Chapter 3

Microbial Interactions in Biofilms: Impacts on Homeostasis and Pathogenesis

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Additional information is available at the end of the chapter

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Abstract

Microbes in nature or in the human body are predominantly associated with surfaces and living in biofilms. Species diversity, high cell density and close proximity of cells are typical of life in biofilms, where organisms 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, 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 microbial interactions in biofilms profoundly affect their overall function, biomass, diversity and pathogenesis. It is now known that every human body contains a personalized microbiome that is essential to maintain host health. Remarkably, the indigenous species in most microbial communities often maintain a relatively stable and harmless relationship with the hosts despite regular exposure to minor environmental perturbations and host defence factors. Such stability or homeostasis results from a dynamic balance of microbial–microbial and microbial–host interactions. Under some circumstances, however, the homeostasis may breakdown, predisposing a site to diseases. The evidence has accumulated that such biofilm or community-based diseases can be prevented or treated not only by targeting putative pathogens, but also by interfering with the processes that drive breakdown of the homeostasis in biofilms.

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

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 [1–3]. 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 species [4, 5]. Even without physical contact, microorganisms living In the same community may secrete small diffusible signal molecules to interact with each other [6]. The human microbiome, including "core" microbiota shared by all individuals and "personalized" microbiota exclusive to the individuals, plays important roles in human health, such as breakdown of complex molecules in food, protection from exogenous pathogens and stimulation of healthy immune development [3]. One of the most striking aspects of these complex communities is their long-term stability in healthy individuals. The indigenous species in a community often maintain a relatively stable and harmless relationship with the host despite regular exposure to minor environmental perturbations and host-defense factors [7]. Such stability or homeostasis is considered critical for 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]. What determines such homeostasis in a community? What factors can change homeostasis and what are the mechanisms behind? How can these changes be detected and prevented? This chapter aims to briefly review the current advances relevant to these questions.

2. Social structure of microbial biofilms

Microorganisms in nature are predominantly associated with surfaces and live in multispecies biofilms, which account for over 99% of microbial life on this planet [9]. Similarly, the host-associated microbes largely reside in biofilm communities on the surfaces of human body, including nonshedding 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 [9–11]. Biofilm formation is a dynamic process that often results in a developmental biofilm life cycle [9, 10]. During the process of biofilm formation, some organisms are early colonizers that express biochemical components allowing them to effectively adhere to a 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]. Biofilms are spatially structured communities that often display a high degree of organization and their functions depend on complex webs of symbiotic interactions [11]. If viewing an intact biofilm under a microscope, then one will immediately find that microbes in biofilms do not randomly stick together, but rather form a well-organized community with numerous specialized configurations [10, 13]. One may also find that microbial cells in biofilms physically interact with each other and maintain intimate relationships [12]. Even without physical contact, microbes living in the same community may secrete small diffusible signal molecules to interact with each other [14]. For example, many bacteria are found to regulate diverse physiological processes through a

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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 signal molecules, extracellular polymers, exoenzymes, antibiotics and virulence factors [16–18]. Structural and physiological complexities of biofilms have led to the idea that microbes in biofilms frequently cooperate for social activities as groups, like multicellular organisms [19]. Indeed, microbiologists have discovered an unexpectedly high degree of multicellular behaviours that have led to the perception of biofilms as "cities" of microbes [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].

