microbiome. The more heritable species were detected at lower abundance with increasing age and sugar consumption.

MEASURING CHANGES IN MICROBIOME COMPOSITION AND ACTIVITIES

Risks of pathogen colonisation

One of the beneficial activities of the microbiotas of the skin and oral cavity is the protection of the host tissue from pathogens (as summarised in Figure 1). Perturbation of commensal communities may be therefore a contributing factor to the pathogenesis of certain inflammatory conditions. In some circumstances, overgrowth of commensal microorganisms with pathogenic potential (pathobionts) or colonisation by external pathogenic organisms (transients) can cause disease. The

ability of transient organisms to colonise is likely to depend on the interactions with the commensals residing at each specific body site. In this respect, microbial communities with more competitive interactions than cooperative interactions are assumed to be more resilient in the sense that cooperation causes coupling between species involving several species to change at the same time and destabilise the system (82). In the mouth, loss of colonisation resistance through antibiotic use can lead to infections by opportunistic pathogens such as *Candida* species and *S. aureus* (as reviewed (83) (60)). In this regard, microbial changes that do not increase the opportunity for pathogens to colonise are unlikely to adversely affect the wellbeing of the host.

The human body as a microbial niche

The skin and oral cavity present distinct environments, and ecological conditions *in situ*, have a large influence on the compositional differences in microbiota between body sites. Oily, moist and dry skin sites regulate nutrients and harbour specific microbial taxa (46, 52, 84). The mouth can be broadly divided into different habitats: the gingiva and hard palate; the tongue and throat; and dental plaque; each one colonised by a microbiome characteristic of the specific site (60). The microbiota present in the oral cavity form biofilms by attaching to the different surfaces, which confer spatial structure and provide the conditions required for different organisms to survive within the community (85). The availability of oxygen is one of the drivers of microbiota composition and in this context, a succession during the formation of dental plaque has been proposed whereby teeth are initially colonised by facultative genera such as *Streptococcus*, with a shift to a microbial community better adapted to anaerobic conditions, as the biofilm matures. Bacterial succession on the tooth surface can also be strongly influenced by nutrient availability, mechanical stress and saliva flow (6, 61, 86) and by binding of bacteria to proteins in the salivary pellicle coating the tooth surface (87).

Interactions with the external environment can also drive selection. For example, an increase in sugar intake or a reduction in saliva flow may induce a reduction in pH that allows the expansion of

aciduric organisms (86). Loss of moisture, changes in temperature and exposure to ultraviolet radiation can also result in microbiota alteration in the skin (88). Similarly, changes in the spatial structure may also influence the microbial community within a given body site (9, 88).

Microbial diversity in health

Several indices have been employed to differentiate microbiomes associated with health and disease. Among these, microbial (ecological) diversity is frequently measured. Ecological diversity can be measured as richness (the number of taxa present) and evenness (the abundance of microbial constituents). Although not universally applicable, higher diversity has been associated with health in specific contexts when considering that more diverse microbes may supply the host with increased functional traits. However, microbial diversity on its own is not an accurate measure for determining disease aetiology or health. Whilst reduced microbial diversity has frequently been observed in conditions such as atopic dermatitis and psoriasis (89)-(90) this is not always the case, for example, in both psoriatic and unaffected elbows (81) richness has been reported to be the same whilst, an increase in bacterial diversity due to the rise of species of minor abundance has been observed in gingivitis and periodontitis (64, 91). The measurement of diversity also does not account for interactions among species and two microbiomes with the same level of diversity may be different. It may therefore be more pertinent to observe the entire community of microbes present and by extension how they are functioning, rather than relying on richness alone as a predictor of disease (92).

The importance of bacterial abundance

Compositional studies of the skin and oral microbiomes have suggested that the load or abundance of organisms can be more significant than their presence in the progression of disease. A 65% increase in the proportion of *S. aureus* in atopic dermatitis sufferers at flare sites and partial correlation between *S. aureus* abundance and disease severity have been reported (99). Similarly, *S.*

epidermidis was significantly more abundant during flares than post flares and in controls, although the underlying reasoning for the increase in *S. epidermidis* was not determined (99). Several studies have reported increased *C. acnes* abundance in acne compared to unaffected volunteers (93). Whilst differences between the absolute numbers of bacteria between inflammatory acne, papules and pustules have been reported there appears to be progressively higher bacterial loads vis-à-vis severity of the disease (94). The use of quantification methods such as quantitative PCR has revealed higher levels of *S. mutans* and *S. sobrinus* in children with caries compared to caries-free children (95). In other oral diseases such as gingivitis, severity is better correlated with the plaque load and maturity than with some specific bacteria (60). It should however be born in mind that NGS is not well-suited to determining differences in bacterial absolute abundance (quantified genetic or microbial load within a sample) such that two samples with identical relative abundance (genetic representation of microbes within a sample ranked against all taxa in the sample) could differ markedly in absolute abundance (96).

Host-microbiota interactions

Skin functions as a two-way barrier, which helps to preserve hydration levels and prevent entry of noxious substances into the body. Skin function may be shaped by the commensal organisms and in this respect, Naik *et al.* (97) demonstrated that germ-free mice had a weakened immune response to the parasite *Leishmania major* compared to mice raised under specific pathogen-free conditions. The impaired response in the germ-free mice could be rescued by colonisation with *S. epidermidis* (97) implying a role for the microbiota in promoting host immunity. More recent evidence suggests that the microbiota is fundamental to skin structure. Conventionally reared mice showed altered gene expression compared with germ-free mice. Meisel et al. (98) reported that 2820 genes were differentially regulated by microbial colonisation, which included genes associated not only with the host immune response but also epidermal differentiation. Crucially, the expression of 9 genes involved in the epidermal differentiation complex (EDC), a collection of genes involved in terminal

differentiation of keratinocytes (reviewed in (99)), was regulated by the microbiota. When the skin of conventionally raised mice was compared to germ-free mice, differences in the balance of proliferation and differentiation were observed. These data support the view that the microbiome may be associated with the development of the skin architecture since the EDC has been implicated in dermatological diseases such as psoriasis (reviewed in (100)). Various studies have shown that the microbiota is associated with the outcome of the healing response when wounding breaches the skin barrier. In broken skin the commensal microorganisms can behave as pathogens and colonisation of wound sites can result in release of microbial metabolites that can further damage host tissues (reviewed in (101)). It is therefore unsurprising that accelerated wound healing has been observed in the absence of microbiota (102, 103) but it is also the case that the commensal microbiota can produce antimicrobial peptides (AMPs) that can inhibit the invasion of wound sites by pathogens (104). There is also evidence that *S. epidermidis* can inhibit the uncontrolled inflammation sometimes associated with wounding. Part of the mechanism for this may involve the inhibition of cytokine release by keratinocytes (57).

With respect to beneficial effects, *S. epidermidis* has been reported to augment tight junction function in keratinocytes (105) where the interaction of keratinocyte monolayers with *S. epidermidis* increased the trans-epithelial electrical resistance (a measure of tight junction function) within a short time of exposure to this bacterium. Furthermore, toll-like receptor (TLR) ligands such as lipoteichoic acid or peptidoglycan may augment tight junction function in keratinocyte monolayers (106). These data suggest that skin commensals, like those of the gut, are probably involved in many aspects of epithelial barrier homeostasis.

MEASURING CHANGES IN MICROBIOME COMPOSITION

Various data analysis methods are used in microbiome research that can objectively assess microbial changes. This section describes the information that each technique provides and how it is applied to characterise health and disease.

Metagenomic profiling

Studies employing both ribosomal profiling and metagenomics have sought to identify microbes linked to either oral or cutaneous disease, whether at the community level or that of individual taxa. Several studies have reported changes in the proportion of bacteria on the skin in psoriasis (25, 26, 89). Gao et al (25, 26) for example reported that Firmicutes were significantly overrepresented in psoriasis lesions compared to uninvolved skin, whilst the *Actinobacteria* and *Propionibacterium* species were reportedly present at significantly lower relative abundance in psoriatic lesions. Apart from bacteria, the fungal genus *Malassezia* has also been associated with psoriasis (89, 107-110). Altered microbial community profiles have also been reported in atopic dermatitis, where an increased proportion of *Staphylococcus*, particularly *S. aureus* and *S. epidermidis*, were observed during disease flares in comparison to baseline or post-treatment, and correlated with increased disease severity (111-113).

In terms of the oral microbiota, changes in microbial composition have long been associated with dental caries and periodontitis. For caries, sequence analysis has confirmed that bacteria other than *S. mutans* are correlated with active caries *(Lactobacillus* and *Bifidobacterium)* and likewise several taxonomic groups of bacteria are associated with periodontitis (28, 114-117). It is also clear that the aetiology of disease also involves a complex interplay between the host and the resident microbial communities that is yet to be fully explored. Applied to the study of psoriasis, such approaches indicate that strain level features and associated functional variation may be pertinent to disease (118).

This exploration of host-microbe interactions have been hindered by the fact that virulence and pathogenic determinants could be partitioned at the sub-species or strain level. It is well established that intra-species genomic features lead to phenotypic variability (113, 119-121). Ribosomal genera-based profiling approaches lack strain level resolution. Several recent computational tools to

taxonomically (122-124) and functionally (125, 126) characterise individual members of the microbiome at strain level resolution in metagenomic datasets have become available.

