REVIEW ARTICLE

Microbial transitions from health to disease

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1 | INTRODUCTION

Over 20 years ago in the pages of this journal, Page and Kornman[1] presented a framework for our knowledge and understanding of the pathogenesis of periodontal disease. This hugely influential, conceptual model divided knowledge in the field into four inter-connecting compartments: the microbial challenge; the immune and inflammatory response; connective tissue and bone turnover; and clinical signs. The microbial challenge, comprising the microbial communities of the subgingival biofilm, interacts with the immune and inflammatory response of the host; in the majority of individuals, most of the time this results in a balanced equilibrium with a proportionate host response characterized by minimal and reversible inflammation and no net tissue destruction. However, this model also indicates that the balance at this interface is fundamentally governed by environmental and acquired risk factors of the individual and host genetics, such that in a proportion of individuals the outcome of this interplay between these two compartments can lead to a deregulated response characterized by exacerbated inflammation, increased gingival crevicular fluid flow, and elevated recruitment of phagocytic cells into the subgingival crevice. In this scenario, the enhanced immune and inflammatory response alters the pattern of signaling to the connective tissue and bone compartment of the model, which can in turn change the normal patterns of tissue turnover associated with healthy homeostasis. The result of this inter-compartmental interplay can thereby lead to tissue destruction in the form of migration of the junctional epithelium down the longitudinal axis of the tooth, epithelial ulceration, and ultimately lowering of the apical surface of the bone. As before, the model indicates that the interface between these two host compartments is strongly governed by both environment and host genetics. Finally, the clinical signs compartment represents the accumulation of all the outcomes of the aforementioned interactions, which will range from health with low inflammation, and gingivitis with elevated inflammation, to periodontitis with associated inflammation and net tissue loss. Importantly, Page and Kornman[1] also reflected that the clinical signs will impact upon the nature of the microbial challenge to acknowledge the importance of the local environment to the composition and activity of the subgingival microbial communities.[2] Thus, the model describes the potential for a repetitive cycle of increasing microbially driven inflammation leading to increasingly adverse clinical signs and a more aggressive and virulent microbial challenge.

2 | DEVELOPMENTS IN THE ETIOPATHOGENESIS OF PERIODONTAL DISEASE

There have been a number of subsequent iterations of the original model but the overall concept of a linear interplay between microbial biofilm, inflammatory response, and connective tissue/bone destruction surrounded by environmental and genetic risk factors has remained the core theme.[3] Over the intervening 2 decades, the periodontal research community has made significant progress in describing the fine details within each of the four compartments described above. A summary of this progress is beyond the scope of this review. However, the highlights include: an exponential increase in understanding of the nature of the oral microbiome, its component members and community organization[4-6]; significant advances in the microbial pattern recognition systems of the host, the associated signaling and cellular responses of innate immunity,

and the mechanisms of establishment of the immune systems in the mouth[7,8]; an appreciation of the control of the inflammatory response and the molecular mechanisms of resolution of inflammation[9]; enhanced comprehension of the cellular control of tissue turnover in health and the mechanisms of disruption in destructive disease[10]; and application of these advances to clinical studies of oral health and disease in both animal models and human subjects.[11]

The provision of this finer level of detail within each of the four compartments has enabled a more detailed description of the molecular and cellular actions at the interfaces. In so doing, it is now becoming possible to view our knowledge map of the etiology of periodontal health and disease as a broad, highly connected, and integrated system that spans the entire spectrum of microbe/host/clinical interactions, rather than as a strictly compartmentalized system of four discrete boxes. The overall concept of this issue of *Periodontology 2000*, that the microbial biofilm can be considered a human tissue of bacteriological origin, is entirely consistent with this integrated system view.

Although researchers in the field have made undoubted progress in filling some of the blank spaces in the original concept design presented by Page and Kornman,[1] there remain significant areas for development. These are especially evident in the identities and roles of the acquired, environmental, and genetic factors, which almost certainly govern the transition points of the system and that ultimately will define susceptibility to the disease. Of course, the range of acknowledged environmental risks for periodontal disease includes modifiable factors, notably tobacco usage and alcohol consumption, as well as a growing list of diseases or conditions including diabetes, obesity, metabolic syndrome, osteoporosis, chronic kidney disease, and low dietary calcium and vitamin D.[12,13] Management of these lifestyle factors and diseases is now firmly embedded in periodontal care. However, with relatively few exceptions,[14] a clear understanding of the mechanism through which these factors may influence disease susceptibility has remained elusive. Similarly, despite some early promise (eg, genetic polymorphisms in the interleukin 6 gene), except for rare, aggressive forms of the disease, our comprehension of the genetic factors that may explain the significant hereditary component of periodontitis is still limited.[12] It has been suggested that the reasons why few true genetic associations for periodontal disease have been identified are two-fold. First, inappropriate sample recruitment: lack of a universally accepted diagnostic criterion for periodontitis; the frequently small sample size studied; and failure to take account of ethnicity differences and environmental effects. Second, inadequate study designs, including, for example, candidate gene association studies with a low sample size, no adjustment for covariates, no study of rare variants, and finally no replication of the results in an independent sample.[15] While this rather harsh critique may have some basis, it may also be the case that hereditary factors other than germ line genetics deserve some consideration.

