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Resilience of the oral microbiota in health - mechanisms that prevent dysbiosis

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Abstract

Dental diseases are now viewed as a consequence of a deleterious shift in the balance of the normally stable resident oral microbiome. It is known that frequent carbohydrate consumption or reduced saliva flow can lead to caries, and excessive plaque accumulation increases the risk of periodontal diseases. However, when these disease drivers are present, while some individuals appear to be susceptible, others are more tolerant or resilient to suffering from undesirable changes in their oral microbiome. Health-maintaining mechanisms that limit the effect of disease drivers include the complex set of metabolic and functional inter-relationships that develop within dental biofilms and between biofilms and the host. In contrast, 'positive feedback loops' can develop within these microbial communities that disrupt resilience and provoke a large and abrupt change in function and structure of the ecosystem (a microbial 'regime shift'), that promotes dysbiosis and oral disease. For instance, acidification due to carbohydrate fermentation or inflammation in response to accumulated plaque select for a cariogenic or periopathogenic microbiota, respectively, in a chain of self-reinforcing events. Conversely, in tolerant individuals, health-maintaining mechanisms, including negative feedback to the drivers, can maintain resilience and promote resistance to and recovery from disease drivers. Recently studied health-maintaining mechanisms include ammonium production, limiting a drop in pH that can lead to caries, and denitrification, which could inhibit several stages of disease-associated positive feedback loops. OMICS studies comparing the microbiome of, and its interaction with, susceptible and tolerant hosts can detect markers of resilience. The neutralization or inhibition of these 'disease drivers', together with the identification and promotion of health-promoting species and functions, for example by pre- and probiotics, could enhance microbiome resilience and lead to new strategies to prevent disease.

1. Introduction

In healthy individuals with the right dietary and oral hygiene habits, the oral microbiota lives in symbiosis with the host, preventing the colonization of foreign pathogens and contributing to host physiology (Hezel 2015). In our article, we define symbiosis as a microbial composition, activity and ecology that keeps a balanced relationship with the host, resulting in a healthy state. Nevertheless, perturbations in the microbiome caused by certain stress factors, such as carbohydrate consumption or plaque accumulation, can lead to the development of oral diseases, e.g. caries or periodontal diseases, respectively (Marsh 1994; 2003). In these oral diseases a shift of species and functions associated with the diseases, i.e. dysbiosis, is observed (Belda-Ferre 2012; Griffen 2012; Jorth 2014; Wang 2013).

Importantly, people do not develop similar levels of oral diseases under identical circumstances. In the Vipeholm study, mentally-challenged subjects received high amounts of fermentable carbohydrate snacks over 5 years (Krasse 2001). Most individuals developed caries, but 20-30% did not, "although they had a frequent intake of between meal sweets for long periods" (Krasse 2001). Likewise, in a Sri Lankan population with no oral hygiene habits nor dental care, while 89% of the population had moderate to fast progression of periodontal breakdown, the other 11% had no periodontal disease beyond gingivitis (i.e., an inflamed gingiva) (Löe 1986). Low and high responders are also observed in experimental gingivitis studies (Trombelli 2004).

Several disease drivers have been identified which can potentially induce disease. When populations are exposed to certain level of disease drivers, a unique opportunity is provided to retrospectively discriminate between susceptible and tolerant individuals. In the last century, the focus of research has mainly been on oral diseases in susceptible individuals. Accordingly, mechanisms that limit disease development in tolerant individuals when disease drivers are present remain relatively uninvestigated. However, a clearer understanding of these health-maintaining mechanisms might allow their active stimulation in susceptible individuals and thus open up new avenues for disease prevention and treatment.

The aim of this review is to describe the processes behind dysbiosis and discuss the mechanisms that could prevent this shift when disease drivers are present.

