



*Review*

## **Recent advances in dental biofilm: impacts of microbial interactions on the biofilm ecology and pathogenesis**

**Yung-Hua Li<sup>1,2,\*</sup>, Xingxing Huang<sup>1,3</sup>, and Xiao-Lin Tian<sup>1</sup>**

<sup>1</sup> Department of Applied Oral Sciences, Dalhousie University, Halifax, Canada

<sup>2</sup> Department of Microbiology and Immunology, Dalhousie University, Halifax, Canada

<sup>3</sup> Shenzhen Maternity and Child Healthcare Hospital, Shenzhen, China

\* **Correspondence:** Email: [yung-hua.li@dal.ca](mailto:yung-hua.li@dal.ca); Tel: +1-902-494-3063; Fax: +1-902-494-6621.

**Abstract:** The human oral cavity is a complex ecosystem harboring hundreds species of microbes that are largely living on the tooth surfaces as dental biofilms. Most microbes in dental biofilms promote oral health by stimulating the immune system or by preventing invasion of pathogens. Species diversity, high cell density and close proximity of cells are typical of life in dental biofilms, where microbes interact with each other and develop complex interactions that can be either competitive or cooperative. Competition between species is a well-recognized ecological force to drive microbial metabolism, species diversity and evolution. However, it was not until recently that microbial cooperative activities are also recognized to play important roles in microbial physiology and ecology. Importantly, these interactions profoundly affect the overall biomass, function, diversity and the pathogenesis in dental biofilms. It is now recognized that every human body contains a personalized oral microbiome that is essential to maintaining the oral health. Remarkably, the indigenous species in dental biofilms often maintain a relatively stable and harmless relationship with the host, despite regular exposure to environmental perturbations and the host defense factors. Such stability or homeostasis results from a dynamic balance of microbial-microbial and microbial-host interactions. Under certain circumstances, however, the homeostasis may breakdown, predisposing a site to diseases. In this review, we describe several examples of microbial interactions and their impacts on the homeostasis and pathogenesis of dental biofilms. We hope to encourage research on microbial interactions in the regulation of the homeostasis in biofilms.

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**Keywords:** biofilms; microbial interactions; homeostasis; microbiome; pathogenesis; community-based diseases

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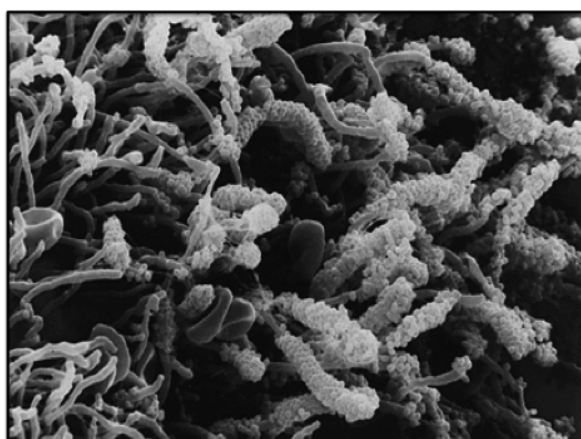
## 1. Introduction

The human body is host to a wide variety of microbial life, termed as the human microflora or microbiota, or more recently microbiome [1,2]. The human microbiome contains hundreds of species and trillions of cells that are predominantly associated with surfaces as communities, such as dental plaque and biofilms on many mucosal surfaces of the human body [1,2,3]. Species diversity, high cell density and close proximity of microbial cells are typical of life in biofilms, where microbes interact with each other and develop complex social interactions that can be either competitive or cooperative among microbial species [4,5]. Even without physical contact, microbes living at the same community may secrete small diffusible signal molecules to interact with each other [6]. The human oral microbiome, including “core” microbiota shared by all individuals, and “personalized” microbiota exclusive to individuals, plays important roles in oral health, such as breakdown of complex food molecules, protection from exogenous pathogens, and stimulation of healthy immune development [3]. One of the most striking aspects of oral microbiome is their long-term relationship with healthy individuals. The indigenous species in dental biofilms often maintain a relatively stable and harmless relationship with the host, despite regular exposure to environmental perturbations and host defense factors [7]. Such stability or homeostasis is considered critical to the host health and wellbeing. Under some circumstances, however, such homeostasis may break down, leading to population shifts in a community and predisposing a site to diseases [8]. How is the homeostasis maintained in a biofilm? What factors can influence such homeostasis? How can these factors leading to the homeostasis breakdown be detected or prevented? This review briefly discusses recent advances relevant to these questions.

