Oral microbial dysbiosis in cardiovascular diseases

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Abstract

The most common diseases in humans are oral infections. Although modern research is mainly concerned with the role of the gut microbiota in systemic diseases, there are also reports indicating that oral infections, and especially periodontal infection, are one of the risk factors for atherosclerotic cardiovascular disease (CVD). The composition of the oral biofilm is a community of several hundred species of bacteria, fungi, spirochetes, viruses and protozoa. It varies depending on the state of health and disease. Pathogenic bacteria in the oral cavity can cause systemic disease by entering the bloodstream or by triggering immune responses at the cellular level. The discovery of the role of the oral microbiome in CVD is leading to new methods of prevention and their treatment. In this review, we discuss the various mechanisms by which oral dysbiosis may contribute to the pathogenesis of CVD as well as available options for their prevention and treatment.

Oral dysbiosis, or the imbalance of bacteria in the oral cavity, has been linked to an increased risk of CVD. Several mechanisms have been proposed to explain how oral dysbiosis may contribute to CVD, including: The production of inflammatory molecules by oral bacteria. The activation of the immune system, which can lead to inflammation throughout the body. The entry of oral bacteria into the bloodstream, where they can travel to other organs and tissues. There are a number of things that can be done to prevent oral dysbiosis and reduce the risk of CVD, including: Good oral hygiene, such as brushing and flossing twice a day. Regular dental checkups and cleanings. Avoiding tobacco use. Eating a healthy diet.

Conclusions. The evidence is growing that oral dysbiosis is a risk factor for CVD. Further research is needed to better understand the mechanisms involved and to develop effective interventions for prevention and treatment. The following are some other important points: The role of oral dysbiosis in CVD is likely to be complex and involve multiple factors. The effects of oral dysbiosis on CVD may vary depending on the individual's overall health status and other risk factors. More research is needed to determine the optimal methods for preventing and treating oral dysbiosis in order to reduce the risk of CVD.

Keywords: cardiovascular diseases; infection; inflammation; oral microbiota
Introduction

The human oral cavity is the main pathway for microorganisms to enter the human body [1]. The oral microbiota is considered to be the most diverse and numerous right after the intestinal system microbiota. This diverse oral microbiome includes more than 700 species of bacteria [2]. Due to the colonization by microorganisms of different areas of the oral cavity, such a high variability of the oral microbiota is observed [1]. The entire community of bacteria colonizing the oral cavity plays an important role in maintaining the homeostasis of the body. Any changes in the abundance and diversity of microorganisms lead to various oral pathologies. Oral dysbiosis is influenced by a number of factors starting with diet, oral hygiene, smoking, tobacco, ending with non-modifiable factors such as impaired salivary glands or congenital genetic defects [3]. Primary oral infections namely dental caries and periodontitis are considered to induce disease entities leading to dysbiosis of the oral microbiota [4]. All of these conditions can lead to inflammation and, what is more, to chronic inflammation. In turn, the inflammation that persists in the human body is due to effective bacterial impairment of the host immune response [3,5]. It is widely known that oral bacteria infection is a risk factor for endocarditis. Moreover, in recent years, much attention has been paid to the impact of dysbiosis on the overall health of the whole body and, in particular, on the impact of CVD [5,6]. A number of studies have indicated links between disorders of the oral microbiome and the incidence of CVD risks such as atherosclerosis and coronary artery disease (CAD).

Physiological elements concerning the oral microbiota

The oral microbiota is defined as a complex ecosystem of diverse bacteria, fungi or viruses [7]. The oral microbiome has been classified as the second largest microbial community found in the human body [8]. Ecological niches are divided into salivary, tongue, tooth surface, gingiva, oral mucosa, palate and subgingival/subgingival areas. Due to the colonization of several areas, the microflora is made up of different species and the different sections show varying levels of microflora activity, as well as having different susceptibility to causing disease [9]. Bacteria are the predominant microorganisms inhabiting the oral microflora [7]. There are 700 species in the human oral cavity, which contribute to the maintenance of the normal physiological environment of the oral cavity and at the same time affect the health of the host [6]. In turn, there are six main types residing in the oral microflora: Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Fusobacteria, Spirochaetes [6,10,11].
Potential types of bacteria have been assigned to each ecological niche [Fig 1.]. However, the composition of the oral microflora is not fixed, it dynamically evolves with the host. There are a number of factors that determine the variability that occurs in each area. The oral microflora can be modulated by external as well as internal factors. Factors modulated by the host include diet, oral hygiene, smoking or the use of pharmacotherapy. In contrast, independent causes of changes in microflora diversity are host immune response, hormonal fluctuations or genetics [12]. The oral microbiome is not only single-species organisms, it is also a multicellular bacterial structure encapsulated in an extracellular polymeric substance referred to as a bacterial biofilm [13].