In this paper, we summarize some recent findings to suggest a more central role for the subgingival microbiome as a key risk factor for susceptibility to periodontal disease that may, in part, explain some of the hereditable components of the disease and the outcome of the interactions at the interface between the microbial challenge and the host response. We draw upon two areas of research investigation to support the proposal: first, increased understanding of the acquisition of the oral microbiome and the role of genetics in this process, and, second, a growing understanding of the stability of microbial populations in both health and disease.

3 | ACQUISITION OF THE ORAL MICROBIOME

As pointed out by Wade[16] this issue of *Periodontology 2000*, microbial community profiling studies have demonstrated that a single individual may harbor approximately 300 species of oral bacteria. The identities and proportions of these organisms are stable but vary considerably among different people (as well as between different sites in the same individual) to the point where there may be potential for forensic use in identifying individuals.[17] It is now clear that transmission from the mother/primary carer plays a key role in defining the composition of the early oral microbiome. In a study of 47 mothers and their full-term, normal birthweight, pre-dentate children, Mason et al[18] demonstrated that the infants shared 85% of their microbiota with their mothers, suggesting that the maternal oral microbiome plays the major role in introducing microbial species to the child. This early microbiome appeared to form the foundation upon which newer microbial communities develop as more colonization niches emerge. The expansion of biodiversity (eg, following tooth eruption) may be attributable not simply to the introduction of exogenous, new species, but to an increase in the abundance of the pre-dentate organisms. In this regard it is significant that two-thirds of the species found in the pre-dentate mucosal microbiome were also seen in the subgingival microbial community of the parent, demonstrating the potentially influential role that the pre-dentate microbiome derived from the mother plays in the development of the periodontal microbial community. A similar mother and infant study by Drell et al[19] demonstrated that the infant gut microbiota harbors a distinctive microbial community that exhibits low similarity with the microbiota that colonizes the mother's gut. By contrast, infants' oral microbiome, as well as mothers' breast milk microbiota, mammary areola microbiota, and oral microbiota, exhibited a high similarity to each other. Interestingly, the mechanism that underlies this maternal-infant transfer may not solely be caused by early exposure following birth. As reported by Kaan et al[20] this issue of *Periodontology 2000*, there is a growing volume of literature that suggests the oral microbial ecosystem may be shaped in utero, by, most likely, maternal antigen exposure to the immune system of the infant, which thereby prepares the infant for postnatal microbial encounters. Additional longitudinal prospective studies are required to confirm the role that vertical microbial transfer from mother to infant plays in the establishment of the oral and

subgingival microbiome of the adult. However, it is already clear that this process of establishment of the microbiome in the infant is not simply governed by maternal exposure, and that the genetic landscape of the recipient is also a key determinant.

The influence of the host genetic background on the acquisition of the oral microbiome was examined recently in an elegant investigation involving a genome-wide association study on longitudinal data collected from 752 twin pairs.[21] The study demonstrated that the microbial population diversity of the oral microbiome in monozygotic twins was significantly lower than that for dizygotic or unrelated individuals. This was independent of whether the twins lived together or separately. Furthermore, modeling of these data showed that a number of microbiome phenotypes were more than 50% heritable, consistent with the hypothesis that human genes influence microbial populations: two loci on chromosomes 7 and 12 appeared to be most heritable in the acquisition of the oral microbiome.[21]

In summary, there is accumulating evidence to indicate that transgenerational transfer of the oral microbiome, governed in part by host genetics and potentially by in utero conditioning, may have a role in the development of the periodontal microbiome. By extrapolation, one can therefore ask the question as to whether transfer from a diseased parent of the oral microbiome, which has the necessary component parts to form a community with the capability of driving disease in that individual, will also predispose the recipient child to disease development in combination with the necessary genetic determinants of disease susceptibility? Few investigations have addressed this question to date although some evidence for hereditability of the disease has been suggested in relation to the JP2 clone of the oral pathogen *Aggregatibacter actinomycetemcomitans*. This particular genomic variant of the organism, characterized by a 530 base pair deletion in the leukotoxin gene operon, appears to be restricted to individuals of west African descent. The presence of this clone in the population correlates highly with one form of localized periodontal disease in adolescents.[22,23]