## 2. Dental Plaque As a Highly Structured Biofilm

Microorganisms in nature are predominantly associated with surfaces and live in multispecies biofilms, which account for over 99% of microbial life on the earth [9]. Similarly, the host-associated microbes largely reside in biofilm communities on the surfaces of human body, including non-shedding surfaces, such as teeth, and shedding surfaces, such as the mucosa of the mouth, upper respiratory tract, digestive tract and urogenital tracts, although large numbers of microbial cells may be washed or shed off from these surfaces by mechanical and biological movements [9,10,11]. Formation of dental plaque is a dynamic process that often results in a developmental biofilm life cycle [6]. During the process of dental biofilm formation, some organisms are early colonizers that express biochemical components allowing them to effectively attach to the tooth surface [10]. Others are the later colonizers, which often contain components enabling them to adhere to the early colonizers, bringing metabolic and other competitive advantages into the community [9,12]. Dental plaque biofilms are spatially structured communities that often display a high degree of organization and their functions depend on complex webs of symbiotic interactions [6,11]. Upon viewing an intact

biofilm under a microscope, one will immediately find that microorganisms in dental biofilms do not randomly stick together, but rather form a well-organized community with numerous specialized configurations (Figure 1). One may also find that microbial cells in biofilms physically interact with each other and maintain intimate relationships. Even without physical contact, microbes living in the same community may secrete small diffusible signal molecules to interact with each other [6,13,14]. For example, many bacteria are found to regulate diverse physiological processes through a mechanism called quorum sensing, in which bacteria secrete, detect and respond to small signal molecules for coordinated activities in a cell density-dependent manner [15]. During quorum sensing, bacterial cells cooperate to obtain group-specific benefits such as production of extracellular polymers, exoenzymes, antibiotics and virulence factors [16,17,18]. Structural and physiological complexities of biofilms have led to the idea that microbes in biofilms frequently cooperate for social activities, like multicellular organisms [13,19]. Indeed, microbiologists have discovered an unexpectedly high degree of multicellular behaviors that have led to the perception of biofilms as “cities” of microbes [6,20]. Through cooperation, microbes can impact their environments in many ways that are simply impossible for individual cells. Clearly, microbes in such “cities” can achieve strength by increasing their cell density and interactions or by collectively producing virulence factors required for the pathogenesis [17–20].



**Figure 1.** Scanning electron microscope shows a matured dental plaque biofilm that is well-organized microbial community with a number of specialized configurations such as “corn cob” or “test-tube brush”. The dental biofilm is characterized by high cell density and species diversity. Microbial cells in the biofilm physically interact with each other and maintain intimate relationships, which likely promote cell-cell interactions and other coordinated activities in the biofilm. Adapted with permission [6].

### 3. Microbial Interactions in Dental Biofilms

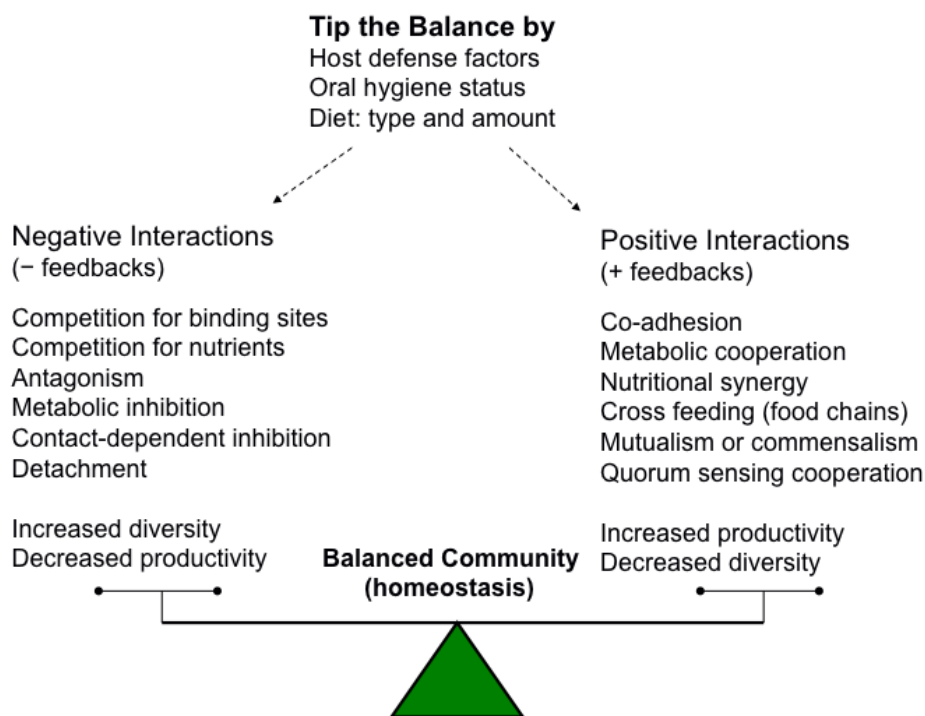
Dental plaque is a well-recognized biofilm community that is characterized by its vast diversity (> 700 species) and high cell density ( $10^{11}$  cells/g wet wt.), which facilitate organisms to interact with one another [6,9,12]. This suggests that microbial cells in dental biofilms likely display

