

# Consumer safety considerations of skin and oral microbiome perturbation

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1 **Consumer Safety Considerations of Skin and Oral**  
2 **Microbiome Perturbation**

3

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25 perturbation, personal care, hygiene, cosmetic, risk assessment.

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## 44 **Summary**

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

60

## 61 **INTRODUCTION**

62

### 63 **The human microbiome**

64

65 The last two decades have seen the effective application of culture-independent methods to study  
66 the human microbiota (the microbial cells) or microbiome (the associated DNA) (1). This has led to  
67 a deeper and more comprehensive analysis of the diverse range of organisms that inhabit the body,  
68 where a substantial proportion are not readily amenable to culture (2). In the process some but  
69 certainly not all knowledge gaps have been addressed. High-throughput sequencing is currently  
70 performed using a range of platforms including Illumina and Ion Torrent, which can rapidly  
71 sequence millions of fragments of DNA in parallel (3). Hypervariable regions of the bacterial 16S  
72 rRNA genes, or whole genome DNA is targeted to analyse complex microbial communities. For  
73 16S amplicon sequencing in particular, bioinformatic analyses have been applied to cluster the  
74 generated sequences according to their similarity to define different operational taxonomic units  
75 (OTU), which are then compared to databases to reveal community composition. However, tools  
76 such as DADA2 are being increasingly used to obtain exact sequence variants (4) giving greater  
77 resolution (5). The often short sequencing reads and the large data volumes generated through NGS  
78 presents challenges and taxonomic classification and relative abundances can vary depending on the

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

88

### 89 **The challenge of establishing causality**

90

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

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

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

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

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

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

140

#### 141 **Targeting specific microbes with personal care products**

142

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

156 **Aims and Objectives**

157  
158 Whilst differentiating between association and causality remains a key issue in microbiome  
159 research, the fact that in some cases interactions between the microbiome and the host play a role in  
160 health and disease has been established (as previously reviewed (50)). It is therefore important that  
161 the effect of personal care regimes on the microbiome receives adequate consideration.  
162 Understanding of the factors that cause fluctuations in the microbiome is likely to contribute to the  
163 development of novel approaches to understand potential links to undesirable health conditions, and  
164 to the identification of microbiome-based biomarkers. It is in this context that the U.S. National  
165 Academy of Sciences have discussed the need to incorporate interactions between the microbiome  
166 and chemicals in assessing human health risks associated with environmental chemical exposure  
167 (51). As understanding of the functional significance of the human microbiome progresses, and the  
168 exploration of host-microbial interactions advances, understanding the effects of intentional  
169 manipulation of the human microbiome in the context of human safety should be addressed.

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

176 **PROTECTION OF THE ORAL AND SKIN MICROBIOME FUNCTIONS TO PROMOTE**  
177 **HEALTH**

178  
179 **The human microbiome in health and wellbeing**

180  
181 Microbiotas associated with the oral mucosa and the skin help programme the human immune  
182 system to recognise pathogens (52, 53), reduce the risk of invasion by undesired organisms (54),  
183 produce vitamins and other metabolites such as short-chain fatty acids (55). In skin, Phenol Soluble



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

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

### 205 **Microbiome Composition versus function**

206  
207 Initiatives such as the Human Microbiome Project (HMP) (13) (53) (55) and other studies (14, 52,  
208 61) have enhanced our understanding of baseline skin and oral microbial composition but the search  
209 for attributes that define a healthy human microbiome continues. As part of the HMP, where 200

210 healthy individuals were examined, the “core” microbiome of different body sites, including saliva,  
211 plaque, tongue and other oral tissues, ranged from zero to eight operational taxonomic units (OTUs)  
212 when analysed for percentage prevalence of 100% compared to a higher range of 19-75 OTUs when  
213 the percentage was lowered to 50% (62). Interpretation of the core microbiome to measure the  
214 similarity of samples depends on the taxonomic resolution employed since samples may decrease in  
215 apparent similarity when analysed to genus or OTUs compared to phylum level (61) (63) (64).  
216 Whilst a specific group of microorganisms may be shared between individuals, inter-individual  
217 variation may still be considerable at the species-level, and for the presence of rare microorganisms  
218 (8, 14, 61). Care is therefore required when classifying microbiome composition as healthy or  
219 otherwise, especially in the absence of species-level classification. This is of particular importance  
220 in the oral cavity where different species within the same genera can have contrasting associations  
221 between health and disease.