3. Microbial interactions in biofilms

Microbial biofilms are characterized by species diversity, high cell density and close cell-cell proximity [6, 9, 12]. This suggests that microbial cells in biofilms likely display intermicrobial interactions that contribute to the formation of a highly structured community, allowing cells to carry out metabolic activities that may enhance the overall function of the community [21]. The significance of intermicrobial interactions was first realized and thoroughly described for microorganisms residing in the oral cavity [10, 12]. Dental plaque is a well-recognized biofilm community characterized by its vast diversity (>700 species) and high cell density (1011 cells/g wet wt), which allow organisms to develop complex interactions [12]. Cooperative interactions among organisms 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 [10, 12]. Biofilm matrix also plays important roles in promoting bacterial adhesion, trapping nutrient molecules, forming microenvironments and protecting microbial cells from lethal challenges or antimicrobial agents [22, 23]. Cooperative metabolic interactions are even more common among microbial species, involving nutritional synergy or complementation enabling organisms to breakdown complex salivary components [6, 12]. Cross feeding is another type of cooperation in which microbes obtain available nutrients, allowing formation of 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 neighbouring species, for example *Veillonella* sp., 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]. Clearly, cooperative interactions among species probably play important roles in biofilm development and metabolic activities.

However, microbes in most ecosystems often face major challenges of limited space and nutritional resources, which inevitably results in competition among species. To survive and pass their genes to the next generation, microbes have to cope with constant battles of resource competition [26]. The potential pool of microbial competitors is vast, and a wide range of mechanisms can be responsible for the emergency and radiation 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 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 [28]. 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 [8, 30]. This indicates that some regulatory mechanisms must exist and play critical roles in balancing microbial cooperative and competitive activities in microbial communities.

Based on recent community structure and dynamic studies using metagenomics and 16S pyrosequencing, 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 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 also common in natural and host-associated microbial communities [30]. For example, *Streptococcus mutans* in dental plaque can produce an array of bacteriocins that kill other related species in the community, a typical win–loss interaction (+/−) [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

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species of microorganisms interested. Relatively few studies have been carried out to investigate microbial interactions and their impacts at community level until recently when genomics and metagenomics techniques are available to study communities [31]. However, detecting these various types of interactions in natural microbial communities is far from straight forward. Novel approaches to the investigation of community- or even 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. Roles of 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 minor environmental perturbations and numerous host-defence factors [8, 33]. The ability of microbes to maintain the community stability is referred to as homeostasis (**Figure 1**). The homeostasis is believed to stem not from any indifference among the component species but rather results 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 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 favour 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 or feedbacks, such as cooperation and synergism, play important roles in increasing community productivity, the positive microbial interactions can come at costs to the

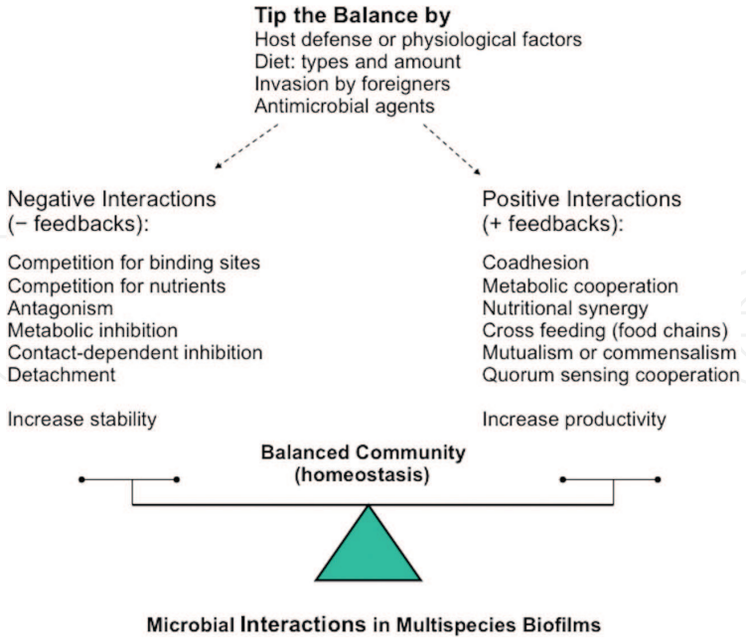

Figure 1. A schematic diagram describes microbial–microbial interactions and their roles in maintaining the homeostasis in a community. Microbial interactions include negative interactions (− feedbacks) such as competition and antagonism and positive interactions (+ feedbacks) such as cooperation, synergy and mutualism. Positive interactions likely increase the productivity of the community but potentially destabilize the community, while negative interactions often dampen cooperative activities but favour 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. Dashed arrows indicate the potential of these factors to tip the balance of the community.

