Diseases linked to the bacterium *Porphyromonas gingivalis*

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SVEUČILIŠTE U RIJECI

ODJEL ZA BIOTEHNOLOGIJU

Prijediplomski studij

"BIOTEHNOLOGIJA I ISTRAŽIVANJE LIJEKOVA"

Lucia Fabijančić

Bolesti povezane s bakterijom *Porphyromonas gingivalis*

Završni rad

Mentor: doc. dr. sc. Željka Maglica

Rijeka, 2023.

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Abstract

Porphyromonas gingivalis is a Gram-negative bacterium that is classified as an oral pathogen and, together with the bacteria *Treponema denticola* and *Tannerella forsythia*, forms a complex that promotes periodontal diseases such as periodontitis and gingivitis. The main virulence factors of *P. gingivalis* are cysteine proteinases, gingipains, fimbriae, capsule, lipopolysaccharide layer, and outer membrane vesicles. Some of these virulent factors promote the recruitment of the innate and acquired immune system cells, but still, *P. gingivalis* can penetrate the bloodstream and potentially participate in the promotion of other diseases such as cancers, cardiovascular diseases, metabolic disorders, and neurological diseases. Molecular mechanisms that can affect the occurrence of these diseases mostly include the activation of the pro-inflammatory response via the NF-κB pathway. Although there are indications of a connection between the bacterium *Porphyromonas gingivalis* and the previously mentioned diseases, their cause-and-effect relationship has not yet been confirmed. However, the possibilities of using drugs that target the removal of the *P. gingivalis* bacterium as a potential method of suppression or prevention of the mentioned diseases should be researched.

Keywords: *Porphyromonas gingivalis*, cancers, cardiovascular diseases, metabolic disorders, neurological diseases

Sažetak

Porphyromonas gingivalis je Gram-negativna bakterija koja se klasificira kao oralni patogen te zajedno s bakterijama *Treponema denticola* i *Tannerella forsythia* čini kompleks koji potiče parodontne bolesti kao što su parodontitis i gingivitis. Glavni faktori virulencije *P. gingivalis* su cisteinske proteinaze, gingipaini, fimbrije, kapsula, lipopolisaharidni sloj i vezikule vanjske membrane. Neki od tih virulentnih faktora potiču regrutaciju stanica urođenog i stečenog imunosnog sustava, no ipak, *P. gingivalis* može prodrijeti u krvotok te potencijalno sudjelovati u promicanju drugih bolesti kao što su tumori, kardiovaskularne bolesti, poremećaji u metabolizmu i neurološke bolesti. Molekularni mehanizmi koji mogu utjecati na pojavu tih bolesti većinom uključuju aktivaciju proupalnog odgovora imunosnog sustava preko NF-κB puta. Iako postoje indikacije o povezanosti bakterije *Porphyromonas gingivalis* i navedenih bolesti, još nije potvrđena njihova uzročno-posljedična veza. Ipak, treba istražiti mogućnosti uporabe lijekova koji ciljaju na uklanjanje bakterije *P. gingivalis* kao potencijalnu metodu suzbijanja ili prevencije navedenih bolesti.

Ključne riječi: *Porphyromonas gingivalis*, tumori, kardiovaskularne bolesti, poremećaji u metabolizmu, neurološke bolesti

Table of Contents

1. Introduction

The human oral cavity is a habitat for a broad range of microorganisms such as bacteria, viruses, archaea, fungi, and protozoa [1]. Around 1000 species of bacteria inhabit the oral cavity [1]. The five most abundant phyla of healthy people's oral microbiota are Actinobacteria, Bacteroidetes (which include the genera *Porphyromonas*), Firmicutes, Fusobacteria, and Proteobacteria [1]. Some of the species among these phyla, namely *Streptococcus mutans, Porphyromonas gingivalis, Tannerella forsythia, Actinobacillus actinomycetemcomitans*, and *Treponema denticola*, are recognized for their pathogenic nature and their ability to contribute to the development of various diseases that manifest in the oral cavity [1–3]. Due to the physiological changes such as pregnancy and aging, an individual's oral microbiota composition changes throughout their lifespan [1,2]. Furthermore, the microorganisms which inhabit the oral cavity exhibit variations in their abundance across different sites. For example, the microorganisms from the genus *Porphyromonas*, along with *Actinomyces, Prevotella, Streptococcus,* and *Veillonella* are known to coat the tongue surface [1]. This is important because the tongue's papillae can provide the necessary anaerobic environment [1]. Alongside that, the genera *Porphyromonas* is found also on the supragingival dental plaque in the experiment of Xu et al. [*Figure 1*] [2]. Dental plaque is a whiteish bacterial mass that is attached to the surface of the tooth [4]. Interestingly, the mass of the dental plaque has been observed to correlate with the severity of various oral diseases. [4,5]. Among all components of the oral cavity, the periodontium, and specifically, the gingival sulcus stand out as particularly susceptible to bacterial overgrowth and dysbiosis. Periodontium is the complex that allows the tooth to be connected to the jaw and, therefore, ensures the structural rigidity of the teeth [6]. It is composed of four tissues: the cementum, the alveolar bone, the periodontal ligament, and the gingiva [6]. The most common inflammatory diseases of the

periodontium are gingivitis and periodontitis which will be further described in the next two subheadings.