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

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

## 241 **FACTORS THAT CONTRIBUTE TO PERTURBATION OF THE SKIN AND ORAL** 242 **MICROBIOME**

### 243 **Microbiome stability as an indicator of health**

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

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

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

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

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

#### 295 **Consumer products can alter microbiome composition or function**

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

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

### 325 **Microbiome individualisation**

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

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

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

## 353 **MEASURING CHANGES IN MICROBIOME COMPOSITION AND ACTIVITIES**

354

### 355 **Risks of pathogen colonisation**

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

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

### 369 **The human body as a microbial niche**

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

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



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

389

### 390 **Microbial diversity in health**

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

### 406 **The importance of bacterial abundance**

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

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

#### 424 **Host-microbiota interactions**

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

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

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

## 457 **MEASURING CHANGES IN MICROBIOME COMPOSITION**

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

## 461 **Metagenomic profiling**

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

473 In terms of the oral microbiota, changes in microbial composition have long been associated with  
474 dental caries and periodontitis. For caries, sequence analysis has confirmed that bacteria other than  
475 *S. mutans* are correlated with active caries (*Lactobacillus* and *Bifidobacterium*) and likewise several  
476 taxonomic groups of bacteria are associated with periodontitis (28, 114-117). It is also clear that the  
477 aetiology of disease also involves a complex interplay between the host and the resident microbial  
478 communities that is yet to be fully explored. Applied to the study of psoriasis, such approaches  
479 indicate that strain level features and associated functional variation may be pertinent to disease  
480 (118).

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

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

### 487 **Profiling of functional potential**

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

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

#### 521 **Metatranscriptomic analyses**

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

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

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

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

### 555 **Metabolomic analyses**

556 Microbial metabolites can have a direct impact on oral or skin health (*e.g.* short chain fatty acids  
557 and sulphides in periodontal diseases, organic acids in dental caries) or they can enter and modulate  
558 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.

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

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

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

650

## 651 **CONCLUSIONS**

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

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

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1115

## 1116 AUTHOR BIOS

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1119 microbiota and pro/prebiotics with the Medical Research Council at Addenbrookes Hospital. His  
1120 interest in the human microbiome has diversified since moving to Manchester in 1999, to include  
1121 body sites such as the skin and the oral cavity. He also maintains research programmes in other

1122 areas of applied microbiology and particularly enjoys multi-disciplinary research. He has supervised  
1123 29 PhD graduates and published over 100 papers.

1124

1125 **Catherine A O'Neill** received both her bachelor's degree and PhD from the University of Wales,  
1126 Bangor where she studied bacterial biochemistry. Subsequently, she was a research fellow at the  
1127 University of Leeds for 5 years before securing her first tenured appointment as a lecturer at the  
1128 University of Manchester. Subsequently she was promoted to senior lecturer and then finally to  
1129 Professor of Translational Dermatology, the post she currently holds at the University of  
1130 Manchester. Professor O'Neill's interests are in using bacteria and their products for the treatment  
1131 of skin in health and disease. The laboratory has a very translational focus and has a record in  
1132 translating basic findings into human studies via the technology transfer into commercial vehicles.  
1133 Professor O'Neill has been involved in this area since 2011.

1134

1135 **Alejandro Amezcua** PhD, graduated from the University of Nebraska-Lincoln (US), with more  
1136 than 20 years of experience in various positions in academic (North Carolina State University, US)  
1137 and industrial research (Unilever), currently working as Science & Technology Director within  
1138 Unilever's R&D group, interested in microbiome innovation in consumer goods and risk-based  
1139 approaches to assure product safety because of the importance of balancing the efficacy-safety  
1140 continuum, using safety-by-design approaches as the foundation for safe innovation, He has been  
1141 working in the microbiome innovation field for 4 years and in the consumer safety and  
1142 microbiological risk assessment fields for 15 years.