Profiling of functional potential

Whilst understanding the community structure of a microbiome and the relationship between specific taxa and health or disease can be informative, knowledge of community function will probably be most useful in understanding the effect of perturbing the microbiome. Shotgun metagenomics provides the potential to access strain level taxonomic features and the potential functional characteristics of the community which has until recently been computationally challenging. This approach can be used for the investigation of functional traits, although it can only reveal the functional potential of communities. It can also be used to profile viruses, which are not amenable to ribosomal-based profiling. The oral microbiome have assessed disease states such as caries or periodontal disease compared to healthy controls. Shi *et al.* (127) and Wang *et al.* (128) reported that community function around bacterial chemotaxis and cell motility are increased in disease compared to periodontal health. It has also been shown that in periodontal disease there is an increase in metabolic pathway genes associated with fatty acid metabolism (129), as well as an increase in genes associated with the metabolic degradation of nutrients (127) and those required for growth in anaerobic conditions (129). Healthy communities have been shown to exhibit increased functions in the areas of fatty acid biosynthesis, aspartate and homoserine metabolism, membrane transport and signal transduction. Metagenomic studies of the skin are more difficult due to the low bacterial density and small sample surfaces available (130). Mathieu *et al.* (131) consider the skin microbiota as a complete organism, reporting a predominance of catabolic genes and the ability of the skin bacteria to use the sugars, lipids and iron that are found on human skin. They also found genes related to antibiotic resistance, as well as some linked to acid resistance, clearly a mechanism for tolerance of the natural acidity of the skin. Oh *et al.* (17) have described a “functional core” of around 30% of the community that can vary depending on the diversity and biogeography of the

differing skin microenvironments, which drives the functional capacity that is required by that community. For example, dry sites were found to favour functional traits surrounding the citrate cycle, and sebaceous sites showed increased function around glycolysis and ATP/GTP/NADH dehydrogenase I. Whilst these metagenomic approaches exceed a simple inventory of taxa and provide information on function and health/disease interrelationships, making judgements of community functional traits by reference genome comparison should be undertaken with care. There is a large genomic diversity that is just starting to be understood, for example the association of only some *C. acnes* strains with acne vulgaris (123) (130). Further complicating the search for a functional understanding of the microbiome is the identification of new genes from metagenomic analysis approaches that are associated with health or disease, but which cannot be assigned to any functional pathway.

Metatranscriptomic analyses

Shotgun transcriptomics can be used to determine the active functions of a microbiome (132), especially as the community composition of a microbiome alone is not necessarily reflective of its active community members (133). This is an emerging research area with less data available, and challenges remain, for example in sampling sufficient mRNA material to enable analysis. However, the transcriptomic profile of a community is dynamic and can easily change in the same biological sample at different times as the microbiome responds continually to changing environmental and host conditions. Metatranscriptomic studies applied to human microbiome are more limited in comparison to metataxonomic/metagenomics surveys.

In comparison to the oral microbiome, metatranscriptomics of the skin is more challenging due to the limitations of microbial biomass in the sample material. Kang and colleagues (132) analysed the metatranscriptomics of patients with acne vulgaris versus healthy controls. *C. acnes* was reportedly the most transcriptionally active organism and was predominant in both the healthy and diseased samples. Further analysis of the gene expression profile of *C. acnes* in the samples identified that

the organism's activity on acne-affected skin was distinct from its activity on healthy skin. Specifically, vitamin B12 biosynthesis pathway was observed to be significantly downregulated in acne. Additionally, a model of how vitamin B12 modulates the transcriptional and metabolic activities of *C. acnes* in acne pathogenesis was suggested. The model underlined how shotgun metatranscriptomic approaches can enhance the understanding of disease pathogenesis. One of the limitations of meta-transcriptome data is the final metabolic products generated by a microbial community are not captured (133). In this respect, techniques such as proteomics, metabolomics, and lipidomics can help to have a deeper functional characterisation of the microbiome.

Metatranscriptomics has been used in conjunction with metagenomics to investigate saliva from individuals with caries and periodontitis to compare with saliva from orally disease-free individuals. Belstrom *et al.* (69) identified 15 differentially expressed KEGG Orthologs (KOs) between periodontitis or caries samples when compared with orally healthy controls. These included eight carbohydrate metabolism-associated KOs that were downregulated in periodontal disease and two KOs that were upregulated in caries associated with glycan biosynthesis and carbohydrate metabolism. In addition, the same study observed that lipid metabolism was increased in healthy samples when compared with dental caries and concluded that longitudinal studies may reveal that screening salivary metabolic gene expression can identify oral diseases preclinically. However, it is also clear that development of such diagnostics is at a very early stage and that overcoming the very significant differences in complexity between the salivary and plaque microbiomes would be a substantial technical and clinical challenge.

Metabolomic analyses

Microbial metabolites can have a direct impact on oral or skin health (*e.g.* short chain fatty acids and sulphides in periodontal diseases, organic acids in dental caries) or they can enter and modulate host metabolic processes. As such, metabolite exchange between the microbiome and host

559 represents one mechanism through which these systems communicate. Variation in the bacterial
560 species present can modulate the genetic library of the microbiome, changing its overall functional
561 capacity, its metabolite production, and the downstream impact on host health. However, different
562 species are known to possess similar or even the same metabolic traits. This functional redundancy
563 means that studying composition alone may be insufficient to accurately determine the overall
564 biotransformation capabilities of the microbiome and therefore its potential to modify host health.
565 Metabolic profiling (metabolomics/metabonomics) has emerged as a powerful tool for studying the
566 microbiota because it can ascertain the metabolic profile via low molecular weight compounds in a
567 sample. These metabolic signatures contain thousands of molecular small molecular weight
568 compounds reflecting biochemical events. This includes host metabolic processes but also those
569 performed by the resident microbes and products arising from interactions between the two. Studies
570 using metabolomics to directly assess the functional status of the skin microbiota are limited.
571 However, several studies have characterised the skin metabolome in a wider context. These have
572 used a variety of sample types including skin swabs, hydrogel micropatches (134), punch biopsies
573 and sweat. In one study analysing epidermal skin tissue, several bacterial-derived metabolites (135)
574 and bacterial substrates were observed, including *p*-cresol, a bacterial metabolite of tyrosine. This
575 demonstrates that these tissue samples can be informative for studying the skin microbiome. Skin
576 surface liquid extracts (sweat) represent another sample type of potential utility. These are complex
577 mixtures of secretions derived from eccrine, apocrine and/or sebaceous glands (depending of body
578 location) as well as from the microbiota inhabiting the skin (136). Attempts are being made to
579 optimise and standardise the collection and analysis of sweat and this may prove to be a useful
580 resource for studying the skin microbiota.

581 Metabolic profiling of gingival crevicular fluid (GCF) has been used to study the importance of
582 host-bacterial interactions in periodontal disease. Here, the depletion of anti-oxidants, degradation
583 of host cellular components and accumulation of bacterial products were seen in the disease state

584 (137) (138). Attempts have been made to integrate salivary bacterial and metabolic datasets to
585 identify metabolic products related to specific bacterial groups (139). Oral biofilms have also been
586 studied by capillary electrophoresis-mass spectrometry (CE-MS)-based metabolomics. This has
587 enabled the central carbon metabolic pathways to be investigated in the oral biofilm. One approach
588 is to measure these pathways in supragingival plaque before and after a glucose rinse. Glucose can
589 be degraded by bacteria to several metabolic products, including acetate, formate, lactate, and
590 succinate. Assessing the metabolic content of this plaque after the rinse provides information on the
591 functional capacity of the biofilm.

592 **Mathematical modelling**

593 Oral and skin microbial community dynamics are shaped by three broad factors: the host, the
594 environment and the community. The human host provides the microenvironment for the
595 community and may alter this environment through hygiene and other behaviours. The genetic
596 makeup of the host also influences the community's microenvironment. The surrounding
597 environment offers a large species pool from which immigration into the local community may take
598 place. Finally, community composition (richness, evenness and interactions) as well as history (e.g.
599 previous exposure to perturbations) may impact its dynamics.

600 A community model expresses in mathematical terms how selected factors influence community
601 dynamics. Community models thus allow prediction of the response of the community to short-term
602 (pulse) perturbations and altered conditions (press perturbations). Models can be coarse-grained or
603 detailed, describing populations or individuals. A general distinction can be made between
604 phenomenological models that predict community behaviour on the basis of immigration and
605 mortality rates, interaction strengths, growth rates and other parameters, and metabolic models that
606 take underlying molecular mechanisms of interactions into account. The generalized Lotka-Volterra

607 equation and its variants (140-142), but also individual-based models such as the neutral model
608 (143) and its extensions are examples of the former.

609 In the oral cavity, these models have to deal with the complication that most community members
610 can exist in both a free-floating planktonic state, as well as part of a biofilm, which may have
611 different growth rates, different access to nutrients and engage in different interactions. Previously,
612 Schroeder and colleagues (144) proposed a discrete and continuous version of a model that
613 describes the dynamics of both planktonic and sessile communities in drinking water pipes and
614 which may be adapted to model community dynamics in the oral cavity. The programming
615 language “gro”, which was designed for individual-based modelling of spatially structured
616 microbial communities, may also be of interest in this respect (145). This facilitates the modelling
617 of cell behaviours planktonically or in microcolonies or biofilms. A range of factors including
618 growth rates, cell-signalling, diffusing and chemotaxis can be factored in.