inter-microbial interactions that contribute to the formation of a highly structured community, allowing microbial cells to carry out metabolic activities that may enhance the overall function of the community [21]. The significance of inter-microbial interactions was first realized and thoroughly described for microorganisms residing in the human oral cavity. Cooperative interactions among microbes in dental biofilms have been well studied, including bacterial co-aggregation and co-adhesion that facilitates bacterial colonization on saliva-coated teeth and effectuates temporal and spatial formation of highly organized biofilm architectures. Biofilm matrix also plays important roles in promoting bacterial adhesion, trapping nutrients, forming microenvironments and protecting microbial cells from lethal challenges including antimicrobial agents [22,23]. Cooperative metabolic interactions are even more common among microbial species in dental biofilm, involving nutritional synergy or metabolic complementation enabling organisms to breakdown complex salivary components (Figure 2). Cross feeding is another type of cooperation in which microbes obtain available nutrients, forming food chains in the community [24]. For example, oral streptococci are well known by their ability to generate lactic acid from sugar fermentation, whereas some neighboring species, such as *Veillonellae* spp., are unable to ferment sugar but use lactic acid as a preferred carbon source to generate energy [25]. Many bacteria in biofilms also use quorum sensing mechanisms to regulate biofilm development and other coordinated activities, including symbiosis, formation of spore or fruiting bodies, bacteriocin production, genetic competence, virulence and pathogenesis [14–18].

The processes controlled by quorum sensing are diverse and reflect the specific needs of particular communities. In many bacteria, quorum sensing represents a central mechanism to regulate cooperative activities, enabling bacteria to reap benefits that would be unattainable to them as individual cells [6,13,14]. Clearly, cooperative interactions among species probably play important roles in biofilm development and metabolic activities.

However, microbes in most ecosystems, including the human oral cavity, often face major challenges of limited space and nutritional resource, which inevitably results in competition among microbial species [26]. To survive and pass their genes to the next generation, microbes have to cope with constant battles of resource competition. The potential pool of microbial competitors is vast, and a wide range of mechanisms can be responsible for the emergency and establishment of dominant microbial populations. Microbial ecologists have long recognized two types of competition: exploitation competition that occurs indirectly through resource consumption, and interference competition that causes a direct, antagonistic effect on competitors [5,27,28]. There is good evidence that both exploitative and interference competition are prevalent in dental biofilms, strongly influencing the homeostasis and outcome of natural selection of microbes in biofilms. Microbial competition for common resources is a typical exploitative competition and can be strong in many natural ecosystems [29,30]. However, microbes cannot be viewed as passive nutritional sinks, but rather have evolved numerous strategies to augment their acquisition of resources. Many microbial activities, such as motility, attachment, antibiotic production, and secretion of extracellular polymers, can tip the competition balance, resulting in outcomes that may differ from those predicted in planktonic cultures [27]. Particularly, biofilms often form gradients in nutrient concentrations, oxygen tension, pH and waste products due to the thickness [12]. These factors can significantly affect the outcomes of microbial competition and compositions in a biofilm community. Interestingly,

despite high levels of competition among species, the majority of the resident organisms in a host-associated community can co-exist and maintain a relative stability in the community (Figure 2). This indicates that some regulatory mechanisms must exist and play critical roles in balancing microbial cooperative and competitive activities in microbial communities.



**Figure 2.** A schematic diagram describes microbial-microbial interactions and their roles in maintaining the homeostasis in a community. Microbial interactions include negative interactions (– feedbacks), such as competition and antagonism, and positive interactions (+ feedbacks), such as cooperation, synergy and mutualism. Positive interactions likely increase the productivity of the community but potentially destabilize the community, while negative interactions often reduce cooperative activities but favor species diversity and community stability. These interactions form complex networks that finely balance the homeostasis of the community. However, a number of ecological factors can tip the balance of these microbial interactions, disturbing the stability of a community. Dished arrows indicate the potential of these factors to tip the balance of the community.