1143

1144 **Laura J Price** received her Applied Biology BSc (Hons) from Staffordshire University in 2001.  
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1147 Unilever in 2004, where she is currently a Microbiology Risk Assessor. Her role is to independently  
1148 assess the consumer safety of new technologies and formulations designed by Unilever R&D. With  
1149 the increasing interest in the microbiome as a target for consumer products designed to improve  
1150 health and wellbeing, she is part of the Human Microbiome project, which is developing knowledge  
1151 on how best to safety assess new technologies. Over the last 4-5 years the project has delivered a  
1152 risk assessment framework, methods and data. The interactions of the microbiome and immune  
1153 system, and dysbiosis manifesting as human disease, are what particularly interest her.

1154

1155 **Karoline Faust** is a biologist turned bioinformatician who graduated at the Humboldt University in  
1156 Berlin and earned her PhD at the Université Libre de Bruxelles under the supervision of Prof. van  
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1158 She is currently an Assistant Professor, heading the group of Microbial Systems Biology at KU  
1159 Leuven since 2016. Her main research interests include the construction and analysis of microbial  
1160 networks, the analysis of microbial sequencing data and the investigation of microbial community  
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1163

1164 **Adrian Tett** is a Senior Research Associate in the Computational Metagenomics group (CIBIO,  
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1168 Institute of Food Research. His current work focusses on the microbial communities and subspecies  
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1170 approaches to explore the population structure, evolutionary history and subspecies diversification  
1171 in abundant yet poorly characterised members of the human microbiome.  
1172

1173 **Nicola Segata** Ph.D., is Associate Professor at the CIBIO Department of the University of Trento  
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1175 moved to Harvard School of Public Health for his post-doctoral training where he started studying  
1176 the human microbiome with computational metagenomics approaches. He came back to University  
1177 of Trento (Department CIBIO) where he started his laboratory in 2013. His laboratory employs  
1178 experimental meta'omic tools and novel computational approaches to study the diversity of the  
1179 human microbiome across conditions and populations and its role in human diseases. His work is  
1180 supported by the European Research Council and by several other European agencies. The projects  
1181 in his laboratory bring together computer scientists, microbiologists, statisticians, and clinicians and  
1182 focus on profiling microbiomes with strain-level resolution and on meta-analysing very large sets  
1183 of metagenomes with novel computational tools.

1184  
1185 **Jonathan R Swann** obtained a PhD in Biochemistry from the Department of Biomolecular  
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1187 research associate at Imperial College in the area of molecular epidemiology. In 2010 he joined the  
1188 School of Chemistry, Food and Pharmacy at the University of Reading as a Lecturer in  
1189 Metabonomics. In this role, he developed metabolic phenotyping strategies to study the impact of  
1190 nutrition, the gut microbiota, and parasitic infections on mammalian health and disease. In 2015,  
1191 Jonathan joined the Division of Computational and Systems Medicine at Imperial College as a  
1192 Senior Lecturer in Human Development and Microbiomics. He was appointed Associate Professor  
1193 in 2017. He leads a metabonomic-based research programme to understand the influence of gene-  
1194 environment interactions on the mammalian metabolic system and their implications for  
1195 development, health and disease. His research has a specific focus on the microbiome.  
1196

1197 **Adrian M Smith** was awarded a BSc in Biomedical Sciences from Sheffield Hallam University in  
1198 2001 and an MSc in Bioinformatics from the same institute in 2002. He worked briefly for GSK  
1199 before taking up his current position as Bioinformatician for Unilever R&D in 2005. He has had an  
1200 interest in Microbiomics for 9 years due to the initial disruptive nature of the science, and the speed  
1201 at which it continues to develop and reveal previously hidden microbial secrets. Most recently he  
1202 has had a particular focus on the development of bioinformatics analysis pipelines and visualisation  
1203 tools for microbial 'Omics data analysis.  
1204