619 Metabolic models require the accurate reconstruction of each community member's metabolism
620 (146), which is a major hurdle because of lack of reliable and complete genome annotations and the
621 large percentage of unknown gene functions. Metabolic reconstructions may be quickly generated
622 automatically with tools such as ModelSEED or RAVEN (147) (148). This type of modelling
623 present some disadvantages such as the requirement for a tedious manual curation to ensure an
624 accurate reconstruction (149) and the assumption that community members are in a metabolic
625 steady state. This assumption is relaxed by some dynamic metabolic models which require kinetic
626 parameters such as compound uptake rates (146). The dynamic individual-based metabolic
627 modelling tools COMETS (150) and BacArena (151) additionally take spatial structure into
628 account, which is important to model biofilms. Metabolic models can also integrate meta-omics
629 data as additional constraints on metabolic fluxes (152). For example, gene expression data has
630 been used to validate metabolic models (153). Despite their promise, to the best of our knowledge,
631 metabolic models have only been applied to communities consisting of a small number of species.

Metabolic models of species grown alone and in pairs can be exploited to predict ecological interactions (154). For instance, gut microbial interactions were predicted based on the semi-curated reconstruction of 773 gut species (155). The extension of dynamic and spatial metabolic models to more complex microbial communities is a promising field for future research.

Community-level metabolic networks are a simpler form of metabolic models, where metabolites and reactions are represented as nodes and edges, respectively, but where stoichiometric coefficients are not taken into account (156). They offer a framework for the straightforward integration of meta-omics data as node or edge weights (157). While metabolic networks can handle larger communities, they do not allow quantitative modelling (158).

Quantitative community models have parameters, which need to be determined through measurements in well-controlled conditions. For instance, growth assays in mono- and co-culture can provide growth rates and interaction strengths. Once a model is parameterized, it needs to be validated experimentally. Such a validation consists of comparing the outcomes of experimental perturbations with the outcomes predicted by the model. The model may undergo several rounds of adjustment and validation until it reaches sufficient accuracy, or it may fail to be predictive because important but unknown factors are not taken into account or the community dynamics are chaotic or predominantly stochastic. A model that predicts community dynamics to an acceptable level of accuracy can be applied to simulate the effects of yet untested perturbations on the community.

CONCLUSIONS

Perturbations of the microbiome can have positive and negative consequences for human health. However, more knowledge is required to understand the extent of change that corresponds to the maintenance of health and the establishment of disease states. Microbiome research is still in its early stages and further studies to elucidate the nature of the functional and structural interactions

among microorganisms and with the host are required. Analysis of the gut microbiome is advancing faster than that of the skin and oral microbiomes, where increasing research investment would help to understand better the dynamics of those two specific body niches. Although mankind has been manipulating its microbiome, often beneficially, through diet, hand washing and oral hygiene practices both modern and historic, for hundreds if not thousands of years, the risks of manipulating the microbiome through new technology innovation should be properly assessed and the development of appropriate methods is required. Numerous factors should be considered when assessing the safety of novel approaches to microbiome perturbation, and approaches need to be developed to ensure that a compositional change delivers benefits whilst not compromising the stability, diversity and immunological state required for healthy functionality of the microbiome. These are summarised in Table 1 and Figure 1. To increase our understanding of the safety of microbiome changes, multi-disciplinary research needs to move to a mechanistic understanding to allow measurable elements specific to the oral and skin microbiome to be identified.

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REFERENCES

1. Lederberg J, McCray AT. 2001. 'Ome sweet 'omics - A genealogical treasury of words. Scientist 15:8-8.

2. Tlaskalová-Hogenová H, Stepánková R, Hudcovic T, Tucková L, Cukrowska B, Lodinová-Zádníková R, Kozáková H, Rossmann P, Bártová J, Sokol D, Funda DP, Borovská D, Reháková Z, Sinkora J, Hofman J, Drastich P, Kokesová A. 2004. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. Immunol Lett 93:97-108.

3. Yohe S, Thyagarajan B. 2017. Review of Clinical Next-Generation Sequencing. Arch Pathol Lab Med 141:1544-1557.

4. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581-3.

5. Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J 11:2639-2643.

6. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner ACR, Yu WH, Lakshmanan A, Wade WG. 2010. The human oral microbiome. J Bacteriol 192:5002-5017.

7. Segata N, Kinder Haake S, Mannon P, Lemon KP, Waldron L, Gevers D, Huttenhower C, Izard J. 2012. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome Biol doi:10.1186/gb-2012-13-6-r42.

8. Utter DR, Mark Welch JL, Borisy GG. 2016. Individuality, stability, and variability of the plaque microbiome. Front Microbiol 7.

9. Welch JLM, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. 2016. Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci USA 113:E791-E800.

10. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation in human body habitats across space and time. Science 326:1694-1697.

11. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA. 2009. Topographical and temporal diversity of the human skin microbiome. Science 324:1190-1192.

12. Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA. 2008. A diversity profile of the human skin microbiota. Genome Res 18:1043-1050.

13. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, Fitzgerald MG, Fulton RS, Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu

R, Magrini V, Martin JC, Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, Wollam AM, Worley KC, Wortman JR, Young SK, Zeng Q, Aagaard KM, Abolude OO, Allen-Vercoe E, Alm EJ, Alvarado L, Andersen GL, Anderson S, Appelbaum E, Arachchi HM, Armitage G, Arze CA, Ayvaz T, Baker CC, Begg L, Belachew T, Bhonagiri V, Bihan M, Blaser MJ, Bloom T, Bonazzi V, Paul Brooks J, Buck GA, Buhay CJ, Busam DA, Campbell JL, et al. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486:207-214.

14. Oh J, Byrd AL, Park M, Kong HH, Segre JA. 2016. Temporal Stability of the Human Skin Microbiome. Cell 165:854-866.

15. Prescott SL, Larcombe DL, Logan AC, West C, Burks W, Caraballo L, Levin M, Etten EV, Horwitz P, Kozyrskyj A, Campbell DE. 2017. The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. World Allergy Organ J 10:29.

16. Gallo RL. 2015. S. epidermidis influence on host immunity: more than skin deep. Cell Host Microbe 17:143-4.

17. Oh J, Byrd AL, Deming C, Conlan S, Program NCS, Kong HH, Segre JA. 2014. Biogeography and individuality shape function in the human skin metagenome. Nature 514:59-64.

18. Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, Shafiq F, Kotol PF, Bouslimani A, Melnik AV, Latif H, Kim JN, Lockhart A, Artis K, David G, Taylor P, Streib J, Dorrestein PC, Grier A, Gill SR, Zengler K, Hata TR, Leung DY, Gallo RL. 2017. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. Sci Transl Med 9.

19. Kennedy EA, Connolly J, Hourihane JO, Fallon PG, McLean WHI, Murray D, Jo JH, Segre JA, Kong HH, Irvine AD. 2017. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. J Allergy Clin Immunol 139:166-172.

20. Morar N, Cookson WO, Harper JI, Moffatt MF. 2007. Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol 127:1667-72.

21. Melnik BC, Zouboulis CC. 2013. Potential role of FoxO1 and mTORC1 in the pathogenesis of Western diet-induced acne. Exp Dermatol 22:311-5.

22. Patra V, Mayer G, Gruber-Wackernagel A, Horn M, Lembo S, Wolf P. 2018. Unique profile of antimicrobial peptide expression in polymorphic light eruption lesions compared to healthy skin, atopic dermatitis, and psoriasis. Photodermatol Photoimmunol Photomed 34:137-144.

23. Palmer RA, Friedmann PS. 2004. Ultraviolet radiation causes less immunosuppression in patients with polymorphic light eruption than in controls. J Invest Dermatol 122:291-4.

24. Janssens AS, Pavel S, Tensen CP, Teunissen MB, Out-Luiting JJ, Willemze R, de Gruijl FR. 2009. Reduced IL-1Ra/IL-1 ratio in ultraviolet B-exposed skin of patients with polymorphic light eruption. Exp Dermatol 18:212-7.

25. Fahlen A, Engstrand L, Baker BS, Powles A, Fry L. 2012. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. Arch Dermatol Res 304:15-22.

26. Gao Z, Tseng CH, Strober BE, Pei Z, Blaser MJ. 2008. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. PLoS One 3:e2719.

27. Griffiths CE, Barker JN. 2007. Pathogenesis and clinical features of psoriasis. Lancet 370:263-71.

28. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, Leys EJ, Paster BJ. 2008. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clinl Microbiol 46:1407-1417.

29. Meuric V, Laine F, Boyer E, Le Gall-David S, Oger E, Bourgeois D, Bouchard P, Bardou-Jacquet E, Turmel V, Bonnaure-Mallet M, Deugnier Y. 2017. Periodontal status and serum biomarker levels in HFE haemochromatosis patients. A case-series study. J Clin Periodontol 44:892-897.