4 | STABILITY OF THE ORAL MICROBIOME IN HEALTH

Stability is a key feature of the human oral microbiome.[24,25] It is known to be less susceptible to major changes or disruptions by external environmental factors at the individual level. When two individuals were sampled over the course of an entire year, 95% of the operational taxonomic units of the oral bacterial population were found to be stable over the course of the study, while only the minor components of the microbiome were found to be involved in fluctuations.[26] This was in complete contrast to the gut microbiome, which was significantly perturbed by dietary influences, antibiotic usage, and other lifestyle factors. In another study comparing 22 different body sites in 236 healthy adults as part of the Human Microbiome Project, the oral microbiome was found to be the most temporally stable microbial community in the body.[27] This stability, at least in health, may be a function of the dominant and continuous influence

of saliva in the nutrition of oral bacteria and only a minimal impact of the diet—except in the case of overwhelming quantities of readily metabolized fermentable dietary sugars—as described by Belstrøm et al.[28]

The intraindividual stability of the oral microbiota has been seen to be consistent, despite the interindividual variations in a population. A core healthy human oral microbiome consisting largely of genera such as *Streptococcus*, *Fusobacterium*, *Prevotella*, *Rothia*, and *Neisseria* among others has been identified,[29,30] and this has been observed to be stable within individuals for up to a year or longer when tested longitudinally.[24] To date, little information is available on the stability of the subgingival microbiome during episodes of disease although there has been recent progress using animal models of disease, which we will return to later.

5 | ECOLOGICAL CONSIDERATIONS IN THE COMPOSITION OF THE MICROBIOTA

The oral cavity is a diverse environment with many different surfaces, topographies, and local environmental conditions. Consequently, the microbial population structure at each ecological site will vary depending on nutrition, pH, host defense factors, and other variables. Hence, when performing a comparison of the microbiology at different locations it is essential to take into account the local environmental conditions. Importantly, these environmental variables may differ independent of the presence or absence of disease. For example, a comparison of the microbial populations in the supragingival vs subgingival tooth surface does not necessarily infer an association with disease. The differences correspond equally to the changes anticipated when comparing ecological sites with different nutritional sources (saliva vs gingival crevicular fluid), different oxygen tensions, differences in pH, and so on.[31] Indeed, even within individual subgingival pockets, the anaerobic microbial population can vary depending on the influence of variations in oxygen sensitivity.[32] The subgingival site, depending upon its depth, may clearly reflect previous disease experience, but the composition of the microbiota at that site does not necessarily correspond to the disease-associated microbial community.

Ximenez-Fyvie et al[33] have discussed variations in the proportions of specific Actinomyces bacterial groups in supragingival and subgingival sites in healthy and diseased patients, as well as specifically within a cohort of adult patients suffering from periodontitis.[34] These studies prove the importance of assessing locations that are ecologically similar to compare the degree and progression of the disease. Even although most studies focus on the characterization of the subgingival microbial population in periodontal disease, it has been observed that even the supragingival region can exhibit distinct microbial signatures for gingivitis and periodontitis, despite it being a site of reduced local inflammation.[33]

Another confounder in gaining an accurate view of the disease-associated microbiota in a given individual is the severity of disease affecting the microbial population being sampled. Ge et al[35]

demonstrated significant differences in subgingival bacterial diversity between deep diseased sites and shallow healthy sites. Even when sampling multiple sites within the same individual, it is important to sample and compare similarly diseased sites (eg, similar or comparable pocket depths), which can influence the accurate representation of the association of the microbiome with disease and inflammation.[36]

More studies involving longitudinal sampling within the same individual are needed to better understand the outcome of the disease. Few studies have looked at the subgingival microbiome composition and its variation before and after periodontal therapy.[37-39] Although technically challenging in study design, longitudinal sampling coupled with an understanding of the disease experience would represent the gold standard.