2. The oral microbiota in health: diverse and stable

Bacteria are the main inhabitants of the oral cavity. In healthy adults, the majority of species belong to the bacterial phyla Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes and Fusobacteria (HMP-Consortium 2012). In addition, archaea, protozoa, viruses, and fungi are present. Of the 700+ species of oral bacteria identified (Human Oral Microbiome Database 2016), a healthy individual is colonized by between 100-200+ bacterial species (Dewhirst 2010; Griffen 2012). Inter-individual variation in microbiota composition results from differences in the environment, genetics, age and lifestyle of the host (Kilian 2016) (figure 1A and B). Importantly, certain microbiota functions can be fulfilled by groups of different microbes (functional redundancy) (Jorth 2014; Lozupone 2012). For instance, some core functions of the oral microbiota are conserved between healthy individuals even when there are large differences on a taxonomic level (HMP-Consortium 2012).

In the oral cavity, indigenous species can reach all surfaces via the flow of saliva, but the corresponding environments determine which species are able to adhere and colonize successfully. As a result, the biofilms that form at different habitats of the oral cavity (e.g., teeth, gingival crevice, tongue and buccal mucosa) have a distinct microbial composition (HMP-Consortium 2012) (figure 1C). The composition changes as the biofilm matures (e.g., the hours after oral hygiene) (Benítez-Páez 2014), which is most relevant for the tooth surfaces that do not shed (figure 1D). Biofilms on the teeth, called dental plaque, are associated with the most common oral diseases – caries and periodontal diseases. The oral epithelia shed several times a day, which restricts biofilm accumulation.

Compared to the other microbial communities of the body, the oral microbiota in health is often considered most stable over time (Zhou 2013). Several studies demonstrated that a stable core microbiota is maintained in the oral cavity of healthy individuals over periods up to 7 years (David 2014; Rasiah 2005). When saliva of a single adult was sampled daily for over a year, only 195 OTUs in saliva – a fraction of the total found in one year – were stable (i.e., present in 95% of samples) but these comprised 99.7% of total bacteria detected (David 2014). The other 0.3% of bacteria detected consisted of a high variety of low abundance species, which seem to appear and disappear over time. In addition, oral microbial communities have been found to recover after the use of antibiotics in adult individuals, whereas the gut microbiota could suffer longer term alterations in composition (Zaura 2015). The continuous presence and the composition of saliva appears to have crucial roles in maintaining the stability of the oral microbiota.

2.1 Role of saliva in ecological stability

A healthy adult produces approximately 1L of saliva per day (Hezel 2015). The importance of saliva is reflected by the fact that individuals with salivary deficiencies are prone to oral diseases (Samnieng 2012). Saliva contains a broad range of antimicrobial components (e.g., lysozyme, lactoferrin, histatins, defensins, and secretory IgA) (Wilson 2005), provides pH buffering, and is continuously refreshed, while swallowing discards food remains, detached cells and microbial waste products.

Salivary glycoproteins regulate attachment of different types of microbes to oral surfaces by either stimulating or blocking their adherence (Everest-Dass 2012; Gibbons 1990). Additionally, they provide a consistent source of nutrients for the oral microbiota (Wilson 2005). Mucins, which are large and complex glycoproteins, make up around 25% of the total protein content and are broken down by mixed consortia of microorganisms in a concerted manner which promotes the characteristic diversity and stability of the oral microbiota (Bradshaw 1994).

The salivary glands also concentrate plasma nitrate into the saliva resulting in high salivary nitrate concentrations (100–500 μM during fasting, which is ~10 times higher than in plasma, and 5-8 mM after a nitrate-containing meal) (Hezel 2015). Nitrate is an electron acceptor used in the respiration of nitrate-reducing bacteria, leading to the production of nitric oxide, a molecule with antimicrobial properties (Schreiber 2010).

Perturbations by food intake are generally relatively short compared to fasting periods in which the saliva is refreshed creating a constant environment. The salivary components thereby confer long term stability to the composition and activity of the oral microbiota.

3. Resilience to disease drivers

In ecology, resilience is the capacity of an ecosystem to deal with perturbations without shifting to an alternative state in which core species and key functions are lost (Folke 2004; Holling 1973). Resilience can be divided into ‘resistance’ and ‘recovery’: the ‘resistance’ determines the magnitude of perturbation that an ecosystem can handle before its state changes, while the ‘recovery’ is the rate at which it returns to its original state. These ecological concepts can be applied to the oral ecosystem to help understand how a stable microbial community is maintained over time.