Microorganisms in the biofilm can communicate using "quorum sensing" system [14]. With this system, microorganisms easily colonize the host and exhibit adaptive features to changing environmental conditions. The biofilm is also resistant to the host's immune response [15]. These characteristics contribute to the virulence and pathogenic potential of bacteria [16]. The oral microbiota in the form of a biofilm also has important functions in maintaining the body's homeostasis. The role of the microbial community is wide-ranging, including metabolic function through, for example, control of fat storage and general influence on metabolic regulation. The gut microbiota also influence the immune response through the formed barrier of the skin and mucous membranes. It also contributes to the balance between inflammatory and anti-inflammatory processes, and prevents the growth of pathogenic microorganisms (it exhibits antibacterial activity). The oral microbiome, through its functions, is an integral element in maintaining the body's homeostasis. A disturbance in the diversity of microorganisms residing in the oral cavity can contribute to the development of disease [10].

The oral microbiota is a complex ecosystem of diverse bacteria, fungi, and viruses that inhabit the mouth. It is the second largest microbial community found in the human body, after the gut microbiota. The oral microbiota is made up of a variety of species, each with its own unique role to play. Some bacteria are beneficial, helping to keep the mouth healthy by breaking down food, preventing the growth of harmful bacteria, and stimulating the immune system. Other bacteria can be harmful, causing diseases such as tooth decay and gum disease.

The composition of the oral microbiota is constantly changing, influenced by a variety of factors, including diet, oral hygiene, smoking, and medications. Stress and hormonal changes can also play a role. The oral microbiota is not only important for maintaining oral health, but it also has a number of other functions, including: Regulating
the immune system; Producing vitamins and other nutrients; Breaking down food; Protecting against harmful bacteria; Preventing tooth decay and gum disease. A healthy oral microbiota is essential for overall health. Disturbances in the diversity of microorganisms residing in the oral cavity can contribute to the development of disease.

Fig.1. Selected predominant bacteria of the microbial community at specific oral sites based on [9,17,18].

**Oral dysbiosis**

An imbalance in the diversity of species inhabiting the oral cavity has been termed oral dysbiosis. Changes in the composition of microorganisms present in the oral cavity correlate with the occurrence of systemic diseases including cardiovascular diseases. The oral microbiome is an ecosystem composed of many different microorganisms. Bouzid et al. [19] conducted a metagenomic study of the saliva microbiome using next-generation 16S ribosomal RNA (rRNA) sequencing techniques. They used oral microbiome profiling among 20 people with CAD and among 10 people without atherosclerotic plaques in the carotid arteries, but after stroke or myocardial infarction. The study conducted showed the same composition of the oral microbiota of patients and healthy control subjects.

Representative microorganisms forming the saliva microbiome included: Streptococcus, Veillonella, Granulicatella, Selenomonas, Neisseria, Haemophilus, Prevotella, Porphyromonas, Fusobacterium, Leptotrichia. However, the researchers noted
that the Eikenella microorganism affected CAD status. They also found a significant negative correlation between syntactic score and Eikenella count in patients with CAD. This study highlights that Eikenella count in oral samples from CAD patients compared to controls may be an important indicator of CAD progression. Numerous studies confirm the association between oral dysbiosis and the severity of cardiovascular disease. Oral dysbiosis induces periodontal and plaque disease which eventually leads to more serious diseases. A study conducted on patients with abdominal aortic aneurysm (AAA) examined the correlation between the severity of periodontitis and abdominal aortic aneurysm. Among the 60 patients studied, Porphyromonas gingivalis overgrowth was observed in saliva and supragingival and subgingival sites in all patients.