Despite these limitations of experimental design that influence much of the microbiological literature in the field, a general consensus is emerging that, during the progression of periodontal disease, the oral microbiota undergoes a major transition, during which the microbial community structure is shifted to an increase in total bacterial diversity. This is accompanied by an increase in the total number of disease-associated bacteria that start dominating the population, which otherwise are present in low numbers in a state of health.[40] Strikingly, this transition of the oral microbiota to dysbiosis during disease is contrary to what is observed in microbially mediated diseases in other environments of the body such as the gut. During inflammatory disease conditions at this site, dysbiosis is accompanied by a decreased level of microbial diversity, in particular by a reduction in the anaerobic microbes, which are otherwise associated with a state of health. Low diversity dysbiosis is also the cause of other conditions such as obesity or inflammatory bowel disease.[41,42]

6 | FACTORS THAT DRIVE ORAL MICROBIAL TRANSITIONS

Three key drivers may be considered important to the shift in microbial populations during periodontal disease. First, certain groups of organisms that subvert the inflammatory response are known to be responsible for influencing a community-wide change on the overall bacterial population. An example is *Porphyromonas gingivalis*, an organism long associated with the development of periodontal disease. This bacterium has been suggested to exert a "keystone" effect on the oral microbial population during periodontal disease by triggering a state of dysbiosis and inflammation.[43] *Porphyromonas gingivalis* is involved in both immune subversion and maintaining inflammation in the host tissues by facilitating communication between the C5aR arm of the complement system and toll-like receptor 2 molecules.[44] Studies in mice have also shown that *P. gingivalis* is not just the sole orchestrator of this shift but is also greatly assisted by the involved activity of the commensal bacterial population. This was demonstrated in germ-free mice, where the absence of the commensal microbiota failed to initiate periodontal disease

and alveolar bone loss.[45] More recently, using a combination of metagenomic and meta-transcriptomic approaches in human oral samples, elevated activity of the commensal microbial population, which is not traditionally associated with disease, was observed in the expression of putative virulence factors associated with activities such as stress tolerance and adhesion.[46] This further supports the hypothesis that the entire community acts as a collective pathogenic unit.[47,48]

Another potential driver for disease in the oral cavity is the largely inflammophilic nature of the oral microbial population.[49] As stated previously, "periodontal pathogens" are present in oral ecological sites, even in states of health at very low abundance, and these could be responsible for triggering these persistent (albeit low) baseline levels of inflammation, even during healthy conditions. It can be argued that provoking the inflammatory response provides two benefits to an inflammophilic organism: first, through the initiation of tissue destruction, a protected site for colonization is produced, which may enable the organism to outcompete other less inflammophilic organisms; and second, the accumulation of nutrients such as haemin-containing compounds and proteins from tissue exudates/plasma will facilitate the survival of specific types of anaerobic bacteria, thus generating a competitive survival advantage in the ecosystem. Therefore, the inflammophilic nature of the oral microbiome drives a "self-feeding" vicious cycle of tissue damage and bacterial survival and growth.[43]

The third driver for the microbial role in periodontal disease is the ability of the oral microbial population to form biofilms. Since these organisms have the propensity to form plaque-like ecosystems, this enables multiple species to coexist in the form of tight complexes with mutually dependent nutritional and survival characteristics. One of the earliest studies to identify such clustering patterns used a combination of genomic DNA probes and DNA-DNA hybridization methodology to identify five microbial complexes, named red, orange, green, yellow, and purple, with varying patterns of association with health and disease.[50] These microbial clusters tend to operate in a highly mutualistic manner and one member of the cluster is often able to provide protection to all the other members from the inflammatory response of the host. Red complex bacteria have been reported as being involved in consortium in the disruption of homeostasis in the host through activities such as inhibition of interleukin 8 and toll-like receptor 4 signal regulation.[51,52] Some oral bacteria such as *Streptococcus mutans* are also known for production of specific bacteriocins that directly target and inhibit competitive species that enable its dominance in the plaque[53] and it is evident that similar strategies are at play in the subgingival microbiota.[54,55] The early stages of plaque formation also involve another feature of oral bacteria—coaggregation––with high levels of specificity between mixed species such as *Streptococcus* and *Veillonella*, or *Streptococcus* and *Actinomyces*.[52,56] Kirst et al[57] used multiple datasets of 16S rRNA gene sequencing data of oral microbial samples and identified two distinct clusters (or "periodontotypes") in the subgingival microbiome, both based on population and functional profiles, a healthy cluster predominated by *Rothia* and *Streptococcus* species

and another cluster more associated with chronic periodontitis consisting of *Fusobacterium* and *Porphyromonas* species.[57] Using cluster analysis on 16S rRNA gene sequencing data of the oral samples of 85 individuals, Boutin et al[58] were able to identify two different microbial "ecotypes" in the oral cavity, one associated with health and/ or mild disease and a second ecotype associated with illness, which could be divided into three subgroups based on the degree of disease progression.