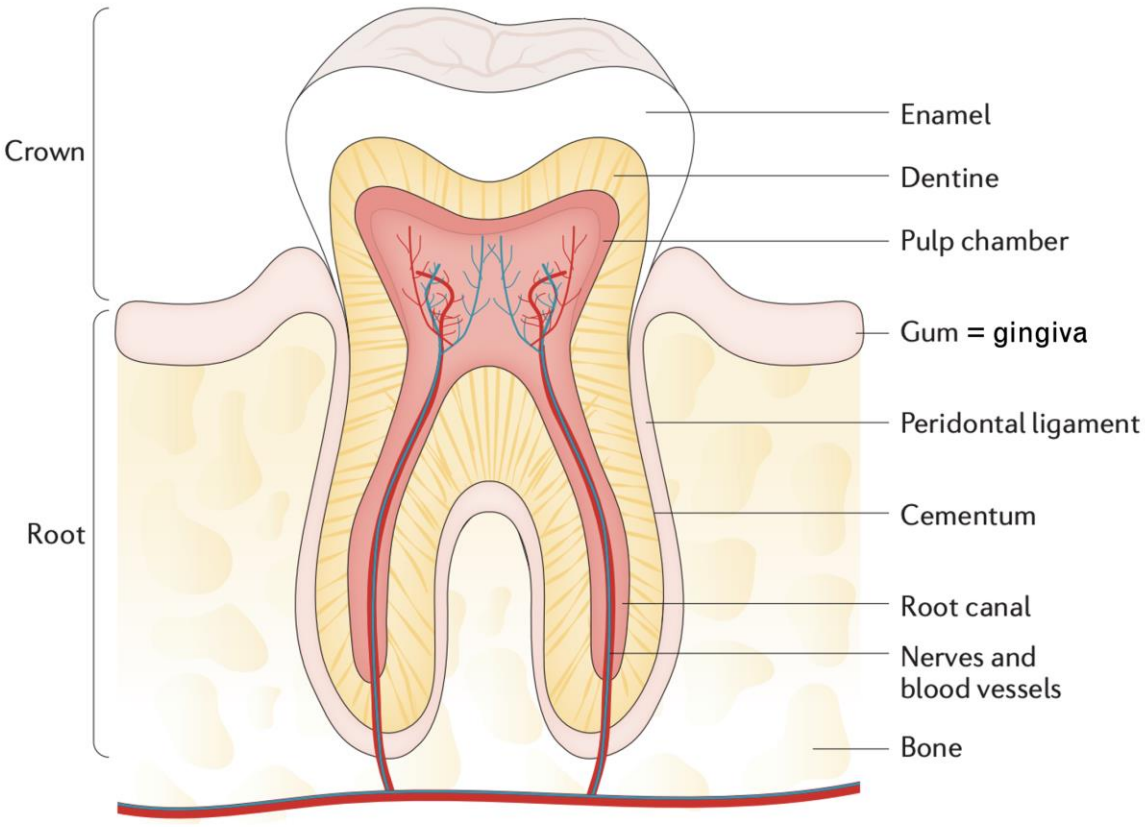

Figure 1. Anatomy of the normal tooth. Supragingival is the term used to easier describe the tooth above the gum (=gingiva). Subgingival is, on the other hand, the term that describes parts of the tooth below the gum. The picture is acquired and redesigned from Pitts et al. [3]

1.1. Gingivitis

Gingivitis is a plaque-induced, reversible inflammatory condition of the gingiva [7]. Gingival inflammation is initiated by dental plaque and calculus, calcified dental plaque [8,9]. Calcium phosphate mineral salts which are accumulated between and inside the remains of once-viable bacteria form dental calculus [9]. Among other things, calculus levels are affected by hygiene practices and, therefore, pose a major problem in cultures that cannot practice regular oral hygiene [9]. High prevalence can also be seen in more developed countries such as the USA and Denmark, where this

disease affects up to 60% of teenagers and 40-50% of adults [10]. Alongside that, the buildup of dental plaque can be caused by metabolic factors such as puberty or pregnancy, but also genetic and environmental factors like smoking and taking medications as well as systematic conditions (e.g., HIV infection) [11]. For example, metabolic factors such as excess glucose (hyperglycemia) can activate a proinflammatory cascade which enhances mitochondrial stress by activating RAGE receptors (receptor for advanced glycation end products) [11]. RAGE receptor is the receptor that enhances cell malfunction in some inflammatory disorders, tumors, and diabetes [12].

If left unchecked, gingivitis can eventually progress to periodontitis which is described as the destruction of connective tissue and alveolar bone [8].

1.2. Periodontitis

Periodontitis is a very frequent, irreversible condition, considered as a chronic inflammatory disease where connective tissue from the gingiva becomes destroyed and there is no more adhesiveness to the root surface [5]. The best representation of the broad prevalence of periodontitis is that over 47%, which is an estimated number of 64.7 million adults aged 30 or more in the USA, have periodontitis [10]. Almost the same distribution was observed for mild and severe periodontitis [10]. Certain "keystone pathogens" are known to be able to modulate the host's immune response and are considered responsible for switching the homeostatic state to dysbiosis [13]. One example of a such pathogen is *Porphyromonas gingivalis* which was shown to be present in the subgingival plaque of 85.75% of patients who have periodontitis, with studies showing that even a small amount can manipulate with commensal bacteria and, therefore, induce a state of dysbiosis [13]. This happens due to the accumulation of *P. gingivalis* on the tooth surface which leads to the formation of biofilm, and after that bacteria expand to the gingival sulcus and form periodontal

pockets [14]. Along with *P. gingivalis*, *Treponema dentic*ola and *Tannerella forsythia* are pathogens known for appearing in the red complex which occurs in the later stages of biofilm formation [*Figure 2*] [15]. *P. gingivalis* has a leading role in the progression of microbial imbalance and development of periodontitis, whereas *T. denticola* and *T. forsythia* act as pathobionts, which means that they contribute to the progression of the disease when homeostasis is disturbed [16]. The discovery of the red complex model led to the acceptance of periodontitis as a multi-microbe disease [13]. Representatives of the red complex are usually found along with the members of the orange complex and together they cause serious diseases, such as periodontitis [13]. On the other hand, there has also been some scientific interest in the connection of genetics and epigenetics with the onset of periodontal diseases [17]. For example, polymorphisms in genes IL1B, TLR4, VDR, etc. were found to be associated with aggressive periodontitis as well as an association in genetic polymorphisms in genes IL6, MMP-1, TLR4, IL1B, etc. with chronic periodontitis [17].