Recent studies of microbial community dynamics show that microbial interactions can have three types of outcomes: a positive impact (win), a negative impact (loss) and no impact (neutral) on the microbial species involved [31]. The possible combinations of win (+), loss (–) and neutral (0) outcomes for two interacting partners allow classification of various interaction types. For example, different species of bacteria may cooperate to build a biofilm, which confers protection of the interacting members from antibiotics, a win-win (+/+) relationship known as mutualism. Other examples for cooperation are certain cases of cross feeding, in which two species exchange

metabolic products to the benefit of both. In contrast, competition between two species is a classic loss-loss (−/−) relationship, which indicates that two species with similar niches exclude each other or presenting competitive exclusion. In addition to typical cooperation or competition, predator–prey relationships and host-parasite relationships are considered to be win-loss (+/−) interactions, which are common in natural and host-associated microbial communities [30]. For example, *S. mutans* in dental plaque can produce an array of bacteriocins that kills other related species in the community, a typical win-loss interaction (+/−) [30,32]. In most ecosystems, there are few cases of neutral or no (0) interaction among species in the same community. These microbial interactions are largely based on laboratory studies of pairwise species of microorganisms interested. Relatively few studies have been carried out to investigate *in vivo* interactions and their impacts at community level until recently when metagenomics techniques are available to study an entire community [31]. However, detecting these interactions in natural microbial communities is far from straightforward. Novel approaches to investigate community- or ecosystem-wide networks may open a way towards global models of community and ecosystem dynamics. Ultimately, these studies will help to predict the outcome of community alterations and the effects of perturbations in complex microbial communities.

#### 4. Microbial Interactions in Maintaining the Homeostasis in Biofilms

It has been recognized that host-associated microbial communities are usually characterized by a remarkable stability among the component species, despite regular exposure to environmental perturbations and numerous host defense factors [8,33]. The ability of microbes to maintain the community stability is referred to as homeostasis (Figure 2). The homeostasis is believed to result from a dynamic balance of microbial-microbial interactions and microbial-host interactions [8]. Interestingly, such stability in a microbial community is often associated with a healthy condition. However, despite our rapidly increasing knowledge of the composition of the human microbiome, we know relatively little about what determines the homeostasis in a microbial community and what mechanisms have been involved in maintaining the homeostasis. There are a few *in vivo* studies on the relative significance of microbial interactions in maintaining microbial homeostasis. Most studies have characterized potential interactions *in vitro* with the assumption that they may operate similarly *in vivo*. It has been proposed that the tendency of a microbial community to maintain its homeostasis often increases with species diversity or with a greater biological complexity of the community [7,8,34]. This suggests that some regulatory mechanisms must operate to favor the development of species diversity and complexity of a microbial community. When the homeostasis is disturbed in a community, the self-regulatory mechanisms may come to work and restore the previous homeostasis status in the community. However, it is not always certain what regulatory mechanisms operate to maintain the homeostasis in a community. Recent studies have revealed that most stable microbial communities contain high levels of species diversity with complementing and seemingly redundant metabolic capabilities [35]. Microbial interactions in these communities can promote high species richness and bolster community stability during environmental perturbations. Clearly, species diversity within a microbial community is an important indicator of the homeostasis [7,8]. The need for microbial diversity in health may suggest that every species can carry out a specific function that is required to maintain the homeostasis in a community.

Recent studies of microbial community dynamics show that although positive microbial interactions, such as cooperation and synergism, play important roles in increasing community productivity, positive microbial interactions can come at costs to the community, so potentially destabilizing the community [7,34]. Microbial cooperation is destabilizing the community because it introduces positive feedbacks, which can generate runaway effects [7]. For example, when two species cooperate, an increase in the abundance of one species increases the abundance of the second, which in turn will increase the abundance of the first species and so on. If these increases are not sufficiently checked by other constraints, this can lead to runaway increases in cooperating species that can cause the collapse of interacting populations and destabilization of the community [7]. In contrast, negative microbial interactions or feedbacks, such as competition and antagonism, are considered as major and essential mechanisms in maintaining the homeostasis in microbial communities. This means that adding species that primarily engage in competitive interactions to the community may counter-intuitively help to stabilize the community by dampening positive feedbacks, stopping the community from cooperating its way to collapse [34]. Human and animal hosts may also suppress positive interactions or feedbacks between cooperating species in order to stabilize the community. Hosts could do this possibly by three mechanisms. First, the host immune response could be a stabilizing force. When certain species in a community rapidly increase in abundance, this could provoke a targeted host immune response, stopping positive feedbacks between cooperating species in their tracks. Second, the host could attempt to block cooperative interactions among species by spatially segregating them: when species grow in separate locations, their interactions will be weakened, thereby, preventing positive feedbacks. Third, the host could feed microorganisms to reduce cooperation among species by providing alternative carbon sources, so that these species no longer rely so strongly on their cooperative partners [34]. Analysis of mouse gut microbiome reveals that cooperative interactions are rare in the gut microbiome (only 10% of pairwise interactions are mutually beneficial), possibly because of their destabilizing effect [7].

An additional unexplored factor that could drive community stability is natural selection on both microbiomes and hosts. The human microbiome is the product of long adaptive processes of constituent species, their interactions, and host factors governing their growth [36]. Given the possibility of selection driving communities towards higher stability, it will be important to ascertain not only how species interactions affect stability on average, but also what characteristics of the most stable communities are, and whether they are achievable by evolution. Work on animal and plant communities has shown that factors that decrease community stability can also counter-intuitively be over-represented in most stable communities [34]. These approaches will be critical in understanding evolutionary ecology of microbial communities, helping manipulation of component species in communities to promote stable microbiomes and health in hosts.