30. Kinane DF, Hajishengallis G. 2009. Polymicrobial infections, biofilms, and beyond. J Clin Periodontol 36:404-5.

31. Kinane DF, Stathopoulou PG, Papapanou PN. 2017. Periodontal diseases. Nat Rev Dis Primers 3:17038.

32. Wade WG. 2011. Has the use of molecular methods for the characterization of the human oral microbiome changed our understanding of the role of bacteria in the pathogenesis of periodontal disease? J Clin Periodontol 38 Suppl 11:7-16.

33. Hajishengallis G, Krauss JL, Liang S, McIntosh ML, Lambris JD. 2012. Pathogenic microbes and community service through manipulation of innate immunity. Adv Exp Med Biol 946:69-85.

34. Ryden L, Buhlin K, Ekstrand E, de Faire U, Gustafsson A, Holmer J, Kjellstrom B, Lindahl B, Norhammar A, Nygren A, Nasman P, Rathnayake N, Svenungsson E, Klinge B. 2016. Periodontitis Increases the Risk of a First Myocardial Infarction: A Report From the PAROKRANK Study. Circulation 133:576-83.

35. Potempa J, Mydel P, Koziel J. 2017. The case for periodontitis in the pathogenesis of rheumatoid arthritis. Nat Rev Rheumatol 13:606-620.

36. Muthu J, Muthanandam S, Mahendra J. 2016. Mouth the mirror of lungs: where does the connection lie? Front Med 10:405-409.

785 37. Heo SM, Haase EM, Lesse AJ, Gill SR, Scannapieco FA. 2008. Genetic relationships
786 between respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid
787 from patients in the intensive care unit undergoing mechanical ventilation. Clin Infect Dis
788 47:1562-70.

789 38. Lee HJ, Jeong SE, Lee S, Kim S, Han H, Jeon CO. 2018. Effects of cosmetics on the skin
790 microbiome of facial cheeks with different hydration levels. Microbiol Open 7:e00557.

791 39. Adams SE, Arnold D, Murphy B, Carroll P, Green AK, Smith AM, Marsh PD, Chen T,
792 Marriott RE, Brading MG. 2017. A randomised clinical study to determine the effect of a
793 toothpaste containing enzymes and proteins on plaque oral microbiome ecology. Sci Rep
794 7:43344.

795 40. Park T, Kim HJ, Myeong NR, Lee HG, Kwack I, Lee J, Kim BJ, Sul WJ, An S. 2017.
796 Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis. Exp
797 Dermatol 26:835-838.

798 41. Rocas IN, Alves FR, Rachid CT, Lima KC, Assuncao IV, Gomes PN, Siqueira JF, Jr. 2016.
799 Microbiome of Deep Dentinal Caries Lesions in Teeth with Symptomatic Irreversible
800 Pulpitis. PLoS One 11:e0154653.

801 42. Fitz-Gibbon S, Tomida S, Chiu BH, Nguyen L, Du C, Liu M, Elashoff D, Erfe MC,
802 Loncaric A, Kim J, Modlin RL, Miller JF, Sodergren E, Craft N, Weinstock GM, Li H.
803 2013. *Propionibacterium acnes* strain populations in the human skin microbiome associated
804 with acne. J Invest Dermatol 133:2152-60.

805 43. Xu Z, Wang Z, Yuan C, Liu X, Yang F, Wang T, Wang J, Manabe K, Qin O, Wang X,
806 Zhang Y, Zhang M. 2016. Dandruff is associated with the conjoined interactions between
807 host and microorganisms. Sci Rep 6:24877.

808 44. James AG, Austin CJ, Cox DS, Taylor D, Calvert R. 2013. Microbiological and biochemical
809 origins of human axillary odour. FEMS Microbiol Ecol 83:527-40.

810 45. Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, Paster BJ. 2003.
811 Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy
812 patients. J Clin Microbiol 41:558-63.

813 46. Grice EA. 2014. The skin microbiome: potential for novel diagnostic and therapeutic
814 approaches to cutaneous disease. Semin Cutan Med Surg 33:98-103.

815 47. Seerangaiyan K, van Winkelhoff AJ, Harmsen HJM, Rossen JWA, Winkel EG. 2017. The
816 tongue microbiome in healthy subjects and patients with intra-oral halitosis. J Breath Res
817 11:036010.

818 48. Frias-Lopez J. 2015. Targeting specific bacteria in the oral microbiome. Trends Microbiol
819 23:527-8.

820 49. Mathapathi MS, Mallemalla P, Vora S, Iyer V, Tiwari JK, Chakrabortty A, Majumdar A.
821 2017. Niacinamide leave-on formulation provides long-lasting protection against bacteria *in*
822 *vivo*. Exp Dermatol 26:827-829.

823 50. Meyle J, Chapple I. 2015. Molecular aspects of the pathogenesis of periodontitis.
824 Periodontol 2000 69:7-17.

825 51. NAS. 2018. National Academies of Sciences, Engineering, and Medicine. Environmental
826 Chemicals, the Human Microbiome, and Health Risk: A Research Strategy.
827 doi:10.17226/24960:1-122, Washington, DC: The National Academies Press.

828 52. Grice EA, Segre JA. 2011. The skin microbiome. Nature Rev Microbiol 9:244-253.

829 53. Lloyd-Price J, Abu-Ali G, Huttenhower C. 2016. The healthy human microbiome. Genome
830 Med 8.

831 54. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS,
832 Wade WG, Zaura E. 2016. The oral microbiome - An update for oral healthcare
833 professionals. Br Dent J 221:657-666.

834 55. Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, Brady A, Creasy
835 HH, McCracken C, Giglio MG, McDonald D, Franzosa EA, Knight R, White O,
836 Huttenhower C. 2017. Strains, functions and dynamics in the expanded Human Microbiome
837 Project. Nature 550:61-66.

838 56. Christensen GJ, Bruggemann H. 2014. Bacterial skin commensals and their role as host
839 guardians. Benef Microbes 5:201-15.

840 57. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, Wu Z-R, Hooper LV,
841 Schmidt RR, von Aulock S, Radek KA, Huang C-M, Ryan AF, Gallo RL. 2009. Commensal
842 bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nature Med
843 15:1377-1382.

844 58. Kreth J, Zhang Y, Herzberg MC. 2008. Streptococcal antagonism in oral biofilms:
845 *Streptococcus sanguinis* and *Streptococcus gordonii* interference with *Streptococcus*
846 *mutans*. J Bacteriol 190:4632-4640.

847 59. Kapil V, Haydar SMA, Pearl V, Lundberg JO, Weitzberg E, Ahluwalia A. 2013.
848 Physiological role for nitrate-reducing oral bacteria in blood pressure control. Free Radical
849 Biol Med55:93-100.

850 60. Wade WG. 2013. The oral microbiome in health and disease. Pharmacol Res 69:137-43.

851 61. Hall MW, Singh N, Ng KF, Lam DK, Goldberg MB, Tenenbaum HC, Neufeld JD, G. Beiko
852 R, Senadheera DB. 2017. Inter-personal diversity and temporal dynamics of dental, tongue,
853 and salivary microbiota in the healthy oral cavity. npj Biofilms Microbiomes 3:2.

854 62. Huse SM, Ye Y, Zhou Y, Fodor AA. 2012. A core human microbiome as viewed through
855 16S rRNA sequence clusters. PLoS One 7:e34242.

856 63. Utter DR, Mark Welch JL, Borisy GG. 2016. Individuality, Stability, and Variability of the
857 Plaque Microbiome. Front Microbiol 7:564.

858 64. Kistler JO, Booth V, Bradshaw DJ, Wade WG. 2013. Bacterial community development in
859 experimental gingivitis. PLoS One 8:e71227.

860 65. Martiny JBH, Jones SE, Lennon JT, Martiny AC. 2015. Microbiomes in light of traits: A
861 phylogenetic perspective. Science 350.

862 66. Flores GE, Caporaso JG, Henley JB, Rideout JR, Domogala D, Chase J, Leff JW, Vazquez-
863 Baeza Y, Gonzalez A, Knight R, Dunn RR, Fierer N. 2014. Temporal variability is a
864 personalized feature of the human microbiome. Genome Biol 15:531.

865 67. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. 2012. Diversity, stability
866 and resilience of the human gut microbiota. Nature 489:220-30.

867 68. Oh J, Byrd AL, Park M, Program NCS, Kong HH, Segre JA. 2016. Temporal Stability of
868 the Human Skin Microbiome. Cell 165:854-66.

869 69. Belstrom D, Constancias F, Liu Y, Yang L, Drautz-Moses DI, Schuster SC, Kohli GS,
870 Jakobsen TH, Holmstrup P, Givskov M. 2017. Metagenomic and metatranscriptomic
871 analysis of saliva reveals disease-associated microbiota in patients with periodontitis and
872 dental caries. NPJ Biofilms Microbiomes 3:23.

873 70. Relman DA. 2012. The human microbiome: ecosystem resilience and health. Nutr Rev 70
874 Suppl 1:S2-9.

875 71. Kong HH. 2011. Skin microbiome: genomics-based insights into the diversity and role of
876 skin microbes. Trends Mol Med 17:320-8.

877 72. Babeluk R, Jutz S, Mertlitz S, Matiasek J, Klaus C. 2014. Hand hygiene - Evaluation of
878 three disinfectant hand sanitizers in a community setting. PLoS ONE 9.