In addition, an increase in Porphyromonas gingivalis abundance has been shown to correlate with the diameter and volume of AAA [17]. In particular, patients with periodontal disease and atherosclerosis are thought to have more specific key pathogens, such as Streptococcus mutans and Porphyromonas gingivalis [20,21]. The bacterium Porphyromonas gingivalis also represents one of the factors in atherosclerotic cardiovascular disease (AVCD). Periodontal disease is a source of this pathogenic bacterium. Atherosclerotic plaque fragments (AVCD) were collected from 23 patients with detectable DNA. The study shows that the highest prevalence of periodontal pathogen DNA detection was observed in P gingivalis (82.61%) followed by Actinobacillus actinomycetemcomitans (60.87%) [21]. One of the biggest risk factor contributing to the global burden of disease is elevated blood pressure. [22]. A study by Gordon et al. examined the correlation between changes in the composition of microorganisms inhabiting the oral cavity and the incidence of arterial hypertension (AH). The study included 446 postmenopausal women. Three groups of patients not taking medication were divided based on blood pressure (BP) values. The first group consisted of patients with normal BP, the second group were patients with elevated BP/hypertension in stage I and the third group were patients with stage II hypertension. The last group was represented by patients taking antihypertensive pharmacotherapy. The 65 bacterial species showed a significant difference in abundance in patients with elevated BP or those taking antihypertensive drugs relative to patients with normal BP values. After accounting for multiple testing, the most significant microorganisms among the 65 species were found to be two species, Prevotella Oral and Streptococcus Oralis. The incidence of these two species in women taking antihypertensive drugs was lower than in women with normal BP [23]. The loss of bacterial balance in the
oral cavity, the formation of bacterial clusters in the form of biofilm, can lead to the
development and exacerbation of cardiovascular disease states.

Oral bacterial biofilm has been linked to cardiovascular disease [Table 1]. The
biofilm includes communities of many different microorganisms. The three-dimensional
structure of the biofilm acquires resistance to environmental factors (such as antibiotics),
cause low levels of immune system activation. These factors cause systemic inflammation.
[24,25]. The penetration of bacteria into the walls of blood vessels and their transport
thanks to the presence of phagocytes are ways by which the oral microbiome launches
attacks on blood vessel tissues. These attacks induce acute vascular inflammation leading to
cardiovascular disease [2,26]. Periodontal bacteria such as Streptococcus sanguinis and
Porphyromonas gingivalis are massively implicated in cardiovascular disease [2].
The main aetiological agent of periodontal disease is P. gingivalis. This bacterium, through
the production of lipopolysaccharide, which has the ability to bind to adhesion molecules
such as IL-8, an intercellular adhesion molecule 1, blocks polymorphonuclear leukocytes.
Another pathogenic feature that contributes to CVD is the secretion of the protease
gingipain R. Gingipain R causes activation of factor X, prothrombin, protein C, resulting in
thrombosis and intravascular clot formation [27]. Bacteria enter the oral cavity, are carried
by saliva and colonize the gastrointestinal tract. The reason for intestinal dysbiosis among
HF patients is increased intestinal permeability, which causes intestinal bacteria to migrate.
These actions result in the development of inflammation [28]. Endotoxins secreted by
bacteria residing in the intestines penetrate the circulation through leaky membranes.

Compounds secreted directly by bacteria can also enter the circulation spontaneously
[30]. A cell wall component of Gram- negative bacteria such as Tannerella forsythia,
Porphyromonas gingivalis and Treponema denticola is lipopolysaccharide (LPS), and its
levels are elevated in patients with heart failure [25,29]. LPS affects the production of pro-
inflammatory cytokines [29]. Leaky periodontal vasculature can lead to the movement of
bacterial-derived toxins into the systemic circulation, thereby driving increased expression
of adhesion substances, cytrullination of host proteins and increased lipid peroxidation, all
of which are proatherosclerotic [25]. In their study, Nakano et al, examined a group of 203
patients, some of whom had 82 aortic valve specimens, 35 mitral valve wall specimens as
well as 86 aortic aneurysm wall swabs, 16 of which contained aneurysmal thrombus. In
addition, 58 plaque samples were collected from patients who had their aortic aneurysm
removed or their heart valve replaced. The analyses showed that oral bacterial species, such
as S. mutans and A. actinomycetemcomitans, could be potentially be factors in the
occurrence of CVD [30]. Viridans group streptococci (VGS) and Staphylococcus spp. are microorganisms located in the oral cavity that are the most common bacterial sources of aortic valve inflammation (IE). The presence of VGS is found in 50-80% of positive blood cultures in patients with IE, while Staphylococcus aureus is responsible for 20-36% of positive tests [31–33].

A study by Lysek et al. also looked at the microorganisms Porphyromonas gingivalis. This study aimed to determine the effect of past myocardial infarction (MI) on the severity of periodontal disease (CP) and to determine the titer of protease antibodies secreted by Porphyromonas gingivalis. Ninety-seven post-MI patients and 113 high-risk patients while without a history of CAD were studied. As has been noticed, high titers of P. gingivalis gingipain were associated with an almost 3-fold increased risk of MI [34]. Nakano et al. in their study collected bacterial DNA from plaque and cardiovascular cells (aortic aneurysm wall samples and also aortic and mitral valve samples). The studies show that Streptococcus mutans and Aggregatibacter actinomycetemcomitans were found to be the main oral bacterial species involved in the development of CVD [30].