community, so potentially destabilizing the community [7, 34]. Microbial cooperation is destabilizing the community because it introduces positive feedbacks, which can generate runaway effects. 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, then 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 counterintuitively 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 on average can also counterintuitively be over-represented in most stable communities [34]. These approaches will be critical in understanding evolutionary ecology of microbial communities, therefore, helping manipulation of component species in communities to promote stable microbiomes and health in hosts.

5. Potential factors disrupting microbial homeostasis: tipping the balance

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 [1–3]. The key to human health is an ecological-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 1**). 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 stable communities? Studies of various host-associated microbiomes, such as those in the oral cavity, gastrointestine 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 relationships between microbiome and its hosts during health are often mutually beneficial because the host is providing its microbial communities with an environment in which they can flourish and, in turn, keep their host healthy [34]. The presence of an immune or physiological disorder can tip the balance of a microbial community. As the immune 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 and predisposing 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 increase in female sex hormones can sometime have the capacity to disrupt microbial homeostasis in several ecosystems of the body, predisposing or enhancing opportunistic infections [8].

Acquisition of virulence factors or pathogenic traits via horizontal gene transfer between microbes in biofilms is a common mechanism to trigger population shifts 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, for example, 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 2**) such as *S. mutans* and *Lactobacillus* sp. 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.

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]. 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 play

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key roles in 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 (**Figure 3**), and also increases patients' risk of developing atherosclerosis, diabetes and possibly rheumatoid arthritis [38, 39]. The tooth-associated dental plaque is required but not sufficient to induce periodontitis, because it is the 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 have been directed towards understanding the pathogenic mechanisms and virulence determinants of these three bacterial species. Dysbiotic microbial communities of these keystone pathogens are thought to exhibit synergistic virulence, whereby not only they can endure the host response but can 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].