879 73. Poole AC, Pischel L, Ley C, Suh G, Goodrich JK, Haggerty TD, Ley RE, Parsonnet J. 2016.
880 Crossover Control Study of the Effect of Personal Care Products Containing Triclosan on
881 the Microbiome. mSphere 1.

882 74. Ribado JV, Ley C, Haggerty TD, Tkachenko E, Bhatt AS, Parsonnet J. 2017. Household
883 triclosan and triclocarban effects on the infant and maternal microbiome. EMBO Mol Med
884 9:1732-1741.

75. Urban J, Fergus DJ, Savage AM, Ehlers M, Menninger HL, Dunn RR, Horvath JE. 2016. The effect of habitual and experimental antiperspirant and deodorant product use on the armpit microbiome. PeerJ 4:e1605.

76. Callewaert C, Hutapea P, Van de Wiele T, Boon N. 2014. Deodorants and antiperspirants affect the axillary bacterial community. Arch Dermatol Res 306:701-10.

77. Demmitt BA, Corley RP, Huibregtse BM, Keller MC, Hewitt JK, McQueen MB, Knight R, McDermott I, Krauter KS. 2017. Genetic influences on the human oral microbiome. BMC Genomics 18.

78. Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, Spector TD, Keinan A, Ley RE, Gevers D, Clark AG. 2015. Host genetic variation impacts microbiome composition across human body sites. Genome Biol 16:191.

79. Kumar PS, Matthews CR, Joshi V, de Jager M, Aspiras M. 2011. Tobacco smoking affects bacterial acquisition and colonization in oral biofilms. Infect Immun 79:4730-4738.

80. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernández M, Gamonal J. 2015. Host response mechanisms in periodontal diseases. Journal of Applied Oral Science 23:329-355.

81. Gomez A, Espinoza JL, Harkins DM, Leong P, Saffery R, Bockmann M, Torralba M, Kuelbs C, Kodukula R, Inman J, Hughes T, Craig JM, Highlander SK, Jones MB, Dupont CL, Nelson KE. 2017. Host Genetic Control of the Oral Microbiome in Health and Disease. Cell Host Microbe 22:269-278 e3.

82. Coyte KZ, Schluter J, Foster KR. 2015. The ecology of the microbiome: Networks, competition, and stability. Science 350:663-666.

83. Pankhurst CL. 2009. Candidiasis (oropharyngeal). BMJ Clin Evid 2009.

84. Grice EA. 2014. The skin microbiome: Potential for novel diagnostic and therapeutic approaches to cutaneous disease. Semin Cut Med Surg 33:98-103.

85. Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. 2016. Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci U S A 113:E791-800.

86. Marsh PD, Head DA, Devine DA. 2015. Ecological approaches to oral biofilms: Control without killing. Caries Res 49:46-54.

87. Kolenbrander PE, Palmer RJ, Jr., Rickard AH, Jakubovics NS, Chalmers NI, Diaz PI. 2006. Bacterial interactions and successions during plaque development. Periodontol 2000 42:47-79.

88. Rosenthal M, Goldberg D, Aiello A, Larson E, Foxman B. 2011. Skin microbiota: Microbial community structure and its potential association with health and disease. InfectGenet Evol 11:839-848.

89. Alekseyenko AV, Perez-Perez GI, De Souza A, Strober B, Gao Z, Bihan M, Li K, Methé BA, Blaser MJ. 2013. Community differentiation of the cutaneous microbiota in psoriasis. Microbiome 1:31.

90. Williams MR, Gallo RL. 2015. The role of the skin microbiome in atopic dermatitis. Curr Allergy Asthma Rep 15:65.

91. Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, Podar M, Leys EJ. 2012. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J 6:1176-85.

92. Zaneveld JR, McMinds R, Vega Thurber R. 2017. Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat Microbiol 2:17121.

93. Akaza N, Akamatsu H, Numata S, Yamada S, Yagami A, Nakata S, Matsunaga K. 2016. Microorganisms inhabiting follicular contents of facial acne are not only *Propionibacterium* but also *Malassezia* spp. J Dermatol. 43:906-11

94. Leeming JP, Holland KT, Cuncliffe WJ. 1988. The microbial colonization of inflamed acne vulgaris lesions. Br J Dermatol 118:203-8.

95. Choi EJ, Lee SH, Kim YJ. 2009. Quantitative real-time polymerase chain reaction for *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples and its association with early childhood caries. Int J Paediatr Dent 19:141-7.

96. Tkacz A, Hortala M, Poole PS. 2018. Absolute quantitation of microbiota abundance in environmental samples. Microbiome 6:110.

97. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, Deming C, Quinones M, Koo L, Conlan S, Spencer S, Hall JA, Dzutsev A, Kong H, Campbell DJ, Trinchieri G, Segre JA, Belkaid Y. 2012. Compartmentalized control of skin immunity by resident commensals. Science 337:1115-9.

98. Meisel JS, Sfyroera G, Bartow-McKenney C, Gimblet C, Bugayev J, Horwinski J, Kim B, Brestoff JR, Tyldsley AS, Zheng Q, Hodkinson BP, Artis D, Grice EA. 2018. Commensal microbiota modulate gene expression in the skin. Microbiome 6:20.

99. Henry J, Toulza E, Hsu CY, Pellerin L, Balica S, Mazereeuw-Hautier J, Paul C, Serre G, Jonca N, Simon M. 2012. Update on the epidermal differentiation complex. Front Biosci (Landmark Ed) 17:1517-32.

100. Abhishek S, Palamadai Krishnan S. 2016. Epidermal Differentiation Complex: A Review on Its Epigenetic Regulation and Potential Drug Targets. Cell J 18:1-6.

101. Eming SA, Krieg T, Davidson JM. 2007. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol 127:514-25.

102. Canesso MC, Vieira AT, Castro TB, Schirmer BG, Cisalpino D, Martins FS, Rachid MA, Nicoli JR, Teixeira MM, Barcelos LS. 2014. Skin wound healing is accelerated and scarless in the absence of commensal microbiota. J Immunol 193:5171-80.

103. Zhang J, Guan J, Niu X, Hu G, Guo S, Li Q, Xie Z, Zhang C, Wang Y. 2015. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. J Transl Med 13:49.

104. Wanke I, Steffen H, Christ C, Krismer B, Gotz F, Peschel A, Schaller M, Schittek B. 2011. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. J Invest Dermatol 131:382-90.

105. Ohnemus U, Kohrmeyer K, Houdek P, Rohde H, Wladykowski E, Vidal S, Horstkotte MA, Aepfelbacher M, Kirschner N, Behne MJ, Moll I, Brandner JM. 2008. Regulation of epidermal tight-junctions (TJ) during infection with exfoliative toxin-negative *Staphylococcus* strains. Journal of Investigative Dermatology 128:906-16.

106. Yuki T, Yoshida H, Akazawa Y, Komiya A, Sugiyama Y, Inoue S. 2011. Activation of TLR2 enhances tight junction barrier in epidermal keratinocytes. J Immunol 187:3230-7.

107. Rudramurthy SM, Honnavar P, Chakrabarti A, Dogra S, Singh P, Handa S. 2014. Association of Malassezia species with psoriatic lesions. Mycoses 57:483-488.

108. Fahlén A, Engstrand L, Baker BS, Powles A, Fry L. 2012. Comparison of bacterial microbiota in skin biopsies from normal and psoriatic skin. ArchDermatolResh 304:15-22.

109. Gao Z, Tseng CH, Strober BE, Pei Z, Blaser MJ. 2008. Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. PLoS ONE 3.

110. Takemoto A, Cho O, Morohoshi Y, Sugita T, Muto M. 2015. Molecular characterization of the skin fungal microbiome in patients with psoriasis. J Dermatol 42:166-170.

111. Chng KR, Tay ASL, Li C, Ng AHQ, Wang J, Suri BK, Matta SA, McGovern N, Janela B, Wong XFCC, Sio YY, Au BV, Wilm A, De Sessions PF, Lim TC, Tang MBY, Ginhoux F, Connolly JE, Lane EB, Chew FT, Common JEA, Nagarajan N. 2016. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. Nature Microbiol 1:16106.

112. Kennedy EA, Connolly J, Hourihane JOB, Fallon PG, McLean WHI, Murray D, Jo J-H, Segre JA, Kong HH, Irvine AD. 2017. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. J Allergy Clinl Immunol 139:166-172.

113. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Mullikin J, Thomas J, Blakesley R, Young A, Chu G, Ramsahoye C, Lovett S, Han J, Legaspi R, Sison C, Montemayor C, Gregory M, Hargrove A, Johnson T, Riebow N, Schmidt B, Novotny B, Gupta J, Benjamin B, Brooks S, Coleman H, Ho SL, Schandler K, Stantripop M, Maduro Q, Bouffard G, Dekhtyar M, Guan X, Masiello C, Maskeri B, McDowell J, Park M, Vemulapalli M, Murray PR, Turner ML, Segre JA. 2012. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res 22:850-859.

114. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, Gamonal J, Diaz PI. 2013. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J 7:1016-1025.

115. Hong BY, Araujo MVF, Strausbaugh LD, Terzi E, Ioannidou E, Diaz PI. 2015. Microbiome profiles in periodontitis in relation to host and disease characteristics. PLoS ONE 10.