Recently, the term resilience was applied in the oral cavity as the capacity to recover from perturbations caused by gingivitis in smokers vs non-smokers (Joshi 2014). After the recovery period, smokers had higher levels of disease-associated species and, accordingly, a higher pro-inflammatory response. This indicates that smokers had a decreased resilience (recovery) from experimental gingivitis. In another recent study, a metatranscriptomic approach was used to observe the active oral microbiota before and after a carbohydrate meal (Benítez-Páez 2014). Even though the group size was small (5 subjects), the microbiota of each individual changed in its own way. Interestingly, virtually no changes were observed in one subject who had never suffered from dental caries, indicating a strong resilience (resistance). Resilience could thus discriminate between susceptible and tolerant individuals when disease drivers are present, and can be described as a capability to cope with stress factors (resistance) and recover from perturbations that are potentially triggered (recovery, figure 2). Perturbations can lead to oral diseases when disease drivers are strong or persistent enough.

4. 'Regime shifts' towards oral disease

The disruption of resilience during the development of oral disease can be compared to the ecological phenomenon of 'regime shifts' (i.e., large, abrupt, persistent changes in function and structure) (Folke 2004). In ecological systems, 'regime shifts' take place when perturbations pass a certain threshold. A classic example is given by shallow clear water lakes (reviewed by (Folke 2004)). When the lake receives a high phosphorus input (perturbation driver), e.g. from agricultural waste, phytoplankton can overgrow. The shading by phytoplankton makes the environment less suitable for higher aquatic plant beds. Additionally, bottom-feeding fish, which feed on phytoplankton, increase in number and damage the plant beds. As the plant beds decrease, phosphorus from the sediment becomes available, which further stimulates phytoplankton overgrowth. Altogether, these events reinforce themselves in a positive feedback loop driving change from a clear water regime to a turbid one.

To draw a parallel to the oral cavity, more than two decades ago, a chain of self-reinforcing processes was proposed that can lead to disruption of the oral microbiota and increase the risk of disease (Marsh 1994; 2003). For example, the development of caries and periodontitis can be represented as positive feedback loops (Rosier 2014) (Figure 3 and 4).

While positive feedback loops triggered by disease drivers can cause microbial regime shifts towards oral disease (i.e., dysbiosis) in susceptible individuals, health-maintaining mechanisms prevent this and could enhance resilience in tolerant individuals.

5. Carbohydrate consumption: regime shift to caries vs resilience

5.1 Regime shift: caries development due to carbohydrate consumption and acidification

In caries, the disease drivers are mostly fermentable carbohydrates, when consumed in high amounts and frequencies (Pitts 2017). The microbiota ferments these carbohydrates into organic acids. If the acid surpasses the buffer capacity of dental plaque and saliva, then the local pH will fall. Acid-producing (acidogenic) species that are adapted to the acidic conditions will gain a selective advantage (Marsh 2003; Rosier 2014). Over time, the microbiota shifts towards a community that is more efficient at fermenting carbohydrates (i.e., saccharolytic) and more adapted to growth and metabolism at a low pH (i.e., aciduric) – a shift towards a cariogenic microbiota (Marsh 2003). As the pH reaches a critical level (below around pH 5.5), enamel demineralization exceeds remineralization. If the acidic conditions persist, or are repeated frequently without sufficient time for remineralization, then a caries lesion can develop (Pitts 2017). Frequent carbohydrate intake can therefore lead to a positive feedback loop causing a shift to a saccharolytic, acidogenic and aciduric microbiota that can cause irreversible dental caries over time.

5.2 Resilience to carbohydrate consumption and acidification

Saliva plays an important role in preventing a regime shift to caries. Salivary characteristics, such as flow rate and buffer capacity, differ among individuals, which can lead to differences in resilience towards acidification (Cunha-Cruz 2013).