7 | STABILITY AND RESILIENCE OF DYSBIOSIS

As stated previously, very few studies in humans have examined the stability of the dysbiotic microbiota in periodontal disease. However, studies involving animal models of disease suggest that it is not just the healthy oral microbiome that has an inherent stability. Once altered, a newly formed dysbiotic population also exhibits a high degree of stability. Studies in specific pathogen-free mice using an oral gavage model of *P. gingivalis* have demonstrated longitudinal stability (Figure 1) as well as the transferability of this dysbiotic, diseased microbial population, both between cohabiting mice in the form of a horizontal transfer, and also from parent to offspring in the form of a vertical transfer (Figure 2).[59] The transmissible dysbiotic community

is not just stable at the population level but also results in the same manifestation of destructive periodontal disease as the source, as observed in the mice in the form of alveolar bone loss. Similar examples of the transferability of a diseased microbiota and the disease phenotype have also been observed under other conditions. For example, the obese phenotype was replicated in germ-free mice by the transfer of an "obese microbiota", which was found to be more efficient at energy retrieval from a high-fat diet compared with the recipients of "lean microbiota".[60]

One implication of these findings concerns the frequently debated argument of "correlation vs causation" regarding the role of the dysbiotic microbiome in disease. Is the change in the microbiota in periodontal disease simply a reflection of the changed environment or does the microbiology have a direct effect on disease? In these mouse oral studies, the simultaneous transfer of the diseased state along with the dysbiotic microbiome into healthy animals strongly implicates the shift in the microbial population as a causative agent of periodontal disease in this model, thus suggesting the role of the entire dysbiotic microbiota as a "pathogenic entity".
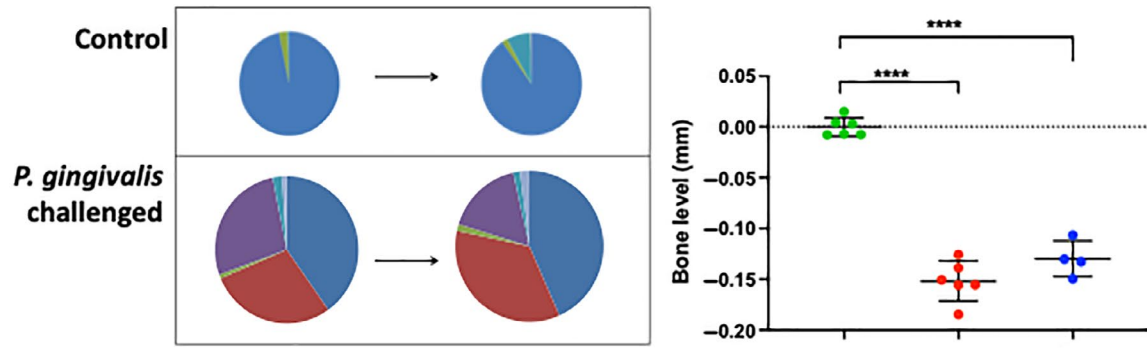
The resilience of the altered diseased microbiome, discussed in more detail by Wade,[16] may provide some explanation of the occasional refractory nature of the disease in humans. In the mouse dysbiosis study, administration of antibiotics only caused a brief perturbation with a temporary, but significant drop in microbial load,
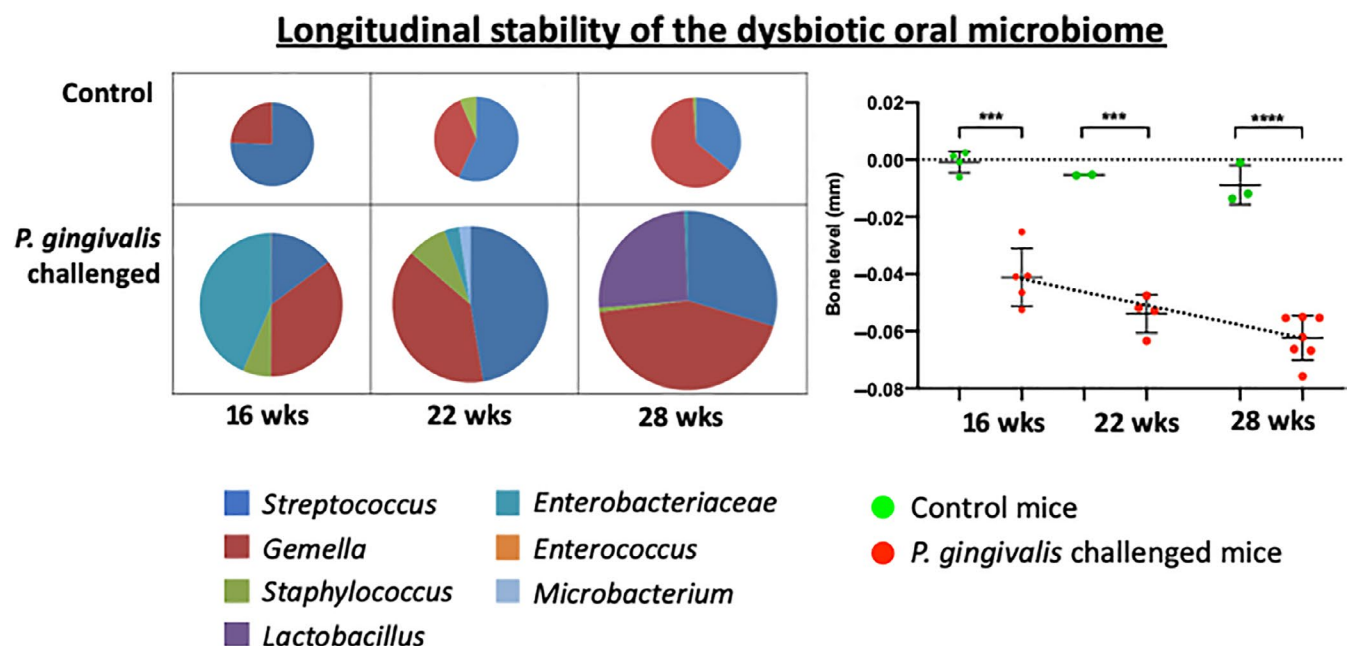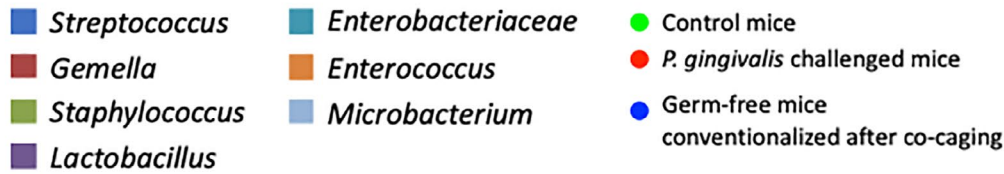