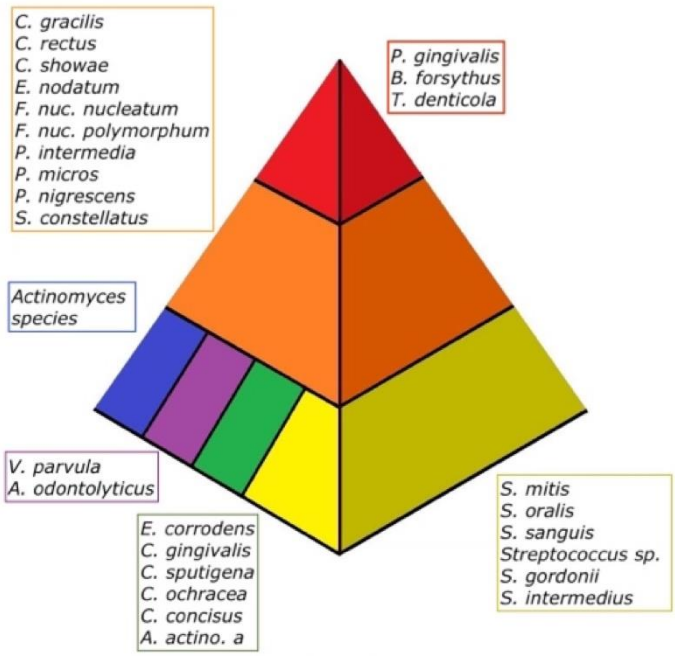

Figure 2. Pyramid of complexes that contribute to the progression of periodontitis and other diseases. Yellow, green, purple, and blue complexes are usually connected with a healthy oral cavity [15]. On the other hand, orange and red complexes contain bacteria that are harmful to the cavity, and they cause diseases. The picture is acquired and redesigned from Mohanty et al. [15]

2. Aim

This bachelor's thesis aims is to describe diseases potentially linked with the oral bacteria *Porphyromonas gingivalis,* other than periodontitis and gingivitis. The focus will be on reviewing the literature about diseases that could be linked with *P. gingivalis*, accompanied by an investigation into the possible underlying mechanisms of such connection. Specifically, this thesis will describe the interplay between *P. gingivalis* and prominent disease categories such as cancers, metabolic diseases, cardiovascular diseases, and neurological diseases. This thesis is trying to contribute to the growing knowledge about the bacterium and its potential contribution to disorders that are important for public health.

3. *Porphyromonas gingivalis*

Porphyromonas gingivalis is a Gram-negative, anaerobic, immotile, rod-shaped bacterium varying in size from 0.5 to 6 μm [*Figure 3*] [13,18]. This bacterium can be found in the oral microbiota of healthy individuals, but it also has a role in the development of the aforementioned disease called periodontitis [16]. As their primary source of metabolic energy, *Porphyromonas gingivalis* ferments amino acids due to limited access to sugars within the periodontal pocket [16]. Furthermore, the bacteria require heme to survive and they acquire it through the breakdown of host proteins, particularly hemoglobin. This is performed by lysine and arginine proteinases known as gingipains [16]. Gingipains are recognized as *Porphyromonas gingivalis'* virulence factors together with lipopolysaccharide (LPS), fimbriae, capsule, and others which will be presented in the next chapter.

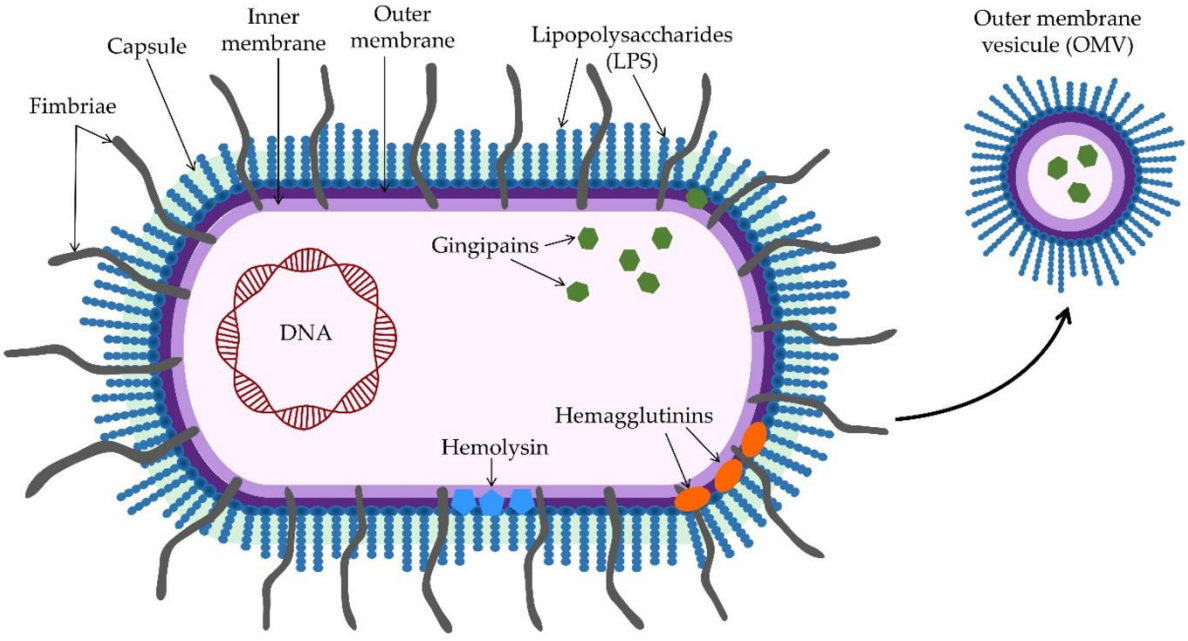

Figure 3. Schematic representation of the structure of *Porphyromonas gingivalis.* This picture also represents the secretion of some virulence factors such as outer membrane vesicles. The picture is acquired from Aleksijević et al. [19]

4. Virulence factors of *Porphyromonas gingivalis*

Virulence factors are molecules that can help to induce bacterial invasion and promote the expansion of the bacterial community [13]. They can help with coaggregation with other bacteria as well as biofilm formation [13,16]. When *Porphyromonas gingivalis* forms biofilm, antimicrobial drugs are 500-1000 times less efficient [19]. The fact that some virulence factors can manipulate the host's immune response, thereby, evading the clearance of the bacteria may contribute to this [13,16]. Some of the *P. gingivalis* virulence factors are gingipains, LPS, fimbriae, capsule, and outer membrane vesicles (OMVs) which are described in the next subheadings [13,16,19].