Compared to the prevention of plaque accumulation associated with periodontal diseases, the role of the immune system in caries is often neglected (Rosier 2014). However, several studies showed differences in host genes involved in immune response or salivary immune components between caries active and caries free individuals (Werneck 2010). For instance, a classical observation is that low caries susceptibility is associated with a high

amount of total antigen-specific S-IgA against Streptococcus mutans (Lehtonen 1984). In accordance with this, the saliva of caries-free individuals was recently shown to have higher concentrations of S-IgA and proportions of S-Ig-coated bacteria than samples from caries-active patients (Mira 2017; Simón-Soro 2015). This indicates that the immune system of caries-free individuals clears bacteria, including ones involved in caries development, more efficiently.

The microbiota itself provides resilience to acidification in several ways. Certain species (e.g., Veillonella spp. and Candida spp.) metabolize lactate into weaker acids (i.e., with a lower pKa), increasing the pH (Mikx 1975; Willems 2016). Candida albicans, for instance, is an aciduric species associated with caries, but its lactate metabolism can limit the total pH drop (Willems 2016). In addition, production of the alkali, ammonia, by oral bacteria, such as representatives of Streptococcus and Actinomyces, inhibits acidification (Liu 2012; López-López 2017). In dental plaque, the two primary routes for ammonia generation are the metabolism of arginine via the arginine deiminase system and the hydrolysis of urea by urease enzymes (Liu 2012). Salivary nitrate and the capacity of the microbiota to reduce nitrate to nitrite also appear to have an anti-caries effect, possibly due the production of ammonia and antimicrobial nitric oxide or the consumption of lactate by nitrate reducing species (Doel 2004; Li 2007). Additionally, at pH 5 or lower, acidic decomposition of nitrite to nitric oxide takes place (Schreiber 2010), which could provide negative feedback to acidification (figure 3, blue box "nitrite → nitric oxide").

Finally, antimicrobial peptides (like bacteriocins) were significantly over-represented in the metagenomes of caries free individuals, compared to caries-experienced subjects (Belda-Ferre 2012). Furthermore, bacteriocins produced by Streptococcus dentisani, isolated from caries-free individuals, inhibited the growth of several cariogenic species (López-López 2017). Host and microbiota functions that prevent a fall in pH, promote pH recovery or inhibit cariogenic species can thus contribute to resilience against caries. Additionally, fluoride, which is held responsible as the main factor for a worldwide decrease in caries, increases resistance to demineralization and recovery by remineralization (see, for example, (Pitts 2017)).

6. Plaque accumulation: regime shift to periodontal diseases vs resilience

6.1 Regime shift: periodontal disease development due to plaque accumulation and inflammation

In periodontal diseases, the first disease driver is accumulation of dental plaque as a result of poor oral hygiene (Marsh 2003) (figure 4). Firstly, the conditions within the plaque biofilm slowly become more anaerobic over time as the biofilm becomes thicker, which increases the levels of anaerobic species. Additionally, to clear the accumulated microbes, the host responds with gingival inflammation (Joshi 2014). This includes an increased flow of gingival crevicular fluid (GCF, i.e., a serum-like exudate), which contains components of host defenses (e.g., immune cells and antibodies), but also many (glyco)proteins. Unintentionally, the GCF proteins can act as a novel source of nutrients for proteolytic species that increase in number during periodontal diseases (Marsh 1994; 2003). As a result of the degradation of proteins and the production of ammonia, the environment becomes more alkaline (Kobayashi 1998). Another component present in GCF is iron, which is essential for bacterial growth, and triggers potential pathogenic mechanisms in oral bacteria associated with periodontal disease (Hajishengallis 2012).