FIGURE 1 Longitudinal stability of the dysbiotic oral microbiome. *Porphyromonas gingivalis*-mediated dysbiosis is stable over time and associated with long-term destructive disease in the murine model. Bacterial composition of the oral microbiome (left), was determined by culture of control and *P. gingivalis*-treated mice at 16, 22, and 28 weeks. The sizes of the pie-charts are indicative of the variations in the total oral bacterial counts in the different groups. The graphs have been plotted using the observed number of colony-forming units of each microorganism in each group. Alveolar bone levels (right) were determined at 16, 22, and 28 weeks in control and *P. gingivalis*-treated mice. Bone loss was expressed as negative values relative to the baseline. Each point represents the mean bone level for an individual mouse with horizontal lines representing the mean bone levels per group ± standard deviation. The dotted line represents the linear rate of bone loss in *P. gingivalis*-treated mice over time (**$P < .05$; ***$P < .005$; ****$P < .0005$). (Adapted from Payne et al [59])
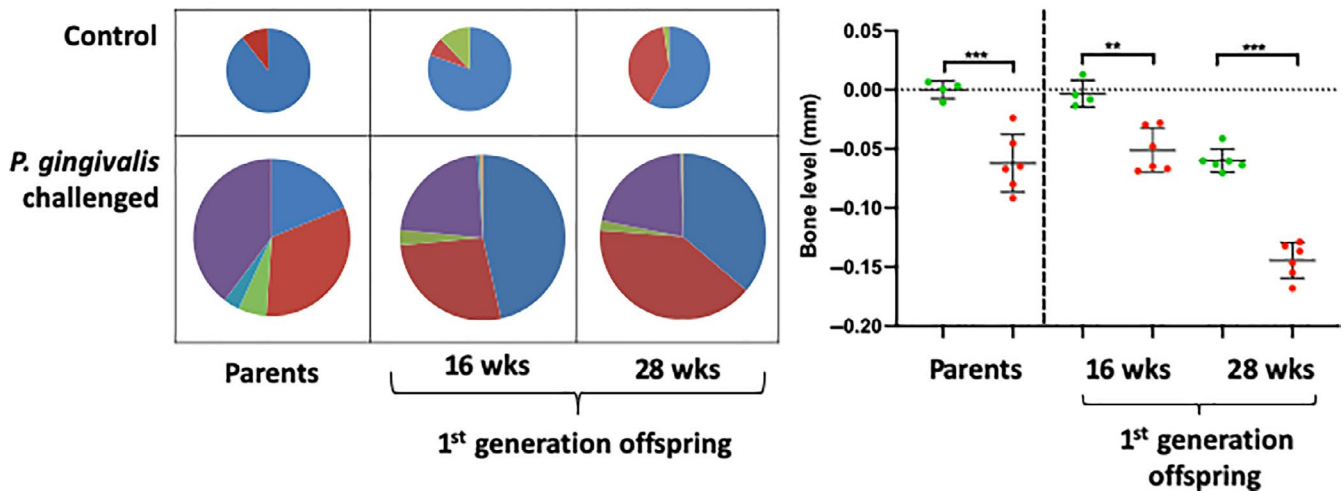
Horizontal Transfer of the dysbiotic oral microbiome