4.1. Gingipains

Gingipains are one of the major virulence factors of *P. gingivalis* [13]. They are cysteine proteinases, also known as "trypsin-like" enzymes which can hydrolyze peptide bonds at specific residues. Lysine-specific gingipains (Kgp) hydrolyze peptides at the C-terminus after lysine residues, whereas arginine-specific gingipains (Rgp) hydrolyze carbonyl groups of arginine residues [13,16,19]. Arginine proteinases can be divided into two groups: RgpA and RgpB [13,16,19]. RgpA has proteolytic and adhesion domains, whereas RgpB performs hydrolysis. Kgp together with RgpA is necessary for the coaggregation of *Porphyromonas gingivalis* with other bacteria. As said earlier, FimA is also important for the aggregation with other bacteria and, therefore, it is assumed that RgpA is somehow associated with the expression of FimA and RgpB [19]. RgpA is also important for the growth of *Porphyromonas gingivalis*, as well as the degradation of collagen [19]. Moreover, gingipains modulate the permeability of blood vessels by inhibiting blood clotting which leads to bleeding of periodontal tissues [13,19]. They can cause dysregulation of host's immune response by

degrading immune factors which usually eliminate pathogens such as α-defensins and ß-defensins, as well as cleave immune cell receptors such as CD14 which are located on the surface of macrophages [13,19]. Lastly, gingipains can break down complement components enabling the elimination of bacteria and, thus, increasing inflammation [13,19]. All of these strategies are used to evade the host's immune response and cause periodontitis [13,16,19].

4.2. Lipopolysaccharide

Lipopolysaccharide, or LPS, is the main component of Gram-negative bacteria's outer membrane [20]. In general, it is composed of three components: the O antigen, the core oligosaccharide, and lipid A. *Porphyromonas gingivalis* produce two types of LPS that differ in the most variable region, the O antigen polysaccharide [*Figure 4*] [21]. It can be composed of tetrasaccharide repeating units, and then it is called O-LPS, or it can have phosphorylated branched mannan called A-LPS [21].

LPS, especially lipid A is known as a pathogen-associated molecular pattern (PAMP) [13]. This is because it can activate the hosts' immune system by the stimulation of Toll-like receptors 4 (TLR4) and TLR2. Because of the variations in lipid A structure, LPS can activate different signaling pathways and, therefore, initiate different immune responses [13].

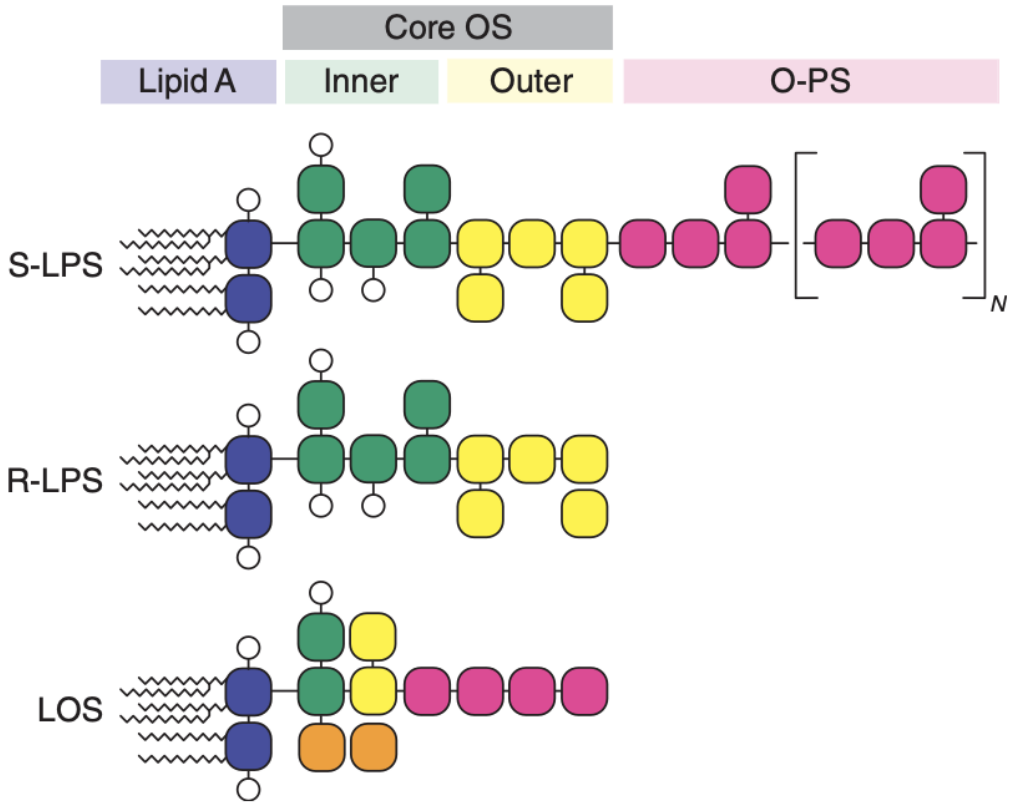

Figure 4. Types of lipopolysaccharides (LPS) in different bacteria [20]. S-LPS stands for "smooth" LPS and is composed of three components: lipid A, core oligosaccharides and O antigen. R-LPS is abbreviation for "rough" LPS and usually does not contain the O antigen (O-PS). LOS stands for lipooligosaccharides and are an important part of the pathogenesis of these bacteria because of the possibility to escape from immunologic surveillance due to high variations [22]. In the contrary, *Porphyromonas gingivalis* has APS (anionic distal polysaccharide) instead of O-PS [21]. The picture is acquired from Tran et al. [20].

4.3. Fimbriae

Fimbriae are *P. gingivalis*' major virulence factors extending from the outer membrane [*Figure 3*] [13,19]. They mediate the biofilm formation by binding to host tissue, cells, and other bacteria via proline-rich proteins, glycoproteins, fibrinogen, statherins, lactoferrin, and fibronectin [13]. Fimbriae are also important for bacterial motility and the invasion of bacteria into the cells [13]. *P. gingivalis* carry the long and short fimbriae which are comprised of the FimA subunit for long fimbriae and the Mfa1 subunit for short fimbriae [13,16]. Even though these subunits differ from each other by amino acid sequence, both are necessary for the pathogenicity of periodontitis [13].