## **5. Factors That Disrupt Microbial Homeostasis in Biofilms**

Human microbiome research reveals that every human body contains a variety of microbial communities that consist of hundreds of microbial species important to human health [3]. The key to human health is an ecologically balanced microbiome that practices commensalism or mutualism within itself and with the host [7]. Microbial-microbial and microbial-host interactions play

important roles in maintaining such a homeostasis in these microbial communities (Figure 2). Despite these interactions, however, the homeostasis in a microbial community can breakdown under certain circumstances, leading to population shifts and predisposing a site to diseases [8]. What factors can disrupt the homeostasis in such a stable community? Studies of various host-associated microbiomes, such as those in the oral cavity, gastrointestinal and vagina, have provided some clues to the type of factors indicating homeostasis disruption in a community, including (1) a significant change in the relationship between a microbial community and the host; (2) acquisition of a virulence factor or pathogenic trait by a resident species in the community; (3) a sudden increase or decrease in relative abundance of one or more species in the community; and (4) more recently, “keystone” species or pathogens that play key roles in the breakdown of host-microbial homeostasis leading to dysbiosis in a community and diseases, based on the keystone-pathogen hypothesis [3,8,33,37].

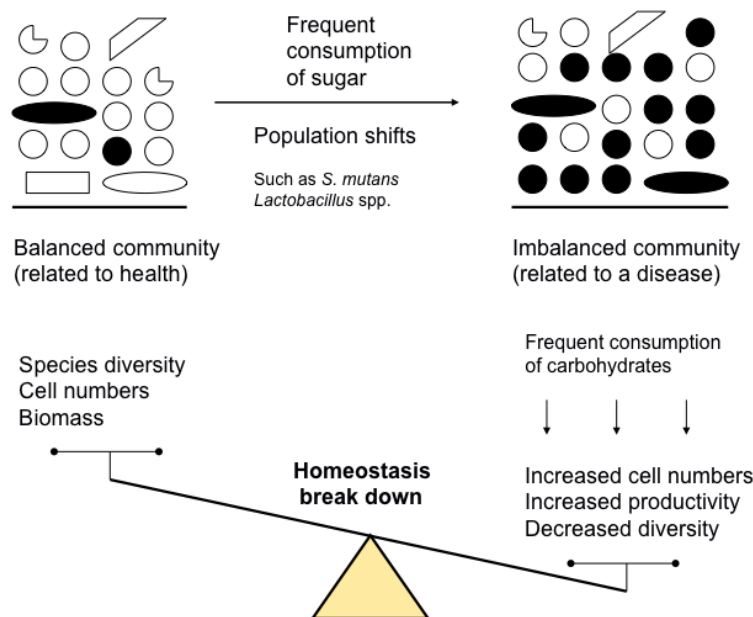
The relationship between microbiome and the hosts during health is often mutually beneficial because the host is providing the microbial community with an environment in which they can flourish and, in turn, keep their host healthy [34]. The presence of the host or physiological factors can tip the balance of a microbial community. As the host defense system regulates microbial-host interactions, a compromised immune system often disrupts the balance relationships between microbes and the host, resulting in the homeostasis breakdown that predisposes a site to disease. For example, immune-deficient or chemotherapy patients have an increased susceptibility to opportunistic infections [11]. Individuals with reduced saliva flow or dry mouth also have an increased susceptibility to dental caries, periodontitis or oral candidosis caused by once-normal resident microbes within the oral cavity [33]. Another example is that increases in female sex hormones can sometime have the capacity to disrupt microbial homeostasis in several ecosystems of the body, predisposing a site to an infection, such as pregnant gingivitis in the oral cavity [8].

Acquisition of virulence factors or pathogenic traits through horizontal gene transfer between microbes in biofilms is a common mechanism to trigger population shifts or dominance by antibiotic-resistant species leading to the homeostasis breakdown in a community. For example, an antibiotic-resistant gene transfer within or between species may lead to dominance by these populations in the community, particularly when the community is exposed to a subinhibitory antibiotic stress condition [38].