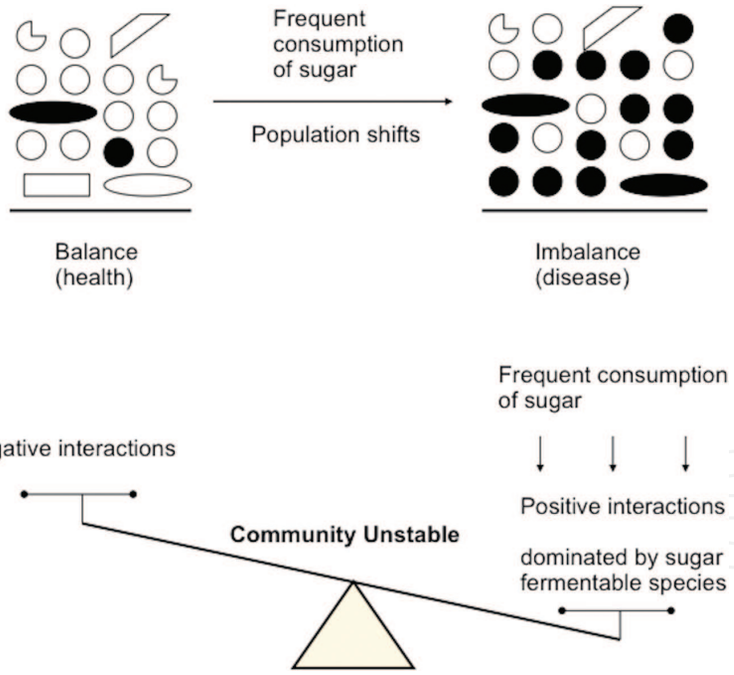

Figure 2. A schematic diagram describes an example of an ecological factor, frequent consumption of sugar (fermentable carbohydrates), to tip the balance of the community. In the human oral cavity, frequent consumption of dietary sugar is a powerful ecological factor that can cause population shifts and tip the balance of a dental biofilm community. Sugar favours the overgrowth of sugar-fermentable and acid-resistant bacteria such as *Streptococcus mutans* (black circles) and *Lactobacillus* sp. (black ovals) in dental biofilms. This will result in population shifts characterized by dominance of *S. mutans* and *Lactobacillus* sp., but reduction or elimination of acid-sensitive bacteria (blank shapes) in the community, leading to the homeostasis breakdown and predisposing the site to dental caries. In this case, fewer species remain in the imbalanced community.