Conventionalization of germ-free mice by co-caging & transfer of the oral microbiome

Legend:
- ■ *Streptococcus*
- ■ *Gemella*
- ■ *Staphylococcus*
- ■ *Lactobacillus*
- ■ *Enterobacteriaceae*
- ■ *Enterococcus*
- ■ *Microbacterium*
- ● Control mice
- ● *P. gingivalis* challenged mice
- ● Germ-free mice conventionalized after co-caging

Vertical Transfer of the dysbiotic oral microbiome

FIGURE 2 Resilience of the dysbiotic oral microbiome. (Upper panel) *Porphyromonas gingivalis*-mediated dysbiotic microbiomes stably transfer horizontally into healthy germ-free mice and lead to periodontal disease in the recipients: bacterial composition of the oral microbiome (left) was determined by culture of control and *P. gingivalis*-treated C3H/Orl mice and conventionalized germ-free mice of identical genotype co-caged with the respective specific pathogen-free (SPF) mice for 14 days. Alveolar bone levels (right) in control (SPF) mice, *P. gingivalis* challenged and conventionalized germ-free mice were determined after 16 weeks of co-caging. (Lower panel) *P. gingivalis*-mediated dysbiotic microbiomes and disease stably transfer vertically between generations: bacterial composition of the oral microbiome (left) was determined by culture of control and *P. gingivalis*-treated parents at 16 weeks and litters of controls and *P. gingivalis*-treated parents at 16 and 28 weeks. Alveolar bone levels (right) were determined at 16 weeks in control and *P. gingivalis*-treated parents and their respective litters at 16 and 28 weeks. The sizes of the pie-charts are indicative of the variations in the total oral bacterial counts in the different groups. The graphs have been plotted using the observed number of colony-forming units of each microorganism in each group. Bone loss was expressed as negative values relative to the baseline. Each point represents the mean bone level for an individual mouse with horizontal lines representing the mean bone levels per group ± standard deviation. (**P <.05; ***P < .005; ****P < .0005) (Adapted from Payne et al [59])