FimA subunits first come out of the cells as lipoproteins, containing both lipids and peptide units [16]. Prior to the polymerization into fimbrial structures, FimA is proteolyzed by arginine gingipain protease. As said, FimA is a subunit of longer fimbriae which makes it perfect for accumulation with other oral bacteria such as *T. denticola*, *S. gordonii*, and *S. oralis* [16]. Also, FimA can activate TLR2 immune response by acting as an antigen. Therefore, immune cells produce IL-10, a cytokine important for the suppression of Th1 cells which are a part of the acquired immune system and are a subset of CD4+ T lymphocytes [13]. These long fimbriae can be up to 3 mm long and 5 nm wide, however, they vary in size from 41 to 49 kDa [13]. Smaller subunits, along with FimA, are FimC, FimD, and FimE. FimE is important for the association of FimC and FimD to FimA, while another protein (FimB) is responsible for anchoring and for the length of fimbriae [13]. Shedding from the surface can be caused by mutations and/or deficiencies in the FimB unit. The FimA subunit can be encoded with six different sequences of the *fimA* gene: type I, Ib, II, III, IV, and V [13]. In periodontitis patients, type I and II genes are the most abundant, and, therefore, have the strongest ability to adhere to the host surface [13]. On the other hand, type IV of the *fimA* gene encodes for poorly fimbriated bacterium which cannot so easily adhere to the surface [13].

Alongside the long fimbriae, short fimbriae usually contain Mfa1 protein subunits which can also vary in size from 60 to 500 nm in length [13]. As well as FimA, Mfa1 must be proteolyzed by gingipains before it can polymerize into short fimbriae [16]. As in the description of FimA, Mfa1 contains extra proteins called Mfa2, Mfa3, Mfa4, and Mfa5 [13]. The function of Mfa2 is to terminate the elongation and Mfa3 acts as a ligand to the host's receptors on the surface. Mfa3, Mfa4, and Mfa5 subunits all work together and cannot subsist without the other two [13].

4.4. Capsule and Outer Membrane Vesicles

A capsule forms an outer envelope outside of the bacterial cells and is usually composed of polysaccharides and water [13,16,19]. The role of the capsule is to be protective against the host's leukocytes, whilst non-encapsulated *P. gingivalis* is not that resistant to phagocytosis by host macrophages and dendritic cells [13,16,19]. Capsule is known as K antigen, meaning it can induce the host's immune response, as well as aggregate with other bacteria and form biofilm [13,19].

Outer membrane vesicles (OMVs) can consist of proteins, phospholipids, lipopolysaccharides, DNA, RNA, and periplasm components [16,19]. They are important for adaptation to stress, and for maintaining contact with other bacterial and host cells [19]. Outer membrane vesicles are important for the increase in survival of the bacteria [16,19]. They can carry drugs, antigens, noncoding RNAs, as well as heme by which OMVs can provide biofilm bacteria with needed nutrients [19].

5. Biofilm formation

In 1897, scientist James Leon Williams first detected biofilms in the oral microbiota [13]. He described them as an accumulation of several pathogenic bacteria which were adherent to the tooth's surface. Some of the bacteria that were found along with *P. gingivalis* were *Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Tannerella forsythia, Treponema denticola,* and *Prevotella intermedia* [13]. The current definition is that microbial biofilms are complex communities that adhere to surfaces and are enclosed in an extracellular matrix (ECM) they self-assembled [23]. Nowadays, it is widely known how biofilm formation progresses in time. First, early-colonizing bacteria must attach to each other or to the surface they are trying to cover with a biofilm [24]. This is followed by the attachment of later colonizers, which form microcolonies. The period of maturation follows, during which the biofilm grows and develops. Finally, the biofilm undergoes a process called biofilm dispersion in which cells are released from the structure into the surrounding media [24]. An important step in biofilm formation is the production of adhesins which are compounds included in ECM. Adhesins are considered bacterial virulence factors since they can attach to the surface and induce inflammation by releasing toxins [24,25]. The best-studied adhesins are fimbriae which are vital for *Porphyromonas gingivalis'* aggregation with other bacteria. As mentioned before, *P. gingivalis* can accumulate with *T. denticola, S. gordonii*, and *S. oralis*. The specific molecules responsible for the aggregation of these bacteria are still under investigation. For example, researchers are investigating if the dentilisin from *T. denticola* impacts binding to *P. gingivalis'* fimbriae. *S. gordonii* and *S. oralis* are also thought to bind to *P. gingivalis* fimbriae via glyceraldehyde-3-phosphate dehydrogenase (abbreviated as GAPDH) [13]. Mutants lacking *fimC, fimD*, and *fimE* bind less efficiently to the GAPDH of *S. oralis* or its type I collagen and fibronectin. The long fimbriae of *P. gingivalis* can bind to human GAPDH

and induce an immune response [13,19]. Furthermore, recent evidence suggests that short fimbriae may differ in the mechanism of biofilm formation from long fimbriae [13,19].

6. Diseases linked with the infection of *Porphyromonas gingivalis*

The release of virulence factors by *Porphyromonas gingivalis* can induce deleterious effects, potentially establishing a correlation between infection and a spectrum of diseases other than gingivitis and periodontitis [26]. This includes a large number of cancers such as oral cancer and esophageal cancer [26–32]. Alongside that, *Porphyromonas gingivalis* is also linked with some metabolic diseases such as diabetes and nonalcoholic fatty liver diseases (NAFLD) [26,33–39]. Along with that, scientists are trying to show the possible linkage between *P. gingivalis* and cardiovascular diseases as well as neurological diseases [5,14,19,26,40–53].

6.1. Cancers

Cancer can be described as an abnormal cell growth in any part of the human body, which means that the cells have lost the ability to stop their growth [27]. In 2018, there were 43.8 million people diagnosed with cancer in the last 5 years [54]. With ongoing rising prevalence, it is expected that by 2040 there will be 29.4 million cancer cases annually [54]. Regarding *Porphyromonas gingivalis*, Heikkilä et al. showed a correlation between periodontitis and cancer mortality in 68,273 patients over a period of 10 years [55].