In the new environment, inflammation-tolerant, anaerobic, proteolytic, alkaliphilic species (i.e., a periopathogenic microbiota) have a selective advantage and increase in number (Marsh 1994; 2003; Rosier 2014). The host responds with more inflammation and a positive feedback loop is formed. This may be further stimulated by bacterial manipulation of the immune system (Hajishengallis 2012). For instance, Porphyromonas gingivalis can instigate a crosstalk between the C5a receptor (C5aR) and Toll-like receptor 2 (TLR2) that increases an inflammatory response, but impairs bacterial killing, which (in mice) facilitates survival of the entire microbial community (Maekawa 2014). In respect to this, periodontitis-associated communities seem not only have evolved to endure the inflammation, but also take advantage of the new environment with more nutrients in the form of tissue-breakdown products (e.g., peptides and heme-containing compounds) (Hajishengallis 2014). These so called 'inflammophilic' biofilms increase with inflammation, while anti-inflammatory treatments diminish the bacterial load in animal models, and could contribute to the positive feedback loop leading to periodontal diseases.

In conclusion, plaque accumulation and the resultant inflammatory host response, can lead to a positive feedback loop that causes gingivitis and in some cases, if the host is susceptible, periodontitis.

6.2 Resilience to plaque accumulation and destructive inflammation

6.2.1 Resilience to plaque accumulation

In ecological systems, species can keep themselves in balanced numbers by negative feedback mechanisms, which can allow long-term stability. A simple example is the cycle of a predator and its prey that allows both species to survive over time: if owls increase, mice will decrease, but if mice keep decreasing, owls will decrease, allowing mice to increase again, and so forth (Holling 1973). Likewise, negative feedback can be provided by the shortage of nutrition, water or shelter, and the increase of a pathogen. Negative feedback mechanisms that can decrease the abundance of a member of the human microbiota after it exceeds a certain threshold are: 1) the lack of nutrition or essential growth factors in its habitat, 2) the accumulation of a specific toxic product of metabolism, and 3) the increase of a bacteriophage specific to that species (Lozupone 2012) (figure 5). Regarding the latter, the vast majority of oral viruses are bacteriophages that could provide negative feedback (Pride 2012). Wang et al. (2016) showed that the levels of certain phages were negatively correlated with periodontal-disease associated bacteria (Wang 2016), suggesting a role in shaping the microbial community.

The complex roles of the immune system in preventing microbial accumulation could be enhanced in tolerant individuals, but this falls outside the scope of this review. In short, different immune components actively kill, inhibit and agglutinate microbes (lyzosyme, defensins, histatins, S-IgA), deprive them of iron (lactoferrin), prevent their adhesion (S-IgA, IgG, IgM), or act as opsonins (complement, IgG, IgM) that increase phagocytosis by immune cells (Wilson 2005).

Species of the microbiota also produce antimicrobial compounds (e.g., bacteriocins and toxic compounds such as hydrogen peroxide and nitric oxide) that suppress the growth of other species (Kreth 2005), providing resistance to plaque accumulation, and genes involved in hydrogen peroxide metabolism have been associated with periodontal health (Wang 2013). Nitric oxide is also a signaling molecule that triggers dispersal of various types of bacterial cells from biofilms (Barraud 2009; Schlag 2007). Salivary nitrate concentrations could generate nitric oxide concentrations that decrease biofilm formation by susceptible species (Schlag 2007), which could provide resistance to, and recovery from, plaque accumulation.

Altogether, resilience to plaque accumulation can be provided by negative feedback mechanisms that are also present in macro-ecosystems. Additionally, the host has several

strategies to limit microbial accumulation, and the microbiota itself produces antimicrobials and biofilm dispersal signals.

6.2.2 Resilience to destructive inflammation

Inflammation can result from plaque accumulation when certain receptors are triggered in a complex interaction of the immune system with the microbiota (Darveau 2010). Differences in immune system phenotypes are likely to be detected in susceptible hosts compared to tolerant hosts (Nascimento 2017).

The host regulates inflammation in several ways, e.g. by adjusting cytokine expression levels and complex cytokine-receptor interactions and signaling, depending on the types and amounts of microbes that are detected. Consequently, some species correlate with anti-inflammatory mediators (e.g., Streptococcus, Neisseria and Veillonella), while others correlate with pro-inflammatory mediators (e.g., Selenomonas, Parvimonas and Campylobacter) (Joshi 2014).