A sudden increase or decrease in relative abundance of one or more species in a microbial community often indicates the homeostasis breakdown of the community [8,33]. A common feature is a significant change in nutrient status, such as introduction of an excess substrate such as sugar or a chemical compound that can disturb the ecosystem [8]. For example, frequent consumption of fermentable dietary carbohydrates in the oral cavity may favor the overgrowth of sugar-fermenting bacteria (Figure 3), such as *S. mutans* and *Lactobacillus* spp. in a dental biofilm [33]. Such carbohydrate metabolism from these bacteria generates large amounts of lactic acid that acidifies the local environment, resulting in selection of acid-resistant bacteria but elimination of acid-sensitive bacteria in the community. The dominance by a few acid-resistant species in the community indicates the breakdown of the homeostasis, predisposing the site to tooth decay [33]. In this case, the microbial community is often dominated by fewer species or reduced species diversity [8]. Clearly, frequent consumption of fermentable carbohydrates is a powerful determinant that disturbs



the homeostasis in dental biofilms. Similarly, antimicrobial agents that kill bacteria are the best-characterized mechanisms resulting in homeostasis breakdown in many host-associated microbial communities [11]. Antibiotic treatment often causes a rapid reduction in sensitive species followed by an emergence of resistant organisms. This inevitably results in population shifts and the homeostasis breakdown in the communities. It is then not surprising that an infectious disease may occur due to the overgrowth of an antibiotic-resistant organism during an improper antibiotic therapy.



**Figure 3.** A schematic diagram describes an example of an ecological factor, frequent consumption of sugar (fermentable carbohydrates), to tip the balance of the community. In the human oral cavity, frequent consumption of dietary sugar is a powerful ecological factor that can cause population shifts and tip the balance of a dental biofilm community. Frequent consumption of dietary sugar promotes the overgrowth of sugar-fermentable and acid-producing-and-resistant bacteria, such as *Streptococcus mutans* (black circles) and *Lactobacillus* spp. (black ovals) in dental biofilm. This will likely result in population shifts in the biofilm that is characterized by dominance of fewer acid-resistant species, such as *S. mutans* and *Lactobacillus* spp. This will reduce or eliminate many acid-sensitive bacteria (blank shapes) in the community, leading to the homeostasis breakdown and thereby predisposing the site to dental caries (tooth decay).

More recently, a novel hypothesis, called the “keystone-pathogen hypothesis”, has been proposed to describe mechanisms underlying the breakdown of host-microbial homeostasis that precipitates dysbiosis (microbiota imbalance) of a community, leading to diseases [37,39–43]. The keystone-pathogen hypothesis holds that certain low-abundance microbial pathogens can orchestrate inflammatory disease by remodeling a normally benign or resident microbiota into a dysbiotic one in a community. Importantly, the keystone pathogens have the capacity of instigating inflammation and triggering dysbiosis even when they are present as quantitatively minor components in the

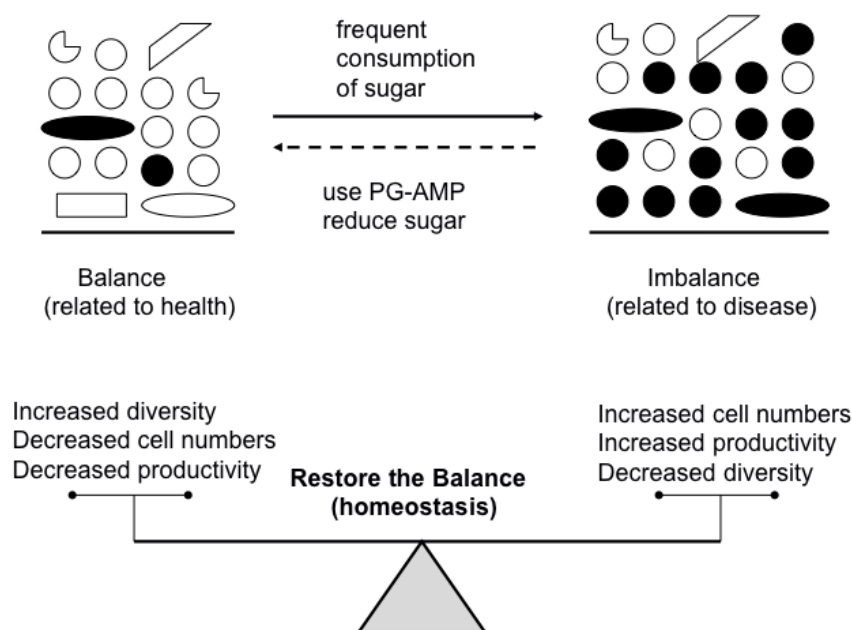
community. Recent studies suggest that keystone pathogens are likely the causes of initiating periodontitis, chronic inflammatory bowel disease, colon cancer and obesity. For example, periodontitis is a biofilm-induced chronic inflammatory disease, which affects the tooth-supporting tissues or periodontium, and also increases a patient's risk of developing atherosclerosis, diabetes and possibly rheumatoid arthritis by local release of pro-inflammatory cytokines that enter the systemic circulation and induce an acute-phase response [38,39]. The tooth-associated dental plaque is required but not sufficient to induce periodontitis, because it is host inflammatory response to this microbial challenge that ultimately can cause destruction of the periodontium. There has been significant progress in the quest to identify specific periodontal pathogens, including the identification of several candidates, mostly Gram-negative anaerobic bacteria that colonize subgingival tooth sites. Foremost among this group are three species that constitute the so-called "red complex", are frequently isolated together and are strongly associated with diseased sites in the mouth: *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* which are the keystone pathogens in subgingival dental biofilms [39]. Much research has been done towards understanding the pathogenic mechanisms and virulence determinants of these bacterial species. Dysbiotic microbial community of these keystone pathogens is thought to exhibit synergistic virulence, whereby they can not only endure the host response but also thrive by exploiting tissue destructive inflammation, which fuels a self-feeding cycle of escalating dysbiosis and inflammatory bone loss, ultimately leading to tooth loss and systemic complications [40,41,42].