6.1.1. Oral cancer

Oral cavity squamous cell carcinoma (OCSCC) is the most common type of cancer in the oral cavity. In adults, the transition to a mesenchymal cell state has been described as a process involved in fibrosis or cancer metastasis because of increased resistance to apoptosis and cytoskeleton reorganization [29]. Primary risk factors, among others, include tobacco,

alcohol consumption, and genetic predisposition. Main genetic factors known to impact the transition are SNAI1, SNAI2, Twist1, Twist2, ZEB1 (zinc-finger E-box-binding homeobox protein), and ZEB2 [30]. Recent studies have shown that infection with *Porphyromonas gingivalis* may increase the chance of OCSCC [26,28]. This can be due to bacterial virulence factors such as lectin-type adhesins, LPS, enzymes, and toxic products of metabolism which can initiate a process of transition from epithelial to mesenchymal cells [*Figure 5*] [29]. Exposure to bacterial virulence factors increases the phosphorylation of the GSK3ß enzyme which increases the ZEB1 levels [26,29]. Alongside that, ß-catenin and FOXO1 pathways are involved in the regulation of ZEB2 levels [26,30]. In addition, *Porphyromonas gingivalis* can inhibit cell apoptosis through the activation of PI3K/Akt and JAK/Stat pathways. Thus, the levels of anti-apoptotic factors are increased whereas the levels of pro-apoptotic factors are decreased [26]. Furthermore, intracellular *P. gingivalis* secretes NDK (nucleoside diphosphate kinase) which phosphorylates HSP27 thus inhibiting the release of cytochrome c and caspase-9 activation [26]. It also inhibits the ATP activation of P2X7 receptors which leads to the decreased production of mature IL-1ß, a pro-inflammatory cytokine, even though the concentration of IL-1ß on transcriptional levels is increased [26,56]. Along with that, the upregulation of B7-H1 receptors on oral cancer cells leads to the evasion of tumor cells from the immune system [26]. Interestingly, OCSCC patients have increased concentrations of pro-inflammatory cytokines such as IL-6, IL-8, TNF-α, IL-1ß, and IL-1α [57]. Goertzen et al. have shown that TNF-α can be involved in the upregulation of genes that are involved in the progression of cancer [57]. On the other hand, tumor invasion is increased with the upregulation of MMPs. Tumor proliferation is induced through the regulation of miR-21/PDCD4/AP-1 signaling pathway and the activation of NF-κB [26].

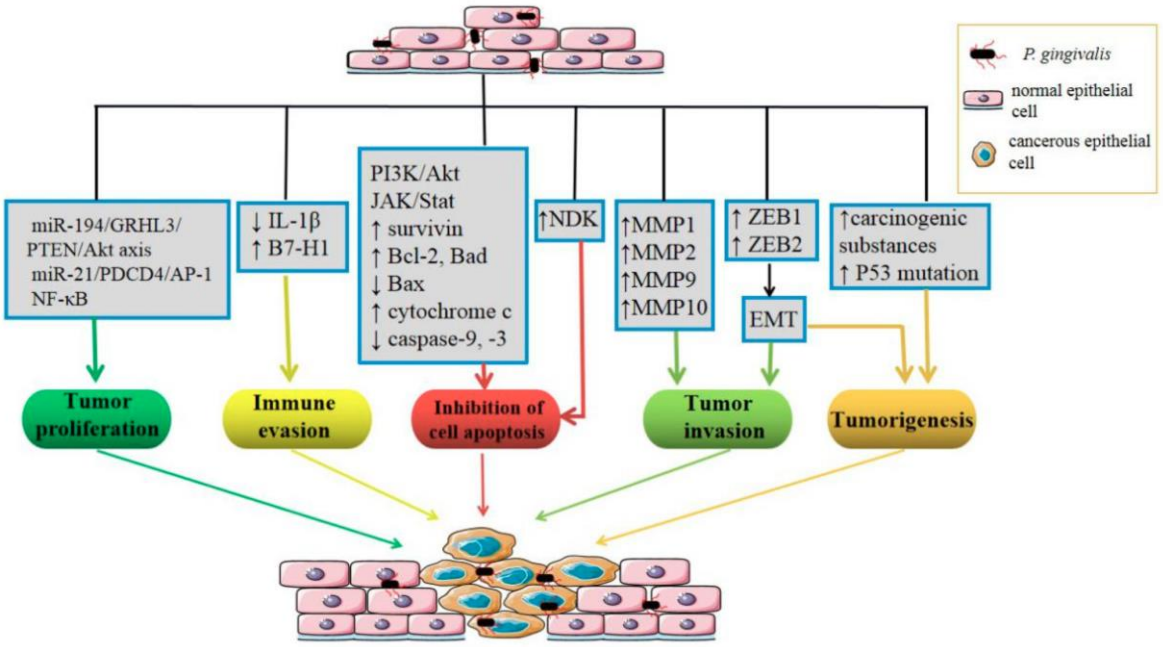

Figure 5. Contribution of *Porphyromonas gingivalis* in oral cancer. Biochemical pathways that affect the development of oral cancer. The picture is adapted from Mei et al. [26]

6.1.2. Esophageal cancer

Esophageal cancer is known as one of the most fatal and aggressive malignancies worldwide [26,31]. Due to the lack of early stages symptoms, this cancer has a 10% 5-year survival rate, and a 15-40% survival rate post-esophagectomy [31]. The main risk factors for this cancer are smoking, alcohol consumption, obesity, a "Western" diet, gastroesophageal reflux disease, age above 50, and some gene mutations [31]. Scientists have also found a positive correlation between infection with *P. gingivalis* and the risk of esophageal squamous cell carcinoma (ESCC) [26]. A possible underlying mechanism is the metabolism of ethanol by alcohol dehydrogenase into acetaldehyde which is its carcinogenic derivative. This may induce DNA damage which can produce DNA adducts and, thus, inducing gene mutations [26,31]. Another mechanism is the production of gingipains which induce pro-inflammatory mediators, such as MMPs. ECM alongside immune components such as C3 and C5 are being destroyed. This enables ESCC to escape from the surveillance of immune cells [32].

Additionally, as described before, NF-κB and miR-194/GRHL3/PTEN/Akt signaling pathways induce ESCC proliferation [26].

6.2. Metabolic diseases

Disorders connected with the abnormal metabolism of proteins, lipids, and glucose are included in the term metabolic disease [26]. The prevalence of these diseases is strongly increasing, with an estimated 415 million people living with diabetes. Unfortunately, it is thought that the actual prevalence is much higher [33]. In the following sections, we will present the link between *P. gingivalis* and diabetes as well as non-alcoholic fatty liver disease (NAFLD).