Current possible approaches or treatments

Diet

The microorganisms present within the oral cavity can freely pass through the gastrointestinal tract via the salivary glands, leading to cardiovascular diseases [3]. Significant differences have been noted between farmers, vegetarian hunters and people on a Western diet in terms of oral microflora [35]. The prevalence of Haemophilus and Neisseria varies between hunters and people on a Western diet. A meat-rich diet is a favorable environment for oral pathogens, so hunters are a higher risk group for oral diseases. Studies in recent years have shown that dietary patterns are important for maintaining the balance between major species [36]. Another aspect worth mentioning is the effect of alcohol polyphenols on alleviating inflammation caused by periodontal pathogens. An excellent source of this compound is apples, cherries, grapes or red wine. In a study by Agric et al. proved that the phenolic compounds of wine (caffeic acid and p-coumaric acid) and the extract of red wine and grape seeds show potential activity against periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis [37]. A healthy diet can help to promote the growth of beneficial bacteria and inhibit the growth of harmful bacteria. A diet rich in fruits, vegetables, and whole grains is ideal.
The effect of diet on the oral microbiota is very interesting. It is important to note that the composition of the oral microbiota is influenced by a variety of factors, including diet, oral hygiene, smoking, and medications. Stress and hormonal changes can also play a role.

**Wine**

The phenolic compounds in wine and grape seeds may have potential activity against periodontal pathogens. This is an exciting area of research, and it is possible that dietary interventions could be used to help prevent or treat oral diseases in the future.

**Hygiene**

Daily oral hygiene may be one of the key ways to prevent periodontal disease and, consequently, inflammatory diseases [38]. Regular and effective tooth brushing, the use of floss or interdental brushes and an irrigator, and the use of an oral rinse maintains normal oral microflora [34]. Good oral hygiene, such as brushing and flossing twice a day, can help to remove plaque and bacteria from the teeth and gums.

Dental surgery: In some cases, dental surgery may be necessary to remove plaque and tartar buildup, or to treat gum disease.

**Vaccines**

A therapeutic response against the immune response of P. gingivalis is the parenteral or intraoral administration of a vaccine with an immunogen directed against bacterial virulence factors, such as gingipain proteinase. Chimera (KAS2-A1) induces IgG1 antibody production and type 2 T helper cell response, a method of preventing periodontitis caused by P. gingivalis [37]. A therapeutic response against the immune response of P. gingivali

One therapeutic approach to address the immune response of P. gingivalis is the parenteral or intraoral administration of a vaccine with an immunogen directed against bacterial virulence factors, such as gingipain proteinase.

Gingipains are enzymes produced by P. gingivalis that are involved in the breakdown of connective tissue in the gums. They are also thought to be involved in the activation of the immune system, which can lead to inflammation and tissue damage.

A vaccine that targets gingipains could potentially prevent or slow the progression of periodontitis. One such vaccine, called chimera (KAS2-A1), has been shown to induce IgG1 antibody production and a type 2 T helper cell response. This response is thought to be protective against periodontitis caused by P. gingivalis.
Other potential vaccine targets include fimbriae, which are hair-like structures that *P. gingivalis* uses to adhere to teeth and gums, and lipopolysaccharide (LPS), a component of the bacterial cell wall that can trigger an inflammatory response.

The development of a successful vaccine against *P. gingivalis* is a complex and challenging task. However, it is an area of active research, and there is hope that a vaccine could be developed in the future to prevent or treat this common and debilitating disease.

**Antibiotics**

Antibiotics can be used to treat infections caused by harmful bacteria. However, they should only be used when necessary, as they can also kill beneficial bacteria.

**Transplantation of oral microbiota**

Considering the high popularity and effectiveness of fecal microbiota transplantation in the treatment of many gastrointestinal disorders, also transplantation of the oral microbiome may prove very useful in the treatment of oral diseases. Although this theory has yet to be tested on humans [38].

The oral microbiome is also a complex ecosystem of bacteria, fungi, and viruses. Disturbances in the oral microbiome have been linked to a variety of diseases, including tooth decay, gum disease, and oral cancer. It is possible that oral microbiota transplantation (OMT) could be used to treat oral diseases. OMT would involve transferring healthy oral bacteria from a donor to a patient with an oral disease. OMT is still in the early stages of research, but it has the potential to be a new and effective treatment for oral diseases.