Apart from being the trigger of inflammation, the microbiota can also actively suppress immune activation. This could be a mechanism enabling microbes to evade the immune system and accumulate to levels that could induce periodontal diseases (Darveau 2010). An example is Porphyromonas gingivalis which expresses a type of lipopolysaccharide (LPS) that decreases Toll-like receptor 4 response and secretes a serine phosphatase that inhibits the secretion of IL-8 (i.e., a pro-inflammatory cytokine), which is thought to impair inflammation (Darveau 2010). Alternatively, in health, the suppression of the immune response by indigenous species may contribute to homeostasis. In light of this, P. gingivalis is also present in health, albeit in lower numbers. Additionally, health-associated commensal species can have comparable mechanisms; for example, Streptococcus salivarius inhibits IL-8 secretion (Cosseau 2008). The microbiota can thus correlate with anti-inflammatory mediators and also actively prevent inflammation by inhibiting pro-inflammatory cytokines. This could contribute to homeostasis and resilience in health by preventing unnecessary and destructive inflammation.

7. Concluding remarks: mechanisms that prevent dysbiosis

In summary, certain health-maintaining mechanisms (green boxes in figure 3 and 4) may prevent a shift to dysbiosis when disease drivers are present. Some of these mechanisms can be triggered by the disease drivers (e.g., acidification that could lead to more nitric oxide production) and act as negative feedback (figure 5). Other mechanisms are continuously present (e.g., the buffering effect of saliva) or take place in episodes (e.g., oral hygiene and fluoride exposure). Altogether, complex interactions between disease drivers, health-maintaining mechanisms and feedback loops will determine the relationship between the microbiota and the host (Figure 6).

Our hypothesis is that certain health-maintaining mechanisms that prevent disease-associated positive feedback loops are enhanced in tolerant individuals. These mechanisms could be microbial, which was the emphasis of this review, but also on the host level (e.g., genetic and epigenetic differences) and human genome association studies could shed light on this issue (Nascimento 2017). By identifying markers that are involved in resilience, susceptible individuals that lack them could be identified before disease develops. Additionally, health-maintaining mechanisms could be actively enhanced in novel strategies of disease treatment (enhancing health rather than reducing disease).

8. Prospects: preventive dentistry

8.1 Identification of markers of resilience

Several decades ago, the ecologist C.S. Holling mentioned that when ecosystems are studied, there is a "tendency to emphasize the quantitative (i.e., a single time point) rather than the qualitative (i.e., fluctuations over time)", while only the latter informs about resilience (Holling 1973). For instance, the numbers of some species fluctuate enormously and others seem to disappear and reappear over time – a single time point does not provide information about this. The same holds for studies involving the oral microbiota. Until now, most OMICS studies have focused on comparison between healthy and diseased individuals from whom samples were taken at a single time point. This does not provide information about their resistance to disease drivers and recovery rate after potential perturbations. For instance, to measure the microbiota's resilience against a sugar pulse, it is necessary to observe its activity before and several time points after the sugar pulse. Only a few recent (small scale) OMICS studies have assessed the response of the oral microbiota to disease drivers, detecting individuals with different susceptibilities (Benítez-Páez 2014; David 2014; Joshi 2014). More

and larger qualitative OMICS studies, which measure fluctuation over time, will provide new insights into microbial and host markers that lead to resilience (Nascimento 2017). Markers of resilience may include health-associated bacterial species or functions, genetic polymorphisms associated to protection from disease (Rosier 2014), certain levels of salivary compounds that prevent disease (Mira 2017) or specific tests directed towards detecting resilience capacity (e.g., pH buffering capacity or Ig-coating levels).

8.2 Enhancement of resilience with pre- and probiotics

8.2.1 Prebiotics

Just like a shift of species and functions is observed after a period of stress, long periods of rest may allow microbiota recovery, which could be enhanced by the frequent administration of a prebiotic–in this review referring to compounds that stimulate beneficial microorganisms or microbial mechanisms. In a recent in vitro study, the continuous administration of arginine enhanced oral microcosm (i.e., in vitro oral microbiota) resilience toward acidification and suppressed outgrowth of the opportunistic pathogen Candida (Koopman 2014). Similarly, 1.5% and 8% arginine toothpaste enhanced ammonia production and decreased lactate production in clinical trials, (Koopman 2016; Wolff 2013), both of which reduce acidification. Furthermore, the microbiota changed towards having a more health-associated composition from a caries point of view (Koopman 2016).