followed by the reacquisition of the original dysbiotic population. We recognize that the stability of a microbial community is not simply maintained by inertia, but by the action of restoring forces within a dynamic system.[61] In the case of the oral microbiome, these may include a complex set of metabolic and functional
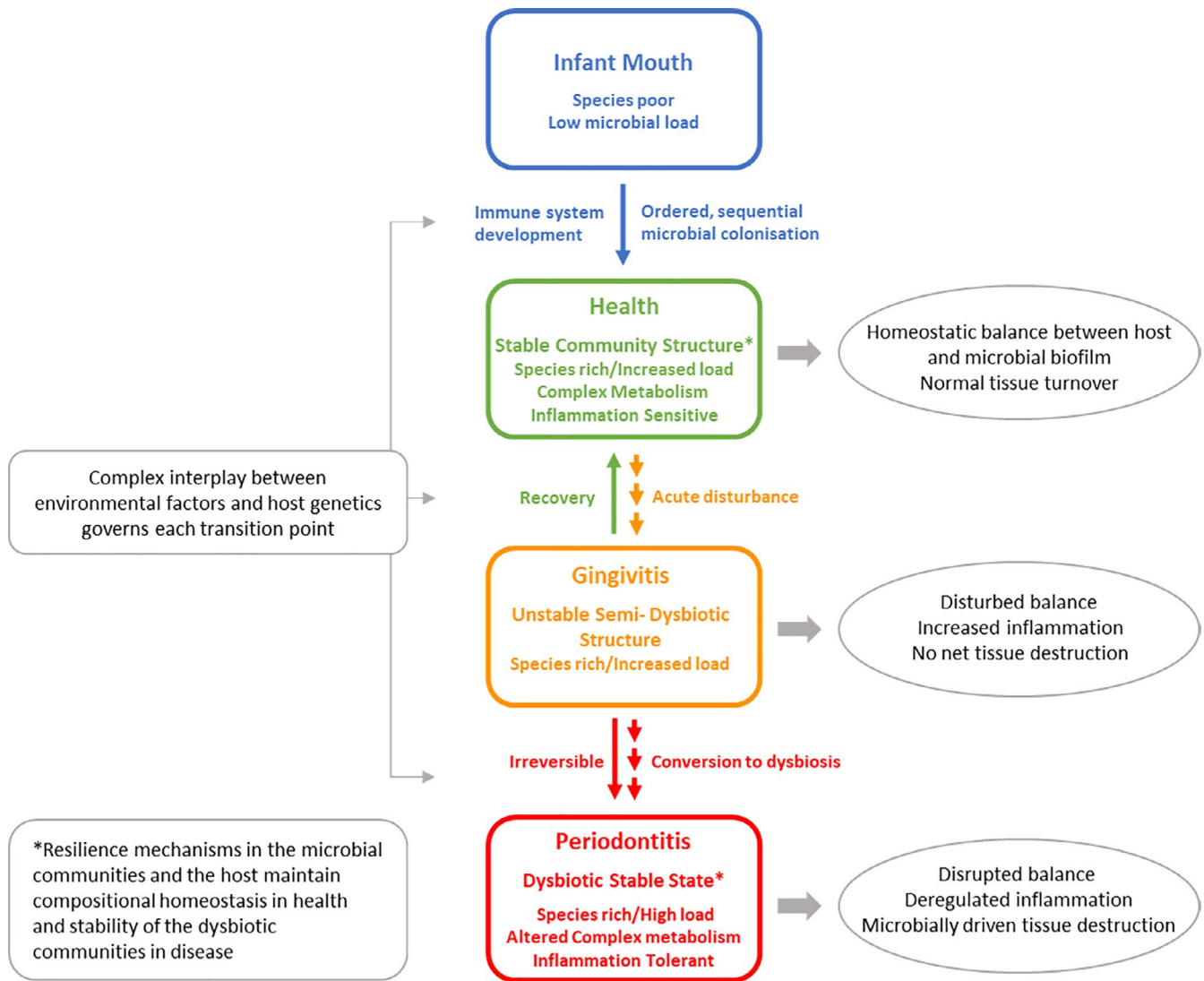
FIGURE 3 Schematic representation of the compositional changes of the microbial communities in the mouth from infancy to adulthood during health and disease. (Adapted from Lozupone et al [42])

interrelationships that develop within dental biofilms and also between biofilms and the host.[62]

8 | SUMMARY

The considerations raised in this work are summarized schematically in Figure 3. After birth, the infant mouth is steadily colonized in an ordered manner over time. The resultant composition of the oral microbiome is heavily influenced by maternal transfer and by the genetic landscape of the infant. Once established, microbial communities exist in a stable state with high diversity, a complex web of nutritional interdependencies, and in homeostatic balance with the adjacent host tissues. Acute disturbance to this relationship occurs if the total microbial burden adjacent to the host tissues increases significantly and/or if there are changes in the efficiency of the surveillance and/or protective mechanisms of the immune and inflammatory systems of the host. The outcome is a change of the healthy community structure to an altered semi-dysbiotic state and development of gingivitis. This population structure is, however, unstable and the equilibrium can be restored to the healthy state by reversal of the disturbance factors. The nature of the change and the ease of reversion is dependent upon the overall composition of the personalized microbiome of the individual and their genetics. In instances where reversion to health is not achieved, and with further continued stresses on the system, the microbiome may convert to a fully dysbiotic system with an elevated species diversity and an inherent tolerance to the inflammatory and immune systems of the host. Once again the conversion is governed by both the microbiome composition and genetic factors of the host. This new system also displays resilience to change, which maintains the stability of the dysbiotic state and leads to microbially driven tissue destruction by the resultant deregulated inflammatory response. Understanding the nature of the parameters that underpin the resilience of healthy and dysbiotic microbial populations may be important to the development of approaches to prevent the progression of disease and to restore health in diseased individuals.

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