**Nanoscale drug delivery system**

Microorganisms are producing more and more resistance mechanisms to the antimicrobial agents and antibiotic therapy used. Recent scientific discoveries have focused on nanoscale drug delivery systems (nano-DDs) and can eliminate limitations of bacteriocins, such as immunogenicity or their sensitivity to proteases. Nano-DDs can affect the solubility and persistence of bacteriocins. In addition, they help bypass the immune system and host response, improving the action of bacteriocins against bacterial resistance mechanisms. In conclusion, nano-DDs enhance the overall antimicrobial activity of bacteriocins, thus making this compound a promising new therapeutic direction [39].

**Probiotics and prebiotics**

It is also worth mentioning the use of probiotics and prebiotics for dysbiosis of the oral microflora. The issue of the action of beneficial bacteria on inflammation at the level of the periodontium and, at a further stage, the action of oral bacteria in the prevention of
systemic diseases has become an interesting aspect [40]. One of the main factors in the development of periodontal inflammation, dental caries is oral dysbiosis. Esteban-Fernández et al. conducted a study on Streptococcus dentisani. The authors reported that S. dentisani was abundant in the gingival sulcus. The results of the study indicate an inhibitory effect of S. dentisani supernatant on periodontal pathogens and more specifically on P. gingivalis and F. fusionum. The mechanism of action of S. dentisani is to block periodontal pathogens through the mechanisms of competition, adhesion and displacement. This oral probiotic also exhibits anti-inflammatory effect through an increase in IL-10 cytokine levels, additionally an inhibitory effect on interferon-γ expression has been observed [37].

Probiotics are live bacteria that can be beneficial for health. They can be taken as supplements or found in some foods, such as yogurt.

**Fluoride**

Fluoride can help to strengthen teeth and make them more resistant to decay.

**Antimicrobial peptides**

Antimicrobial peptides (AMPs) are part of the innate immune system in many species. They are molecules composed of 12 to 100 amino acids with extracellular effects. More than 1,700 AMPs are known; some of these compounds can eradicate bacteria and maintain oral homeostasis. They can also interfere with the host immune response through immunomodulatory properties [41]. These AMPs can be used as targeted therapies for specific oral bacteria. Among them, the most hopeful are leucine-leucine-37 and alpha-defensins [42].

**Applications**

Research conducted on the oral ecosystem can play an important role in discovering the relationship between the oral microbiota and maintaining human health. Violation of the harmonious interaction of the oral microflora with the body leads to a pathological state, the most common symptom is the process of caries, as well as periodontal and oral mucosal diseases. Oral dysbiosis leads to many CVD diseases. The link between an imbalance of oral microorganisms and CVD is confirmed by a growing number of epidemiological studies, systematic reviews and scientific research. Systemic exposure of oral bacteria has been shown to initiate and exacerbate CVD mainly through activation of inflammatory processes. Focusing on the prevention of periodontal disease by targeting the oral microbiome can achieve a beneficial impact on patients' health. Given the high incidence of periodontal disease, caries and the potential impact on CVD, it is important to further identify the mechanism underlying oral dysbiosis and the CVD link. Further uncovering the
role of the oral microbiome in CVD may lead to the development of new approaches to CVD prevention and treatment.

Oral dysbiosis, or the imbalance of bacteria in the oral cavity, has been linked to an increased risk of CVD. Several mechanisms have been proposed to explain how oral dysbiosis may contribute to CVD, including: The production of inflammatory molecules by oral bacteria. The activation of the immune system, which can lead to inflammation throughout the body. The entry of oral bacteria into the bloodstream, where they can travel to other organs and tissues. There are a number of things that can be done to prevent oral dysbiosis and reduce the risk of CVD, including: Good oral hygiene, such as brushing and flossing twice a day. Regular dental checkups and cleanings. Avoiding tobacco use. Eating a healthy diet.

Conclusions. The evidence is growing that oral dysbiosis is a risk factor for CVD. Further research is needed to better understand the mechanisms involved and to develop effective interventions for prevention and treatment. The following are some other important points: The role of oral dysbiosis in CVD is likely to be complex and involve multiple factors. The effects of oral dysbiosis on CVD may vary depending on the individual's overall health status and other risk factors. More research is needed to determine the optimal methods for preventing and treating oral dysbiosis in order to reduce the risk of CVD.

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