Another potential prebiotic is nitrate, but current in vivo evidence in humans is limited. In a recent clinical trial focusing on the cardiovascular benefits of dietary nitrate, oral bacterial profiles were measured (Velmurugan 2016). After six weeks of daily nitrate-rich lettuce juice consumption, 78 bacterial taxa were affected and two nitrate reducing species, Rothia mucilaginosa and Neisseria flavescens, increased notably. Rothia spp. and Neisseria spp. have both been associated with dental and periodontal health (Belda-Ferre 2012; Griffen 2012; Joshi 2014). Furthermore, two weeks of lettuce juice consumption in another recent clinical study reduced gingival inflammation (Jockel-Schneider 2016).

In conclusion, prebiotics can drive beneficial changes in the oral microbiota and could increase resistance to dysbiosis and recovery of health.

8.2.2 Probiotics

The addition of probiotics – microorganisms that confer a health benefit on the host – with beneficial functions (e.g., preventing acidification, plaque accumulation or harmful inflammation) may further contribute to resilience. A recent systematic review of 50 studies (3247 participants) concluded that the current evidence is insufficient for recommending probiotics for managing dental caries, but supportive towards managing gingivitis or periodontitis (Gruner 2016). The identification of new probiotic species which inhabit the oral cavity – as opposed to dairy products or gut-associated bacteria (López-López 2017) – and the development of personal rather than general treatments could improve these results in the future. In respect to this, Kort proposed the triple-A model (acquirement, alteration and administration of the microbiota) for the vaginal microbiota, in which strong selective media enable a person's own beneficial bacteria to be grown for subsequent re-application (Kort 2014). A comparable idea could work for the oral cavity to obtain indigenous probiotic species or communities with certain beneficial functions (e.g., arginolytic pathways to produce ammonia or denitrification pathways to produce nitric oxide).

The application of ecological principles can help us understand how the tight interplay of the oral microbiota and the host dictates health or disease. We hope that the philosophy and ecological ideas developed in the current review provide insights for research directed towards a shift from traditional treatment to preventive and personalized dentistry.

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Figure 1: Factors that determine the composition of the oral microbiota. The main factors that determine the composition of the oral microbiota are shown in the left column. Examples of different variables within these factors are listed in the right column and can vary within the same individual (i.e., over time) and between different individuals. **A) Age (time).** The diversity of the microbiota changes as the host ages (e.g., due to tooth eruption and age-related changes in hormones and the immune system). Additionally, horizontal transfer of microorganisms and micro-evolution of the oral microbiota takes place over time. **B) Host & Environment.** Differences among hosts, such as genetics and the (integrity of the) immune system affect the microbiota composition. Additionally, the environment in which the host is present further affects the composition by e.g. influencing host habits and diet. **C) Habitat.** The habitats of the oral cavity differ in environmental conditions such as oxygen levels, pH, and nutrition. Importantly, the oral mucosal surfaces undergo desquamation (e.g., the buccal mucosa sheds frequently preventing biofilm accumulation), while the dental surfaces do not. Three examples of habitats are given of the many different habitats inside the oral cavity. Changes due to biofilm maturation are most relevant to the teeth and gingival crevice in light of the most common oral diseases (i.e., caries and periodontal diseases), indicated by the thicker arrow in the middle going to row D. **D) Biofilm Maturation.** Physical and chemical perturbations from food, oral hygiene and, in the case of the oral mucosa, shedding remove biofilms from surfaces. Young biofilms differ from mature ones due to changes in density that affect the internal environment (e.g., the interior of the biofilm becomes more anaerobic as it becomes thicker), microbial interactions (e.g., quorum sensing) and the immune response that is triggered by the host. [1]Teeth are only present after certain age and extractions and denture wearing cause variability during the lifespan of a person. [2]GCF: Gingival crevicular fluid. [3]The two way arrow between saliva and maturing biofilms means that the saliva inoculates clean surfaces, while detached microbes from colonized surfaces enter saliva.