1205 **Barry Murphy** has received education at University College Dublin with Post-Doctoral studies at  
1206 the University of Leicester encompassing microbiology, molecular biology and chemistry. A move  
1207 to industry saw him establish and manage DNA sequencing laboratories across Europe before  
1208 moving to Unilever to lead the microbiome capability group. Having held this position for 5 years  
1209 he has an interest in understanding human associated microbial communities to investigate links  
1210 between microbial metabolism and cosmetic conditions.  
1211

1212 **Mike Hoptroff** is a senior project manager at Unilever with responsibility leading Microbiome  
1213 Science and Technology in the UK. He graduated in 1995 from the University of Sheffield and then  
1214 moved to research posts in the UK and USA prior to joining Unilever in 1998. Since joining  
1215 Unilever he has spent 21 years in Microbiology R&D initially as a research scientist and  
1216 subsequently as a project manager. During this time he spent approximately 6 years working on

1217 skin cleansing and hand hygiene (2003-2008), 7 years on scalp microbiology (2009-2016),  
1218 including 4 years leading Microbiology R&D in Unilever China and 3 years on Oral Care  
1219 microbiology research (2016-). Michael has 13 peer reviewed publications and has led the market  
1220 delivery of numerous product technologies.

1221

1222 **Gordon James** originates from Glasgow in Scotland, and was educated at University of Glasgow,  
1223 graduating with a BSc and PhD in Biochemistry in 1987 and 1991, respectively. He then did a  
1224 postdoctoral fellowship at University of Strathclyde in the area of environmental biotechnology,  
1225 during which time he began practicing his favoured disciplines of microbiology and  
1226 biochemistry. Gordon joined Unilever R&D in 1993, and in the time since, his main focus has been  
1227 using his microbial biochemistry skills to probe the human skin microbiome, mainly to unravel the  
1228 origins of axillary (underarm) odour. His current role is to provide scientific leadership to a UK-  
1229 based team specialising in this topic on behalf of Unilever's Deodorants category and the global  
1230 Science & Technology Platform, Human Microbiome.

1231

1232 **Yugandhar Reddy** is a Research Scientist with Beauty & Personal Care, Unilever R&D. I received  
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1234 was a postdoctoral fellow at the department of Microbiology & Molecular Genetics at University  
1235 of Pittsburgh. Prior to joining Unilever, I worked as a Genomics Applications Scientist at Agilent  
1236 Inc. My current interests are the Human Microbiome and its relevance for human health and  
1237 wellbeing as well as building in vitro models to understand microbial community behaviour. In a  
1238 previous role at Unilever I worked at the Safety and Environmental Assurance Center of Unilever  
1239 Plc where I was exploring methods and approaches to risk assess Microbiome related technologies  
1240 and led an S&T program on Microbial Ecology. I have been in this field for about 7 years to date.

1241

1242 **Anindya Dasgupta** has a PhD in Molecular Biology, Albert Einstein College of Medicine, New  
1243 York, USA and is based at Unilever R&D, Bangalore. He is currently exploring scientific insights  
1244 that play a crucial role in skin microbiome. The generation of these insights also help in screening  
1245 of actives and development of products that have a positive impact on the skin microbiome. A key  
1246 factor in this activity is to look at the safety aspect of microbiome modulation.

1247

1248 **Tom Ross** is a Professor in Food Microbiology at University of Tasmania. He was awarded his  
1249 PhD from the University of Tasmania in 1994. Since then he has been employed at University of  
1250 Tasmania since 1994 as a researcher and teacher concerned with the quantitative microbial ecology  
1251 of foods, and leading to my current position. He has supervised ~25 PhD graduates. He has  
1252 published >150 international peer reviewed papers/book chapters with his students and colleagues.  
1253 His research has also led to numerous software tools that translate his research into 'decision-  
1254 support' tools for food safety and preservation that are used by governments and industry  
1255 internationally. Those software tools are risk-based, and quantitative. He has been invited to  
1256 contribute to many FAO/WHO scientific expert panels concerned with microbial food safety risk  
1257 assessment. This background in quantitative risk assessment and microbial ecology led to his  
1258 interest in the potential to modify the human skin microbiome and to assess the potentially  
1259 associated risks.