6.2.1. Diabetes

Diabetes is a chronic disease characterized by hyperglycemia due to the insufficient insulin action (type 2 diabetes), insulin secretion (type 1 diabetes), or both [26]. It is considered that periodontitis and diabetes have a two-way relationship, meaning that periodontitis is likely developed in individuals with diabetes and that people with diabetes have a high tendency to develop periodontitis [33]. The potential mechanisms behind this include increased levels of pro-inflammatory factors which can induce insulin resistance [26]. Virulence factors of *P. gingivalis* such as LPS and fimbriae activate macrophages and endothelial cells. Macrophages then increase the levels of IL-6, IL-1ß, and TNF-α, which are pro-inflammatory cytokines [34]. These cytokines then activate the NF-κB and MAPK pathways which alter the phosphorylation of IRS-1, therefore, the uptake of glucose is reduced and cells become more resistant to insulin [35]. Another pathway that leads to the resistance to insulin is the attenuation of the Akt and GSK3ß pathway [26]. When Akt is activated, it

phosphorylates GSK3ß thus inhibiting it [36]. In this case, when GSK3ß is inactivated, it increases glycogen synthesis, as well as insulin sensitivity [36,37]. On the other hand, when activated, it suppresses insulin-stimulated glycogen synthesis [37].

6.2.2. Non-alcoholic Fatty Liver Disease (NAFLD)

Non-alcoholic fatty liver disease is characterized by an abnormal fat accumulation in the liver [26]. It is one of the most prevalent chronic liver disorders with approximately 25% worldwide prevalence [38]. Risk factors for the progression of NAFLD and NASH (non-alcoholic steatohepatitis which is a necroinflammatory subtype of NAFLD) are obesity, insulin resistance, hypertension, alcohol, type 2 diabetes, and some genetic alterations [38]. As previously described, *Porphyromonas gingivalis* induces both insulin resistance and chronic inflammation. Therefore, it may be involved in the progression of NAFLD even though the exact mechanism connecting periodontitis and the progression of NAFLD is still not fully known [39]. The other possible explanation involves alterations in gut microbiota as a result of swallowing *P. gingivalis* [39]. Endotoxins and LPS from these bacteria can stimulate hepatocytes triggering the production of cytokines and reactive oxygen species. This leads to liver injury and can initiate nonalcoholic fatty liver disease [38,39].

6.3. Cardiovascular diseases

Cardiovascular diseases (CVDs) are a group of disorders that include blood vessels and heart [26]. They are one of the deadliest diseases worldwide, causing approximately 18.6 million deaths per year [26,40]. Implications that *Porphyromonas gingivalis* may be connected with various cardiovascular diseases rest on the fact that *P. gingivalis* was found in arterial tissues of patients with atherosclerosis [41].

6.3.1. Atherosclerotic cardiovascular diseases

Atherosclerotic cardiovascular diseases (ACVDs) are a type of CVDs that include stroke and coronary artery disease [14,26]. The development of atherosclerosis is a pathological clinical feature of ACVDs that occurs due to the buildup of lipids, calcium, macrophages, etc. in the arterial wall [26]. Interestingly, the exact mechanism by which *Porphyromonas gingivalis* can reach the heart and cardiovascular system is still not known, but it has been shown that the bacteria can survive inside the cells of macrophages, smooth muscle cells, epithelial, and endothelial cells [14]. Atherosclerosis involves a highly intricate pathogenesis mechanism where *P. gingivalis* influences multiple critical functions, thereby, affecting disease onset and progression [*Figure 6*] [26]. Firstly, fimbriae and LPS of *P. gingivalis* activate endothelial cells, leading to endothelial dysfunction via the NF-κB or p38 MAPK pathway thus suppressing apoptosis [26,42]. Moreover, *P. gingivalis* can induce oxidative stress and inflammatory response via the NF-κB-BMAL1-NF-κB loop worsening atherosclerosis [26]. Additionally, when *Porphyromonas gingivalis* is opsonized with IgG, it may bind to the FcγRIIa receptor which is located on the platelets [26]. This binding induces a procoagulant effect, leading to platelet activation and aggregation through the activation of GPIIb/IIIa integrin which connects to Hgp44 via the fibrinogen bridge [26]. Moreover, virulence factors of *P. gingivalis* support the migration of monocytes to the endothelial surface which induces the differentiation of monocytes to pro-inflammatory macrophages and foam cells, which are important factor for the progression of atherosclerosis [26]. This process involves the downregulation of ATP-binding cassette transporter A1 and activation of the c-JUN/AP-1 pathway upregulating CD36 expression and promoting lipid accumulation in macrophages and formation of foam cells [26]. Also, *P. gingivalis* may enhance TLR2-CD36/SR-B2 systemic release of IL-1ß further promoting the formation of foam cells and increased lipid uptake by macrophages [26]. Gingipains can induce lipid peroxidation which changes the expression of

low- and high-density lipoproteins contributing to the foam cell formation [19,26]. The proliferation of vascular smooth muscle cells can be induced by a higher expression of S100A9 due to endothelial cell injury, and through the ERK1/2-RUNX2 pathway [26].