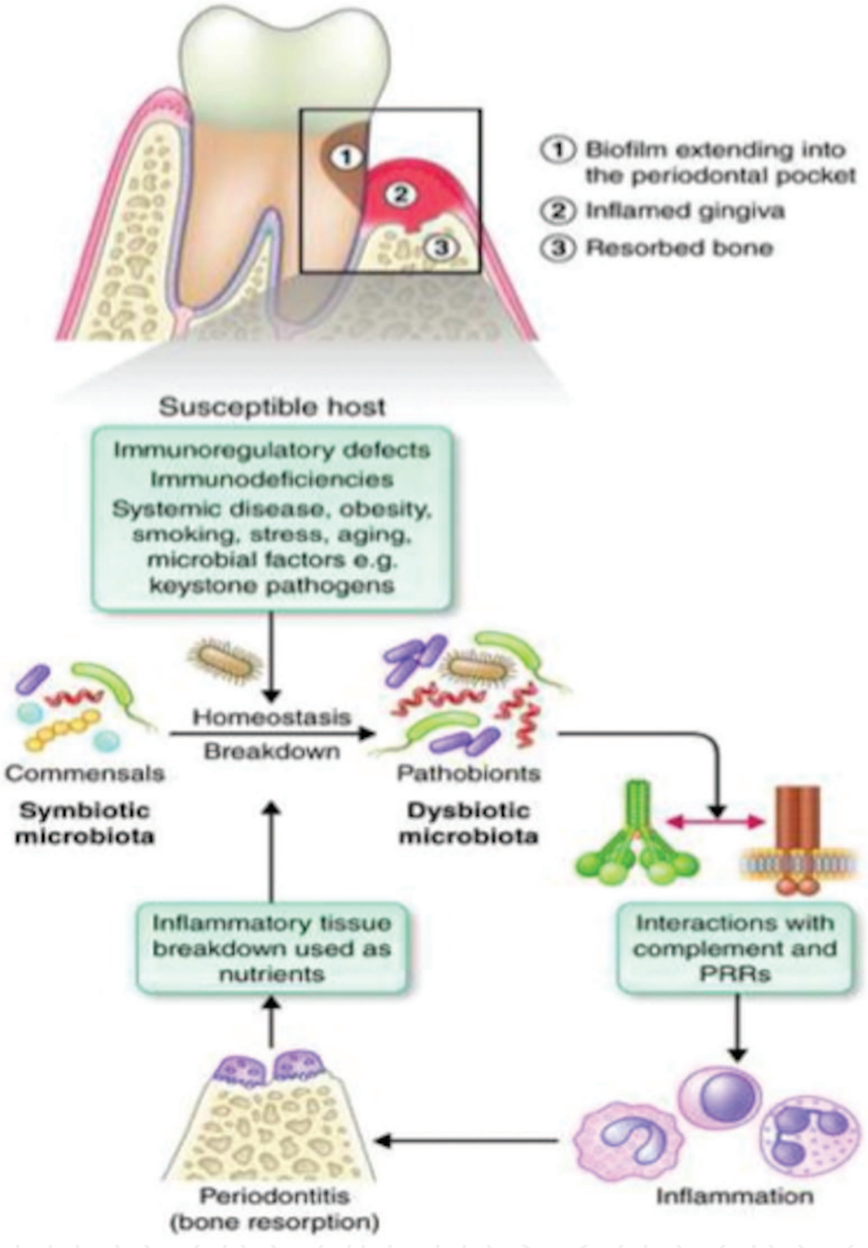

Figure 3. A proposed model describes the roles of pathobionts or keystone pathogens in the initiation and development of periodontitis. In healthy periodontium, a commensal microbe–host relationship is maintained because of a controlled inflammatory state. However, this balanced relationship or homeostasis can breakdown due to defects in the immunoinflammatory state or predisposing conditions or environmental factors, leading to the balance shift towards dysbiosis, a state in which former commensal organisms become proinflammatory pathobionts. In addition, the presence of keystone pathogens can similarly tip the balance toward dysbiosis even in hosts without apparent predisposing factors. The inflammation caused by the dysbiotic microbiota depends in great part on crosstalk signaling between complement and pattern recognition receptors (PRRs). This has two major interrelated effects: it causes inflammatory destruction of periodontal tissue, which in turn provides nutrients (destructed tissues) further promoting dysbiosis. This generates a self-perpetuating pathogenic cycle. It should be noted that host susceptibility might not simply be a determinant of the transition from a symbiotic to dysbiotic microbiota but it may underlie the predisposition of the host to develop inflammation sufficient to cause irreversible tissue damage.

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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 the only way that microbes cause diseases. Evidence has accumulated that some commensal microbes living as the normal residents in a host can also induce diseases or contributes critically to disease development. These commensal microbes that can cause or promote diseases under certain conditions are often called opportunistic pathogens or "pathobionts" [40]. When some species become dominant in their relative abundance in a community, the relationships among the resident members in the community might become imbalanced called dysbiosis, which indicates the breakdown of the homeostasis in the community. The keystone pathogens identified from various ecosystems also play key roles in disturbing the microbial–host homeostasis, leading to dysbiosis, which can be the cause or the consequence of diseases and is largely dependent on microbial–host interactions in a microbial community. Recent studies reveal that factors that can disturb the microbial homeostasis likely result in the dominance by pathobionts in a community, predisposing a site to diseases [39, 40]. A common feature of these diseases is that they are often associated with multiple species of pathobionts, so these diseases are referred to as polymicrobial or community-based diseases [12, 42]. However, only certain species play major roles in driving a commensal community toward the pathogenic shift [41]. Despite multispecies features, a major challenge using antibiotics to treat these diseases is that wide-spectrum antibiotics may indiscriminately kill the resident organisms in the community, resulting in ecological disruption or other negative clinical consequences [43]. Current understanding of polymicrobial or community-based diseases has changed the strategies for diagnosis, prevention and treatment of these diseases.

7. Strategies for diagnosis, prevention and treatment of community-based diseases

7.1. Community-based assays of the microbiomes

It is now known that most biofilm diseases are associated with multiple species of microorganisms. These polymicrobial diseases such as dental caries, periodontitis, otitis media, cystic fibrosis lung infection, inflammatory bowel disease and other biofilm infections are clinically characterized by a chronic process with acute or subacute episodes [41–43]. The homeostasis breakdown leading to dysbiosis in a 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, analyzing the microbiome at the early stages of diseases would allow clinicians to diagnose, predict and prevent potential risk, severity and outcomes of these diseases. In particular, identification of keystone pathogens could have substantial clinical benefits, as it may facilitate the development of targeted treatment by focusing on a limited number of pathobionts in biofilms. Since every human body contains a personalized micro-

biome, analyses of the microbiome will pave the way for more effective diagnosis, prevention and therapies, contributing to the development of personalized medicine.