1260

1261 **Iain L Chapple** is Head of the School of Dentistry; Research Director of the Institute of Clinical  
1262 Science, Birmingham University, UK. He graduated 1986 from Newcastle University. Iain is  
1263 former Scientific Editor of the British Dental Journal; Associate Editor of Journal of Periodontal  
1264 Research and current Associate Editor of the Journal Clinical Periodontology. He has written 8-  
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1268 Chairman of Scientific Advisory Committee; Editor JCP Digest (2014-2016); Secretary General  
1269 (2016-2019). He was British Society of Periodontology President 2014-2015 and awarded the  
1270 Tomes medal - Royal College of Surgeons (2011); the IADR PRG Rizzo Award (2001); IADR  
1271 Distinguished Scientist in 2018; Special citation award -American Academy of Periodontology  
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1273  
1274 **William G. Wade** obtained his BSc in Biological Sciences at the University of East Anglia and a  
1275 PhD in Microbiology at the University of Wales. He began his career as a Lecturer at the Welsh  
1276 National School of Medicine in Cardiff and then moved to a Senior Lecturer appointment at the  
1277 University of Bristol. He was appointed to the Richard Dickinson Chair of Oral Microbiology at  
1278 UMDS (subsequently King's College London) in 1996. In 2013 he moved to Queen Mary  
1279 University of London but returned to King's College London in 2018 to take up his current post of  
1280 Professor of Oral Microbiology within the Centre for Host-Microbiome Interactions. He has played  
1281 a major role in the characterisation of the oral microbiome, culture of previously uncultivated  
1282 bacteria and the development of novel agents for the prevention and treatment of oral diseases. He  
1283 has been active in microbiology research for 40 years.

1284  
1285 **Judith Fernandez-Piquer** received her BSc in Chemical Engineering and BSc in Food Technology  
1286 in Spain, her MSc in Food Safety in the Netherlands in 2007 and her PhD in Food Microbiology in  
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1289 Dairy Australia, Walnuts Australia and the Seafood CRC while at the University of Tasmania.  
1290 Judith has a strong interest in protecting consumer's health. She joined Unilever SEAC in 2014 as a  
1291 risk assessor and led the Human Microbiome project, a programme that aims to enhance the safety  
1292 assessment of microbial reprofiling to support innovative technologies in personal care. Judith  
1293 started her current role as product safety manager with Upfield, a plant-based food company, in  
1294 August 2018.

1295

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	<p><i>S. aureus</i> load correlates with atopic dermatitis flares (18)</p> <p>Early colonization with commensal staphylococci provides protection (REF 18, 19)</p> <p>Abnormal expression of antimicrobial peptides (22)</p> <p>Changes in the proportion of bacteria</p>	<p>Outgrowth of <i>C. acnes</i> and overproduction of sebum associated to acne (21, 93)</p> <p>Associated with specific strains of <i>C. acnes</i> (42, 119, 129)</p> <p>Decrease in the Vitamin B12 biosynthesis pathway (132)</p>	<p>Associated with an imbalance of both bacterial and fungal species, with an increase in <i>Staphylococcus</i> sp. and <i>M. restricta</i> (40).</p> <p>Severity of dandruff dependent on the interactions between the host and microorganisms (43)</p> <p>Decreased <i>Propionibacterium</i> and increased</p>	<p>Associated with <i>Corynebacterium</i> species (44)</p> <p>Malodour caused by short and medium chain volatile fatty acids (44)</p>	<p>Changes in oral microbiota composition (28, 29)</p> <p>Outgrowth of acid-tolerant <i>Streptococcus mutans</i> (30), <i>S. sobrinus</i> (96), <i>Lactobacillus</i> and <i>Bifidobacterium</i> (28, 114-117)</p> <p>Increased glycan synthesis and carbohydrate metabolism and reduced lipid metabolism (69)</p>	<p>Changes in oral microbiota composition (28, 29)</p> <p>Subversion of host response at inflamed site, colonization of inflamed tissue by <i>Porphyromonas gingivalis</i> (33)</p> <p>Plaque load and maturity (60)</p>	<p>Changes in oral microbiota composition (28, 29)</p> <p>Sub-gingival biofilm formation is associated with inflammation and bone loss (31)</p> <p>Translocation of oral microbiome to systemic circulation (34-36)</p> <p>Increased metabolic degradation of nutrients and fatty acid metabolism (126, 128)</p> <p>Increased gene</p>