## 6. Implications in the Pathogenesis of Biofilm Diseases

Traditional studies on infectious diseases have focused extensively on pathogenic microbes that directly damage tissues in hosts. It is increasingly recognized that direct attack is not only way that microbes cause diseases. Some commensal microbes living in a certain location can also induce diseases or contributes critically to disease development. These commensal microbes that can cause diseases under certain conditions are often called opportunistic pathogens or "pathobionts" [37,40]. Recent studies reveal that environmental or host factors that can disturb the microbial homeostasis likely result in the dominance by pathobionts in a community, predisposing a site to diseases [39,40]. When pathobionts become dominant, the community likely shifts to a condition more pathogenic, called dysbiosis, which indicates the breakdown of the homeostasis in the community [43]. In addition, the keystone pathogens identified also play key roles in disturbing the microbial-host homeostasis, leading to the dysbiosis, which can be the cause or the consequence of a disease and largely depends on microbial-host interactions in the community. A common feature of these diseases is that they are often associated with multiple species of pathobionts, so these diseases are referred as polymicrobial community diseases [12,42]. However, only certain species play critical roles in driving a commensal community toward a pathogenic shift [41]. Despite multi-species features, a major challenge using antimicrobial agents to treat this type of diseases is that wide-spectrum antimicrobials may indiscriminately kill the resident organisms in the community, resulting in ecological disruption or other negative consequences [43]. Current understanding of community-based diseases has changed the strategies for diagnosis, prevention and treatment of these diseases.

## 7. Strategies for Prevention and Treatment of the Community-Based Diseases

It is well known that most biofilm diseases are associated with multiple species of microbes, such as dental caries, periodontitis, otitis media, cystic fibrosis lung infection, and inflammatory bowel disease, which are clinically characterized by chronic process with acute or sub-acute episodes [41,42,43]. The homeostasis breakdown leading to dysbiosis in the community is the key step for the initiation and development of these diseases [40]. Because alternations in the microbiota at a given site are potential biomarkers of disease activity, understanding the microbiome at the early stages of diseases would allow clinicians to diagnose, predict and prevent potential risk, severity and outcomes of these diseases. Since every human body contains a personalized microbiome, the analyses of the microbiome will pave the way for more effective diagnosis, prevention and therapies. These will contribute to the development of personalized medicine for better surveillance of this group of diseases at levels of individual patients. In particular, identification of keystone pathogens could have substantial clinical benefits, as it may facilitate the development of targeted treatment of a polymicrobial disease by focusing on a limited number of pathobionts in the dysbiotic community. These diseases may be prevented or treated not only by inhibiting a putative pathogen, but also by



**Figure 4.** A schematic diagram describes an ecological strategy of targeted regulation of cariogenic pathogens, such as *S. mutans* (black circles), in dental biofilms. For an imbalanced dental biofilm community, reducing consumption of dietary sugar, such as use of sugar substitutes, or using target-specific antimicrobial agents, such as application of pheromone-guided antimicrobial peptides (PG-AMPs) that selectively kill *S. mutans*, can effectively reduce or eliminate *S. mutans* in the community. This targeted regulation of cariogenic pathogens may completely or partially reverses the species diversity, which allows the re-growth or re-establishment of acid-sensitive species in the community, eventually restoring the balance or homeostasis in the community that is less pathogenic.

interfering with the factors disturbing the homeostasis in the community (Figure 4). Several strategies of regulation of microbial communities to prevent community-based diseases are further discussed.

### *7.1. Strategies of regulating community ecology*

With our understanding of microbial communities and their associated diseases, there is increasing interest in approaches that modulate the ecology of microbial communities to reduce community-based diseases. Among these strategies, the probiotic approach is a popular method for modulating microbial ecology [44]. The probiotics refers to live organisms that can confer health benefits on the host when administered in adequate amounts [45]. In the past decades, there have been numerous exciting discoveries that reveal beneficial effects resulting from administering probiotics, ranging from direct inhibition of pathogenic microbes to improving host immune functions [46]. The rationale of using probiotics is based on the fact that probiotics can interfere with invasion by foreign pathogens or with pathogenic shifts by keystone pathogens in microbial communities. These may reduce the potential of a microbial community to become a pathogenic one or dysbiosis [44,45,46].