Figure 2: Disease drivers (i.e., stress factors that can potentially induce disease), perturbations and resilience. In this hypothetical graph, the y-axis indicates host-microbiome interactions, which can be divided in three zones (symbiosis, disruption or dysbiosis). Symbiosis keeps healthy interactions between host and microbiota, whereas a disruption is a reversible situation in which microbiome species or functions are altered. Dysbiosis is a host-microbiome interaction that leads to adverse symptoms for the host (e.g., gingival inflammation). Time is shown on the x-axis. Here we give an example of how a tolerant individual could differ from a susceptible individual when disease drivers have the same magnitude (i.e., weak or medium). The thickness of the disease driver arrows (on top) represents its **magnitude** that is also determined by the duration and frequency of its presence. Disease drivers can trigger perturbations towards dysbiosis in some cases. The **resistance (RES)** and **recovery (REC)** determine the impact of the disease driver and the potentially triggered perturbation, respectively. When the resistance is strong (+), the disease driver does not cause a perturbation. When the resistance is weak (- or --), a perturbation is caused and the time it will be present depends on the recovery rate (- or +). Note that recovery can also be the result of active interference such as removing plaque by oral hygiene, which could be presented as an arrow in the opposite direction than the disease drivers.

Figure 3: Positive feedback loop leading to caries. In this positive feedback loop, carbohydrate consumption is the disease driver that can cause a regime shift towards a cariogenic microbiota. The health-maintaining mechanisms that could prevent various stages of the loop are listed in green boxes. Some of these mechanisms are likely to contribute to resilience and be enhanced in individuals that are more tolerant to carbohydrate consumption. Italicized text indicates hypothetical involvement.

Figure 4: Positive feedback loop leading to periodontal diseases. In this positive feedback loop, plaque accumulation as a result of poor oral hygiene is the disease driver that can cause a regime shift towards a periopathogenic microbiota. The grey arrow represents another chain of self-reinforcing events, in which an increase in sulcus size or the formation of a pocket allows for more plaque accumulation. The health-maintaining mechanisms that could prevent various stages of the loop are listed in green boxes. Some of these mechanisms are likely to contribute to resilience and be enhanced in individuals that are more tolerant to a lack of oral hygiene. Italicized text indicates hypothetical involvement.

Figure 5: Negative feedback loop preventing plaque accumulation. This negative feedback loop is based on ecological studies and could prevent the accumulation of members of the microbiota, for instance, on the tooth surface.

Figure 6: Example of how disease drivers (i.e., stress factors that can potentially induce disease), negative and positive feedback, and health-maintaining mechanisms may act over time. For the descriptions of symbiosis, disruption and dysbiosis, see figure 2. In this simplified graph of the complex oral ecosystem, where in reality many different symbiotic and dysbiotic states may exist, we show how disease drivers could act in a systemically healthy individual. If the driver is weak (weak disease driver arrow), no perturbation by positive feedback is triggered (i.e., resistance, also see figure 2). In the case of a medium disease driver (medium disease driver arrow), some positive feedback might destabilize the host-microbiota interaction leading to a short perturbation into disruption, but negative feedback and other health-maintaining mechanisms shortly counteract it (i.e., recovery, also see figure 2) and a symbiotic homeostasis is restored. However, if the disease driver is strong or persistent enough (strong disease driver arrow), the positive feedback might temporary surpass negative feedback and other health-maintaining mechanisms as shown in this graph, leading to dysbiosis (e.g., gingival inflammation). Over time, the health-maintaining mechanisms (e.g., the immune response or oral hygiene) may allow recovery in which adverse symptoms disappear and disease progression stops. However, in contrast to this graph, in a more susceptible host or after frequent exposure to a disease driver, at some point dysbiosis can become stable (e.g., periodontitis is a chronic infection), and new, disease-associated (feedback) mechanisms could contribute to this stability.

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