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

**Assessing the safety of microbiome perturbation**

**Stability**

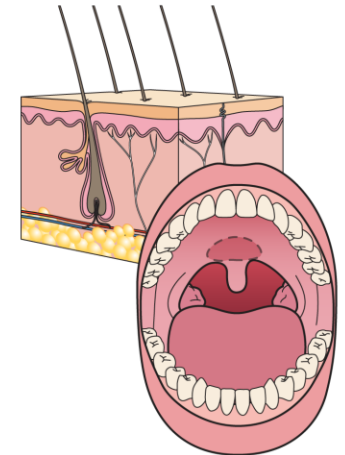
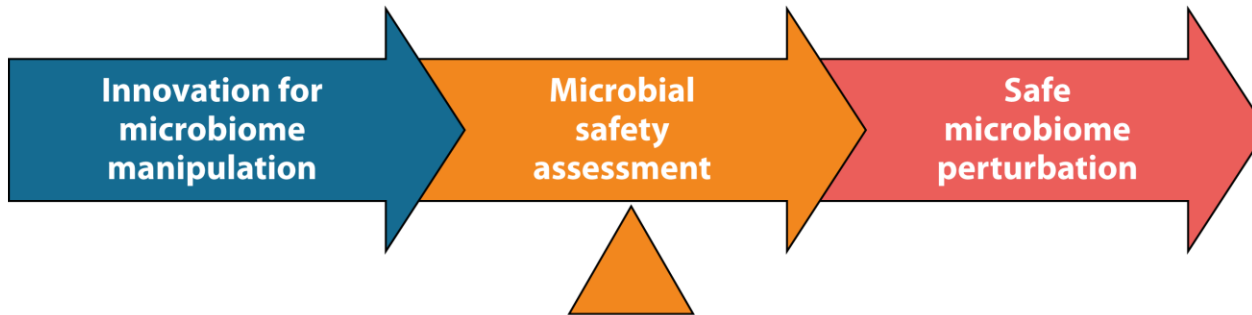
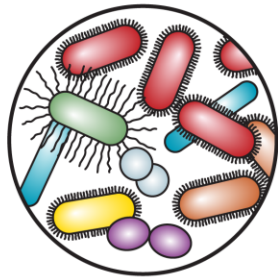
Microbiome composition, specific to each body site, appears to be maintained over the long term

**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



**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

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1298

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





### Assessing the safety of microbiome perturbation

#### Stability

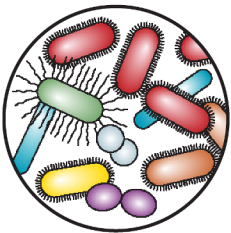
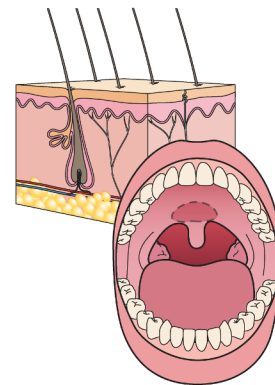
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Innovation for  
microbiome  
manipulation

Microbial  
safety  
assessment

Safe  
microbiome  
perturbation

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