Another strategy is to interfere with microbial quorum sensing mechanisms, since quorum sensing plays important roles in biofilm formation and cell density-dependent virulence [13–18]. In recent years, many natural and synthetic compounds have been identified to act as quorum sensing inhibitors (QSI) that can target bacterial quorum sensing mechanisms and their controlled pathogenic activities [47,48,49]. It is believed that QSIs target bacterial cell-cell signaling and coordinated activities required for infections, thereby, essentially disarming the bacteria and tipping the balance in favor of the host and allowing the immune system to clear the infectious pathogens [47]. QSI therapies that specifically block quorum sensing can make the pathogens become ‘deaf’, ‘mute’ or ‘blind’ rather than directly kill them. Therefore, QSI therapy may achieve the treatment but much less likely cause selective pressure to create resistant microbes [47,48,49].

For some community-based diseases, such as periodontitis and intestine inflammatory diseases, anti-inflammatory agents can be used to break the cycle of inflammation and tissue destruction that often promote the dysbiosis or homeostasis breakdown in a community [42,43]. In particular, these agents combined with some antimicrobials that specifically target the keystone pathogens would provide much better therapy both by targeting the putative pathogens and by interfering with the processes that drive breakdown of the homeostasis in the community [43].

Other strategies in regulating microbial ecology to prevent homeostasis breakdown in some microbial communities include diet regulation such as use of sugar substitutes that reduce carbon source for bacterial fermentation (Figure 4), increasing flow of body fluids such as saliva, use of oxygenating or redox agents that reduce the growth of obligate anaerobes in a biofilm community, and use of non-antimicrobial agents such as fluoride, chelator and metal ions that compromise some metabolic activities of certain microbes [8,33]. For example, fluoride can inhibit enzyme activity required for bacterial metabolism, particularly under low pH, but shows little bacterial killing [8].

## 7.2. Targeted antimicrobial therapy to reduce pathobionts or keystone pathogens in a community

Currently, available antibiotics exhibit broad killing spectra with regard to bacterial genus and species. Indiscriminate killing of microbes by these conventional antibiotics may disrupt the ecological balance of the indigenous microflora, resulting in negative clinical consequences [50]. To circumvent the problem, a new class of such antimicrobials, called pheromone-guided antimicrobial peptides or PG-AMPs (Figure 4), has been developed as potential alternatives [50–53]. The rationale of using such antimicrobials is based on the addition of a targeting domain of a quorum-sensing signal pheromone from a target organism to the killing domain of a known antimicrobial peptide. Both domains are fused via a small linker to generate a fusion PG-AMP without detrimental change of their activities [51]. The targeting domain can guide such a fusion peptide to bind selectively to the target organism, leading to selective killing [50,51,52]. These narrow-spectrum antimicrobials can selectively target specific organisms with little effect on the other members in the community [50,51,52]. Therefore, PG-AMPs have added an exciting opportunity to develop new antimicrobials that target keystone pathogens in a community-based disease. Recent studies explored the possibility of using a *S. mutans* pheromone as a targeting domain to mediate *S. mutans*-specific delivery of an antimicrobial domain [52,53]. It is found that PG-AMPs constructed in this way are potent against *S. mutans* in animal caries model [53]. The PG-AMPs are capable of eliminating *S. mutans* from multispecies biofilms without affecting other non-cariogenic species, indicating the potential of these molecules to be developed into targeted antimicrobial agents. This proof-of-principle strategy suggests that it may be possible to develop PG-AMPs that specifically target other keystone pathogens and modulate microbial ecology in a microbial community [53].

## 8. Conclusion

Research over the last 30 years has generated a substantial amount of knowledge on microbial biofilms. We have learned that microbes form highly diverse communities on surfaces of human body, which are increasingly recognized to have profound impacts on human health and diseases. It has been well established that microbes in such biofilm communities can develop complex social interactions and networks, which play important roles in modulating the community stability or homeostasis important to host health. Despite our rapidly increasing knowledge of the compositions of the human microbiome, we know little about what determines the stability of these communities. However, significant advance has been made to identify factors that affect microbial interactions, ecology and the pathogenesis. Evidence has accumulated that some biofilm diseases can be prevented or treated not only by targeting the putative pathogens, but also by interfering with the processes that drive the breakdown of the homeostasis in biofilms. Studies of the human microbiomes in health and disease will pave the way for development of more effective diagnostic, preventive and treatment techniques in manipulating microbial interactions, and biofilm-associated diseases, ultimately, contributing to personalized medicine.

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## Conflict of Interest

All authors declare no conflict of interest in this review.

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