For a long time, our understanding of microbial communities has been hampered by the intrinsic limitation of conventional culture-dependent techniques. Our views of the complexity and genetic diversity of microbial communities based on cultivation strategies are severely biased. Fortunately, a number of DNA-based assays or genomic approaches have been developed to help overcome such limitation, allowing us to obtain a clearer picture of microbial communities in terms of their structural complexity and genetic diversity. Since intermicrobial interactions in a community often create many new physiological functions that cannot be observed with individual species, community-based assays have emerged to analyze microbial compositions and associated physiology, which has greatly contributed to our understanding of the microbiomes and dysbiosis. Common strategies used to analyze microbial communities or the microbiomes include 16S rRNA gene (pyro)sequencing [44, 45], genomic or metagenomic approaches [46], checkerboard DNA–DNA hybridization [47], PCR-based denaturing gradient gel electrophoresis (DGGE) [48] or denaturing high performance liquid chromatography (DHPLC) analyses [49] and terminal restriction fragment length polymorphism (T-RFLP) analysis [50]. The application of these community-based techniques in the analysis of the human microbiomes has revealed astonishing diversities of largely uncultivated microorganisms present in human samples. These approaches have been expanded to many clinical samples collected from a broader patient pool with a diverse range of healthy conditions and diseases, promoting the discovery of many new species of the human microbiome.

7.2. Modulating community ecology to reduce potential risk of a disease

With our new understanding of microbial communities and their associated diseases, there is an increasing interest in approaches that modulate the ecology of microbial communities to achieve reduction or control of community-based diseases. These diseases may be prevented or treated not only by inhibiting the putative pathogens, but also by interfering with the factors disturbing the homeostasis in microbial communities. Among them, probiotic approach has been a popular method for modulating microbial ecology [51]. The probiotics refers to live microorganisms that can confer health benefits on the host when administered in adequate amounts [52]. 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 [53]. 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 community to become a pathogenic one or dysbiosis [51–53].

Another strategy is to interfere with microbial cell–cell communication via quorum sensing in microbial communities, since quorum-sensing mechanisms play important roles in biofilm formation and cell density-dependent virulence [13–18]. In recent years, scientists actively search for natural and synthetic compounds that act as quorum-sensing inhibitors (QSIs) that can target bacterial quorum-sensing mechanisms and their controlled pathogenic activities [54–56]. It is believed that QSIs target bacterial cell–cell signaling and coordinated activities

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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 pathogen [54]. QSI therapies that specifically block bacterial quorum sensing can make the pathogens become 'deaf', 'mute' or 'blind' rather than directly killing them. Therefore, QSI therapy may achieve the treatment but much less likely cause selective pressure to create resistant microbes [54–56].

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, both of which promote the homeostasis breakdown or dysbiosis in a community [42, 43]. In particular, these agents combined with some antimicrobials that specifically target the keystone pathogens or pathobionts 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 sugar substitutes that reduce carbon source for bacterial fermentation, 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 nonantimicrobial agents such as fluoride, chelating agents such as EDTA, 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, thereby, not significantly affecting community ecology [8].

7.3. Targeted antimicrobial therapy to reduce pathobionts 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 [51]. To circumvent the problem, a new class of such antimicrobials, called pheromone-guided antimicrobial peptides (PG-AMP), has been developed as potential alternatives [57, 58]. The rationale of using such antimicrobial agents 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 [58]. The targeting domain can guide such a fusion peptide to bind selectively to the target organism, leading to selective killing [57–59]. These narrow-spectrum antimicrobials can selectively target specific organisms with little effect on the other members of the community [51, 57–59]. 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 utilizing a pheromone produced by *S. mutans* as a targeting domain to mediate *S. mutans*-specific delivery of an antimicrobial peptide domain [57–59]. It is found that PG-AMPs constructed in this way are potent against *S. mutans* in animal dental caries model [60]. The PG-AMPs are capable of eliminating *S. mutans* from multispecies biofilms without affecting other noncariogenic 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 community-based diseases [58–60].

8. Concluding remarks

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 pathogenesis. Evidence shows 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 open a new avenue for the development of more effective diagnosis, prevention and treatment of community-based diseases, contributing to personalized medicine.

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