116. Kirst ME, Li EC, Alfant B, Chi YY, Walker C, Magnusson I, Wanga GP. 2015. Dysbiosis and alterations in predicted functions of the subgingival microbiome in chronic periodontitis. Appl Environ Microbiol 81:783-793.

117. Peterson SN, Snesrud E, Liu J, Ong AC, Kilian M, Schork NJ, Bretz W. 2013. The Dental Plaque Microbiome in Health and Disease. PLoS ONE 8.

118. Tett A, Pasolli E, Farina S, Truong DT, Asnicar F, Zolfo M, Beghini F, Armanini F, Jousson O, De Sanctis V, Bertorelli R, Girolomoni G, Cristofolini M, Segata N. 2017. Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. NPJ Biofilms Microbiomes 3:14.

119. Mira A, Martín-Cuadrado AB, D'Auria G, Rodríguez-Valera F. 2010. The bacterial pan-genome: A new paradigm in microbiology. Inter Microbiol 13:45-57.

120. Mustapha MM, Marsh JW, Krauland MG, Fernandez JO, de Lemos APS, Dunning Hotopp JC, Wang X, Mayer LW, Lawrence JG, Hiller NL, Harrison LH. 2015. Genomic Epidemiology of Hypervirulent Serogroup W, ST-11 Neisseria meningitidis. EBioMedicine 2:1447-1455.

121. Zhu A, Sunagawa S, Mende DR, Bork P. 2015. Inter-individual differences in the gene content of human gut bacterial species. Genome Biol 16.

122. Luo C, Knight R, Siljander H, Knip M, Xavier RJ, Gevers D. 2015. ConStrains identifies microbial strains in metagenomic datasets. Nature Biotechnol 33:1045-1052.

123. Truong DT, Tett A, Pasolli E, Huttenhower C, Segata N. 2017. Microbial strain-level population structure & genetic diversity from metagenomes. Genome Res 27:626-638.

124. Zolfo M, Tett A, Jousson O, Donati C, Segata N. 2017. MetaMLST: Multi-locus strain-level bacterial typing from metagenomic samples. Nucleic Acids Res 45:e7.

125. Quince C, Connelly S, Raguideau S, Alneberg J, Shin SG, Collins G, Eren AM. 2016. De novo extraction of microbial strains from metagenomes reveals intra-species niche partitioning. bioRxiv doi:10.1101/073825.

126. Scholz M, Ward DV, Pasolli E, Tolio T, Zolfo M, Asnicar F, Truong DT, Tett A, Morrow AL, Segata N. 2016. Strain-level microbial epidemiology and population genomics from shotgun metagenomics. Nat Methods 13:435-438.

127. Shi B, Chang M, Martin J, Mitreva M, Lux R, Klokkevold P, Sodergren E, Weinstock GM, Haake SK, Li H. 2015. Dynamic changes in the subgingival microbiome and their potential for diagnosis and prognosis of periodontitis. MBio 6:e01926-14.

128. Wang J, Qi J, Zhao H, He S, Zhang Y, Wei S, Zhao F. 2013. Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. Sci Rep 3:1843.

129. Xu P, Gunsolley J. 2014. Application of metagenomics in understanding oral health and disease. Virulence 5:424-32.

130. Mathieu A, Vogel TM, Simonet P. 2014. The future of skin metagenomics. Res Microbiol 165:69-76.

131. Mathieu A, Delmont TO, Vogel TM, Robe P, Nalin R, Simonet P. 2013. Life on human surfaces: skin metagenomics. PLoS One 8:e65288.

132. Kang D, Shi B, Erfe MC, Craft N, Li H. 2015. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. Sci Transl Med 7:293ra103.

133. Solbiati J, Frias-Lopez J. 2018. Metatranscriptome of the Oral Microbiome in Health and Disease. J Dent Res 97:492-500.

134. Dutkiewicz EP, Chiu HY, Urban PL. 2017. Probing Skin for Metabolites and Topical Drugs with Hydrogel Micropatches. Anal Chem 89:2664-2670.

135. Kuehne A, Hildebrand J, Soehle J, Wenck H, Terstegen L, Gallinat S, Knott A, Winnefeld M, Zamboni N. 2017. An integrative metabolomics and transcriptomics study to identify metabolic alterations in aged skin of humans *in vivo*. BMC Genomics 18:169.

136. Hussain JN, Mantri N, Cohen MM. 2017. Working Up a Good Sweat - The Challenges of Standardising Sweat Collection for Metabolomics Analysis. Clin Biochem Rev 38:13-34.

137. Barnes VM, Teles R, Trivedi HM, Devizio W, Xu T, Mitchell MW, Milburn MV, Guo L. 2009. Acceleration of purine degradation by periodontal diseases. J Dent Res 88:851-5.

138. Barnes VM, Kennedy AD, Panagakos F, Devizio W, Trivedi HM, Jonsson T, Guo L, Cervi S, Scannapieco FA. 2014. Global metabolomic analysis of human saliva and plasma from healthy and diabetic subjects, with and without periodontal disease. PLoS One 9:e105181.

139. De Filippis F, Vannini L, La Storia A, Laghi L, Piombino P, Stellato G, Serrazanetti DI, Gozzi G, Turroni S, Ferrocino I, Lazzi C, Di Cagno R, Gobbetti M, Ercolini D. 2014. The same microbiota and a potentially discriminant metabolome in the saliva of omnivore, ovo-lacto-vegetarian and Vegan individuals. PLoS One 9:e112373.

140. Fisher CK, Mehta P. 2014. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. PLoS ONE 9.

141. Marino S, Baxter NT, Huffnagle GB, Petrosino JF, Schloss PD. 2014. Mathematical modeling of primary succession of murine intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America 111:439-444.

142. Stein RR, Bucci V, Toussaint NC, Buffie CG, Rätsch G, Pamer EG, Sander C, Xavier JB. 2013. Ecological Modeling from Time-Series Inference: Insight into Dynamics and Stability of Intestinal Microbiota. PLoS Computational Biology 9: e1003388.

143. Rosindell J, Hubbell SP, Etienne RS. 2011. The Unified Neutral Theory of Biodiversity and Biogeography at Age Ten. Trends in Ecology and Evolution 26:340-348.

144. Schroeder JL, Lunn M, Pinto AJ, Raskin L, Sloan WT. 2015. Probabilistic models to describe the dynamics of migrating microbial communities. PLoS One 10:e0117221.

145. Jang SS, Oishi KT, Egbert RG, Klavins E. 2012. Specification and simulation of synthetic multicelled behaviors. ACS Synth Biol 1:365-74.

146. Gottstein W, Olivier BG, Bruggeman FJ, Teusink B. 2016. Constraint-based stoichiometric modelling from single organisms to microbial communities. Journal of the Royal Society Interface 13.

147. Devoid S, Overbeek R, DeJongh M, Vonstein V, Best AA, Henry C. 2013. Automated genome annotation and metabolic model reconstruction in the SEED and Model SEED. Methods Mol Biol 985:17-45.

148. Agren R, Liu L, Shoaie S, Vongsangnak W, Nookaew I, Nielsen J. 2013. The RAVEN toolbox and its use for generating a genome-scale metabolic model for *Penicillium chrysogenum.* PLoS Comput Biol 9:e1002980.

149. Thiele I, Palsson BO. 2010. A protocol for generating a high-quality genome-scale metabolic reconstruction. Nat Protoc 5:93-121.

150. Harcombe WR, Riehl WJ, Dukovski I, Granger BR, Betts A, Lang AH, Bonilla G, Kar A, Leiby N, Mehta P, Marx CJ, Segrè D. 2014. Metabolic resource allocation in individual

microbes determines ecosystem interactions and spatial dynamics. Cell Reports 7:1104-1115.

151. Bauer E, Zimmermann J, Baldini F, Thiele I, Kaleta C. 2017. BacArena: Individual-based metabolic modeling of heterogeneous microbes in complex communities. PLoS Comput Biol 13:e1005544.

152. Kim MK, Lun DS. 2014. Methods for integration of transcriptomic data in genome-scale metabolic models. Comput Struct Biotechnol J 11:59-65.

153. Henry CS, Bernstein HC, Weisenhorn P, Taylor RC, Lee JY, Zucker J, Song HS. 2016. Microbial Community Metabolic Modeling: A Community Data-Driven Network Reconstruction. J Cell Physiol 231:2339-45.

154. Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E. 2011. Competitive and cooperative metabolic interactions in bacterial communities. Nat Commun 2:589.

155. Magnusdottir S, Heinken A, Kutt L, Ravcheev DA, Bauer E, Noronha A, Greenhalgh K, Jager C, Baginska J, Wilmes P, Fleming RM, Thiele I. 2017. Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. Nat Biotechnol 35:81-89.

156. Greenblum S, Chiu HC, Levy R, Carr R, Borenstein E. 2013. Towards a predictive systems-level model of the human microbiome: progress, challenges, and opportunities. Curr Opin Biotechnol 24:810-20.

157. Roume H, Heintz-Buschart A, Muller EEL, May P, Satagopam VP, Laczny CC, Narayanasamy S, Lebrun LA, Hoopmann MR, Schupp JM, Gillece JD, Hicks ND, Engelthaler DM, Sauter T, Keim PS, Moritz RL, Wilmes P. 2015. Comparative integrated omics: identification of key functionalities in microbial community-wide metabolic networks. NPJ Biofilms Microbiomes 1:15007.

158. Muller EEL, Faust K, Widder S, Herold M, Martínez Arbas S, Wilmes P. 2018. Using metabolic networks to resolve ecological properties of microbiomes. Currt Opin Syst Biol. 8:73-80.

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Catherine A O'Neill received both her bachelor's degree and PhD from the University of Wales, Bangor where she studied bacterial biochemistry. Subsequently, she was a research fellow at the University of Leeds for 5 years before securing her first tenured appointment as a lecturer at the University of Manchester. Subsequently she was promoted to senior lecturer and then finally to Professor of Translational Dermatology, the post she currently holds at the University of Manchester. Professor O'Neill's interests are in using bacteria and their products for the treatment of skin in health and disease. The laboratory has a very translational focus and has a record in translating basic findings into human studies via the technology transfer into commercial vehicles. Professor O'Neill has been involved in this area since 2011.

Alejandro Amezquita PhD, graduated from the University of Nebraska-Lincoln (US), with more than 20 years of experience in various positions in academic (North Carolina State University, US) and industrial research (Unilever), currently working as Science & Technology Director within Unilever's R&D group, interested in microbiome innovation in consumer goods and risk-based approaches to assure product safety because of the importance of balancing the efficacy-safety continuum, using safety-by-design approaches as the foundation for safe innovation, He has been working in the microbiome innovation field for 4 years and in the consumer safety and microbiological risk assessment fields for 15 years.

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Karoline Faust is a biologist turned bioinformatician who graduated at the Humboldt University in Berlin and earned her PhD at the Université Libre de Bruxelles under the supervision of Prof. van Helden. She worked as a postdoctoral researcher at KU Leuven and VIB in the group of Prof. Raes. She is currently an Assistant Professor, heading the group of Microbial Systems Biology at KU Leuven since 2016. Her main research interests include the construction and analysis of microbial networks, the analysis of microbial sequencing data and the investigation of microbial community dynamics in silico and in vitro. She therefore works at the intersection of microbial ecology, systems biology and bioinformatics.

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Jonathan R Swann obtained a PhD in Biochemistry from the Department of Biomolecular Medicine at Imperial College London in 2008. Following his PhD, Dr Swann continued as a research associate at Imperial College in the area of molecular epidemiology. In 2010 he joined the School of Chemistry, Food and Pharmacy at the University of Reading as a Lecturer in Metabonomics. In this role, he developed metabolic phenotyping strategies to study the impact of nutrition, the gut microbiota, and parasitic infections on mammalian health and disease. In 2015, Jonathan joined the Division of Computational and Systems Medicine at Imperial College as a Senior Lecturer in Human Development and Microbiomics. He was appointed Associate Professor in 2017. He leads a metabonomic-based research programme to understand the influence of gene-environment interactions on the mammalian metabolic system and their implications for development, health and disease. His research has a specific focus on the microbiome.

Adrian M Smith was awarded a BSc in Biomedical Sciences from Sheffield Hallam University in 2001 and an MSc in Bioinformatics from the same institute in 2002. He worked briefly for GSK before taking up his current position as Bioinformatician for Unilever R&D in 2005. He has had an interest in Microbiomics for 9 years due to the initial disruptive nature of the science, and the speed at which it continues to develop and reveal previously hidden microbial secrets. Most recently he has had a particular focus on the development of bioinformatics analysis pipelines and visualisation tools for microbial ‘Omics data analysis.

Barry Murphy has received education at University College Dublin with Post-Doctoral studies at the University of Leicester encompassing microbiology, molecular biology and chemistry. A move to industry saw him establish and manage DNA sequencing laboratories across Europe before moving to Unilever to lead the microbiome capability group. Having held this position for 5 years he has an interest in understanding human associated microbial communities to investigate links between microbial metabolism and cosmetic conditions.

Mike Hoptroff is a senior project manager at Unilever with responsibility leading Microbiome Science and Technology in the UK. He graduated in 1995 from the University of Sheffield and then moved to research posts in the UK and USA prior to joining Unilever in 1998. Since joining Unilever he has spent 21 years in Microbiology R&D initially as a research scientist and subsequently as a project manager. During this time he spent approximately 6 years working on

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Gordon James originates from Glasgow in Scotland, and was educated at University of Glasgow, graduating with a BSc and PhD in Biochemistry in 1987 and 1991, respectively. He then did a postdoctoral fellowship at University of Strathclyde in the area of environmental biotechnology, during which time he began practicing his favoured disciplines of microbiology and biochemistry. Gordon joined Unilever R&D in 1993, and in the time since, his main focus has been using his microbial biochemistry skills to probe the human skin microbiome, mainly to unravel the origins of axillary (underarm) odour. His current role is to provide scientific leadership to a UK-based team specialising in this topic on behalf of Unilever's Deodorants category and the global Science & Technology Platform, Human Microbiome.

Yugandhar Reddy is a Research Scientist with Beauty & Personal Care, Unilever R&D. I received my BSc and MSc in Microbiology and later Ph.D at the Indian Institute of Science, Bangalore. I was a postdoctoral fellow at the department of Microbiology & Molecular Genetics at University of Pittsburgh. Prior to joining Unilever, I worked as a Genomics Applications Scientist at Agilent Inc. My current interests are the Human Microbiome and its relevance for human health and wellbeing as well as building in vitro models to understand microbial community behaviour. In a previous role at Unilever I worked at the Safety and Environmental Assurance Center of Unilever Plc where I was exploring methods and approaches to risk assess Microbiome related technologies and led an S&T program on Microbial Ecology. I have been in this field for about 7 years to date.

Anindya Dasgupta has a PhD in Molecular Biology, Albert Einstein College of Medicine, New York, USA and is based at Unilever R&D, Bangalore. He is currentjy exploring scientific insights that play a crucial role in skin microbiome. The generation of these insights also help in screening of actives and development of products that have a positive impact on the skin microbiome. A key factor in this activity is to look at the safety aspect of microbiome modulation.

Tom Ross is a Professor in Food Microbiology at University of Tasmania. He was awarded his PhD from the University of Tasmania in 1994. Since thne he has been employed at University of Tasmania since 1994 as a researcher and teacher concerned with the quantitative microbial ecology of foods, and leading to my current position. He has supervised ~25 PhD graduates. He has published >150 international peer reviewed papers/book chapters with his students and colleagues. His research has also led to numerous software tools that translate his research into 'decision-support' tools for food safety and preservation that are used by governments and industry internationally. Those software tools are risk-based, and quantitative. He has been invited to contribute to many FAO/WHO scientific expert panels concerned with microbial food safety risk assessment. This background in quantitative risk assessment and microbial ecology led to his interest in the potential to modify the human skin microbiome and to assess the potentially associated risks.

Iain L Chapple is Head of the School of Dentistry; Research Director of the Institute of Clinical Science, Birmingham University, UK. He graduated 1986 from Newcastle University. Iain is former Scientific Editor of the British Dental Journal; Associate Editor of Journal of Periodontal Research and current Associate Editor of the Journal Clinical Periodontology. He has written 8-textbooks and 18 book chapters. Iain served the IADR Periodontal Research Group (PRG) as President (2006-7); Group Chair (2008-1015); Counsellor (2016). He served the European Federation of Periodontology (EFP) as: Treasurer (2007-2013); Workshop co-chair (2008-current); Chairman of Scientific Advisory Committee; Editor JCP Digest (2014-2016); Secretary General (2016-2019). He was British Society of Periodontology President 2014-2015 and awarded the Tomes medal - Royal College of Surgeons (2011); the IADR PRG Rizzo Award (2001); IADR Distinguished Scientist in 2018; Special citation award -American Academy of Periodontology 2018. Iain has >200 peer reviewed manuscripts in the international literature.

William G. Wade obtained his BSc in Biological Sciences at the University of East Anglia and a PhD in Microbiology at the University of Wales. He began his career as a Lecturer at the Welsh National School of Medicine in Cardiff and then moved to a Senior Lecturer appointment at the University of Bristol. He was appointed to the Richard Dickinson Chair of Oral Microbiology at UMDS (subsequently King's College London) in 1996. In 2013 he moved to Queen Mary University of London but returned to King's College London in 2018 to take up his current post of Professor of Oral Microbiology within the Centre for Host-Microbiome Interactions. He has played a major role in the characterisation of the oral microbiome, culture of previously uncultivated bacteria and the development of novel agents for the prevention and treatment of oral diseases. He has been active in microbiology research for 40 years.

Judith Fernandez-Piquer received her BSc in Chemical Engineering and BSc in Food Technology in Spain, her MSc in Food Safety in the Netherlands in 2007 and her PhD in Food Microbiology in Australia in 2012. Judith has a broad knowledge of risk assessment and the integration of predictive microbiology for exposure assessment in foods. After her PhD, she was involved in projects for Dairy Australia, Walnuts Australia and the Seafood CRC while at the University of Tasmania. Judith has a strong interest in protecting consumer’s health. She joined Unilever SEAC in 2014 as a risk assessor and led the Human Microbiome project, a programme that aims to enhance the safety assessment of microbial reprofiling to support innovative technologies in personal care. Judith started her current role as product safety manager with Upfield, a plant-based food company, in August 2018.

1296 TABLE 1. Habitat parameters, microbiome functions and intervention strategies for human skin and oral cavity

	Skin				Oral cavity		
Conditions with microbiome associations	Atopic dermatitis, psoriasis	Acne	Dandruff	Axillary malodor	Caries	Gingivitis	Periodontitis
Routine perturbations	Cleansing, moisturizing, use of cream, gels, lotions	Cleansing, use of cream, gels, lotions	Cleansing, use of shampoo	Cleansing, use of antiperspirants and deodorants	Toothbrushing, flossing, use of toothpaste, mouthwash		
Microbiome understanding and potential target mode of action for microbial interventions	*S. aureus* load correlates with atopic dermatitis flares (18) Early colonization with commensal staphylococci provides protection (REF 18, 19) Abnormal expression of antimicrobial peptides (22) Changes in the proportion of bacteria	Outgrowth of *C. acnes* and overproduction of sebum associated to acne (21, 93) Associated with specific strains of *C. acnes* (42, 119, 129) Decrease in the Vitamin B12 biosynthesis pathway (132)	Associated with an imbalance of both bacterial and fungal species, with an increase in *Staphylococcus* sp. and *M. restricta* (40). Severity of dandruff dependent on the interactions between the host and microorganisms (43) Decreased *Propionibacterium* and increased	Associated with *Corynebactrium* species (44) Malodour caused by short and medium chain volatile fatty acids (44)	Changes in oral microbiota composition (28, 29) Outgrowth of acid-tolerant *Streptococcus mutans* (30), *S. sobrinus* (96), *Lactobacillus* and *Bifidobacterium* (28, 114-117) Increased glycan synthesis and carbohydrate metabolism and reduced lipid metabolism (69)	Changes in oral microbiota composition (28, 29) Subversion of host response at inflamed site, colonization of inflamed tissue by *Porphyromonas gingivalis* (33) Plaque load and maturity (60)	Changes in oral microbiota composition (28, 29) Sub-gingival biofilm formation is associated with inflammation and bone loss (31) Translocation of oral microbiome to systemic circulation (34-36) Increased metabolic degradation of nutrients and fatty acid metabolism (126, 128) Increased gene

	compared to healthy skin (25, 26) Associated to the fungus *Malassezia* (106)	*Staphylococcus* abundance (43)	activity related to anaerobic growth conditions (128) Depletion of anti-oxidants, degradation of host cellular components and accumulation of bacterial products (136, 137)
Ecological factors specific to the human body site	Bacteriocins and phenol soluble modulins contribute to the maintenance of the niche (56) Skin has a mixture of secretions from different glands and microbiota (137) Host physiological conditions such as sebum and water content are relevant in scalp (43) Higher exposure to moisture, changes in temperature and UV (88) Host factors including skin barrier protein mutations e.g. Filaggrin in AD (20) and mTORC1 changes (increase sebum formation) due, in part, to diet (21) Host immune/inflammatory status (23 - 27)	Food intake, high intake of sugar correlated to production of lactic acid and acidification (REF 30,31) Biofilm formation by attaching to different surfaces (30, 31) Host susceptibility (32) Presence or absence of inflammation (33) Oxygen availability, mechanical stress and saliva flow (6, 61, 86) Antibiotic use (60) Exposure to tobacco smoke (79)	
Selected microbiota functions	*S. epidermis* produces AMPs to control the growth of *S. aureus* (16), serine proteases to inhibit biofilm (16) and fermentation products to inhibit *C. acnes* (46) *C. acnes* converts sebum to free fatty acids, inhibit colonisation and maintains acidic pH of the skin (46)	Some streptococci generate hydrogen peroxide to inhibit *S. mutans* (58) Nitrate-reducing bacteria can influence cardiovascular health and blood pressure (59) Some streptococci support enzymatic reactions for nutritional purposes (60)	

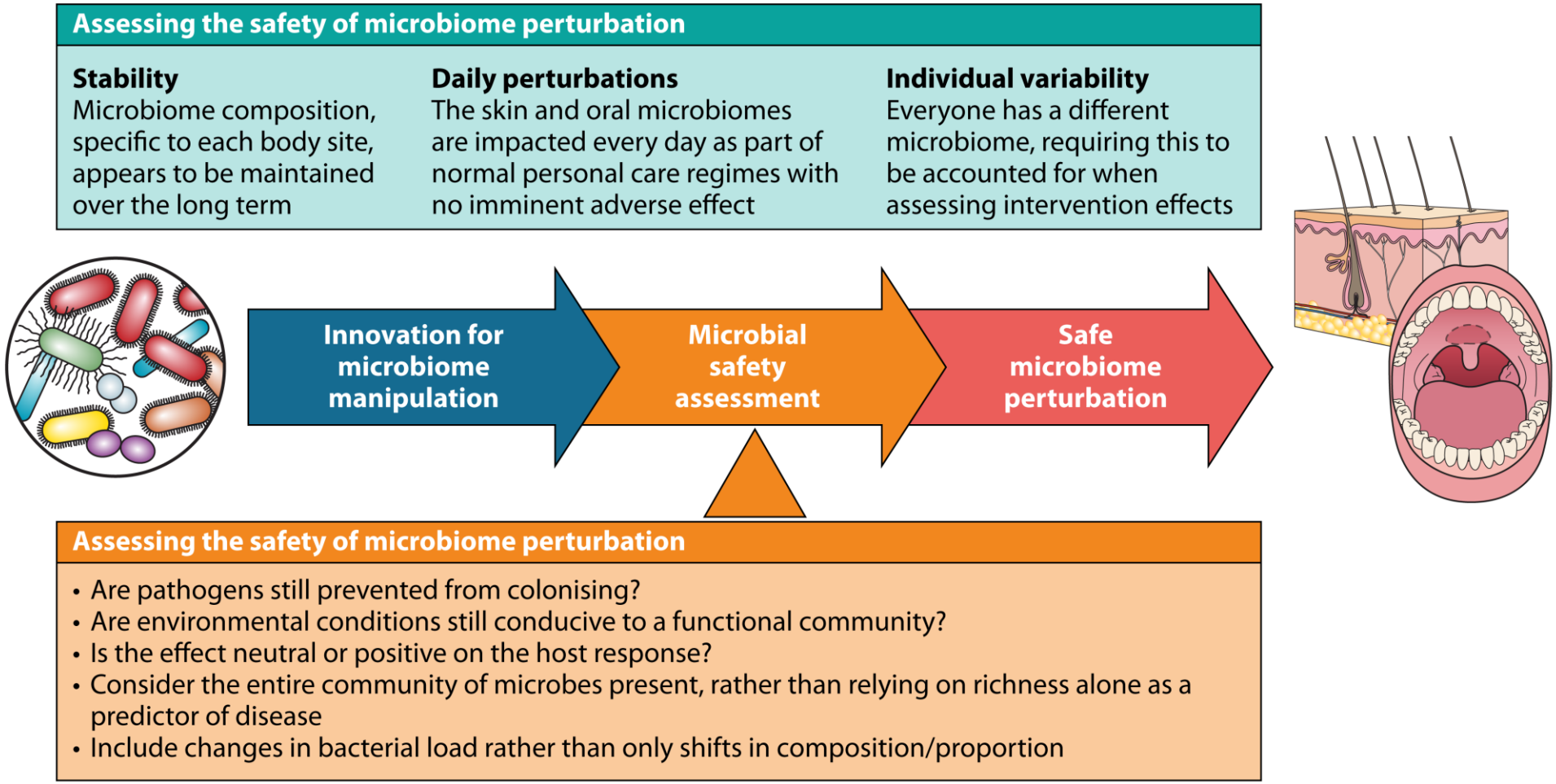

FIG 1. Assessing the safety of perturbations of the skin and oral microbiome

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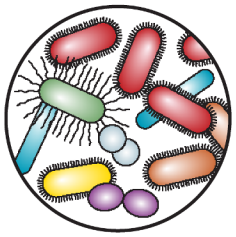
Assessing the safety of microbiome perturbation

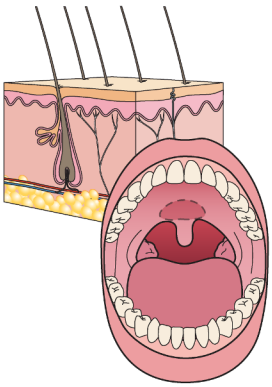
Stability
Microbiome composition, specific to each body site, appears to be maintained over the long term

Daily perturbations
The skin and oral microbiomes are impacted every day as part of normal personal care regimes with no imminent adverse effect

Individual variability
Everyone has a different microbiome, requiring this to be accounted for when assessing intervention effects



Innovation for microbiome manipulation → **Microbial safety assessment** → **Safe microbiome perturbation**



Assessing the safety of microbiome perturbation

- Are pathogens still prevented from colonising?
- Are environmental conditions still conducive to a functional community?
- Is the effect neutral or positive on the host response?
- Consider the entire community of microbes present, rather than relying on richness alone as a predictor of disease
- Include changes in bacterial load rather than only shifts in composition/proportion