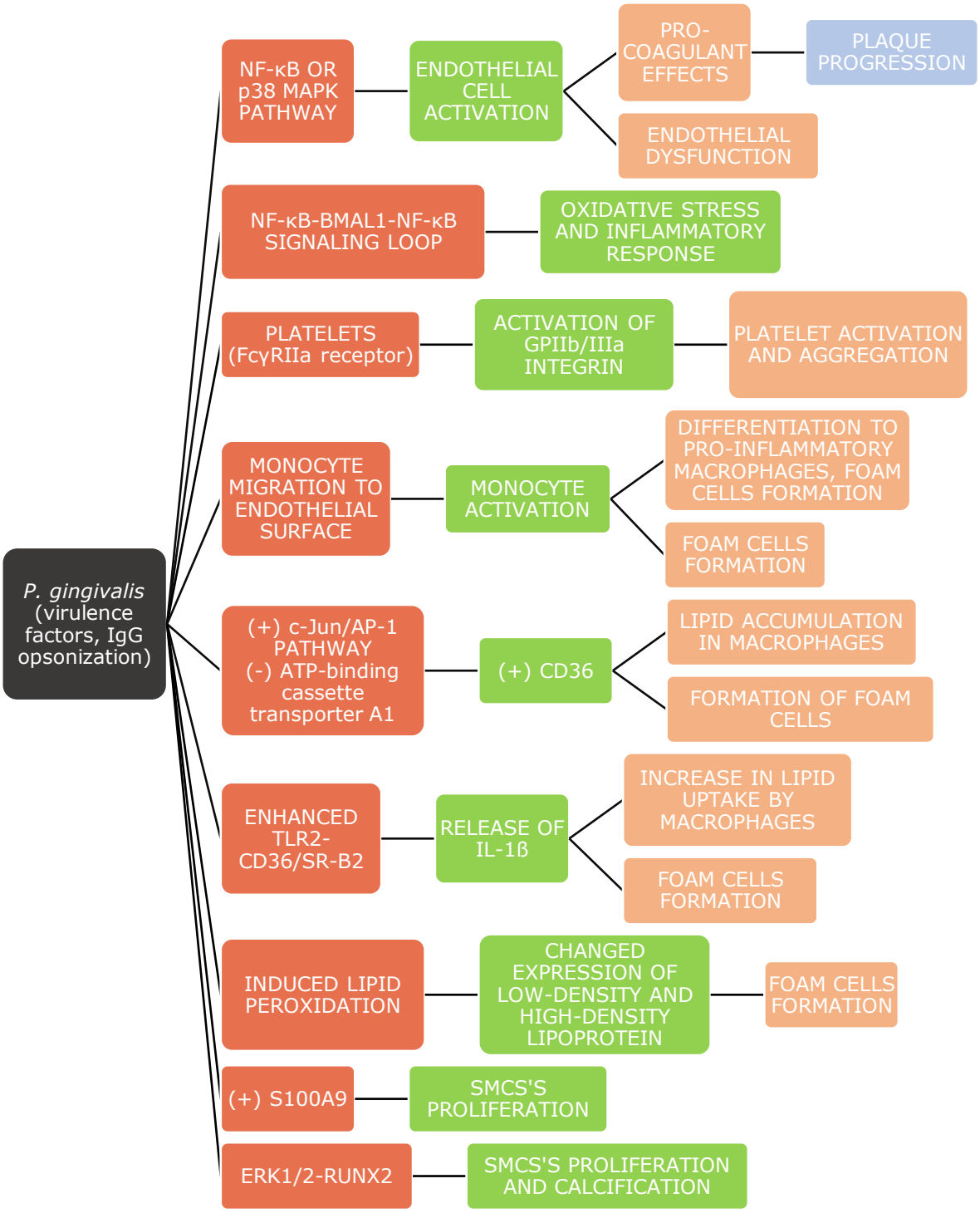

Figure 6. Molecular mechanisms by which P. gingivalis affects the development of atherosclerotic cardiovascular diseases. Data was taken from Mei et al. [26]

6.3.2. Myocardial infarction

Myocardial infarction, MI is a state of deficient blood flow into the heart caused by necrosis of cancer cells which results in the formation of scars [26]. *Porphyromonas gingivalis* was found in about 40% of samples from MI patients with atherosclerotic plaque [26]. Besides that, a mechanism behind the linkage of *P. gingivalis* and MI is still being investigated. Researchers have explored the mechanism of the affect on the cardiomyocytes which includes the activation of Bax and overexpression of metalloproteinase-9 (MMP-9) [19,26]. This leads to the apoptosis of cardiomyocytes and myocardial rupture due to a severe negative impact on their repair after MI [26]. This process is also encouraged by overexpression of high mobility group box 1 protein [26]. These proteins are known as nuclear proteins which can increase the inflammation from necrotic cells [43].

6.3.3. Abdominal Aortic Aneurysms

Abdominal Aortic Aneurysms (AAA) is a term used for a condition in which the abdominal aorta is wider than normal [26,58]. In this disease, experiments on rodents found that injection with *P. gingivalis* resulted in wider abdominal aorta [26]. There are multiple ways by which *Porphyromonas gingivalis* may promote AAA. One of them is the activation of TLR-2 which induces inflammation [26,58]. Alongside that, AAA growth can be induced by neutrophil extracellular traps, recruitment of neutrophils, and abdominal aortic aneurysms thrombus [26]. Also, as previously described, *P. gingivalis* can increase the expression of MMP [26]. Along with that, oxidative stress contributes to the development of AAA which may be triggered by the elevation of myeloperoxidase-DNA complexes [26].

6.3.4. Hypertension

Hypertension is a disease characterized by high systolic and/or diastolic blood pressure [44]. It is among the world's major causes of premature death [44]. Hypertension can be diagnosed when a person has systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg [26,44]. It was reported that *Porphyromonas gingivalis* induces the release of pro-inflammatory cytokines, inflammation mediators (C reactive protein, IL-6, and TNF-α), as well as angiotensin II in coronary artery endothelial cells [26]. This leads to the endothelial dysfunction and arterial hypertension [26]. In addition, studies are showing that immune response, especially Th1 can be responsible for elevated blood pressure [26].

6.4. Neurological diseases

The brain is an organ that is considered immunologically privileged due to the physical blood-brain barrier (BBB) [5]. Regardless of that, neurological disorders, especially dementia and Parkinson disease are in the top 15 diseases with an increase in the past decade as a global burden [49]. Together with dementia, Alzheimer disease, and Parkinson disease, other neurological disorders are multiple sclerosis, epilepsy, and headache disorders [49].

6.4.1. Alzheimer disease and other dementias

Alzheimer disease is a prevalent neurodegenerative condition that manifests in elderly individuals aged 65 years and above and contributes to nearly 2/3 of reported dementia in this age group. [48]. It is positioned among the top ten causes of mortality in the USA [48]. Alzheimer disease's pathogenic mechanism is primarily described as a neurodegenerative process that is characterized by the deposition of β-amyloid protein and

neurofibrillary tangles that result in cognitive and behavioral impairments [5,26,45,46,48,50].

Regarding *P. gingivalis*, Singhrao et al. have done experiments on mice where they infected them with the red complex bacteria and *Fusobacterium nucleatum* and demonstrated that *P. gingivalis* is capable of entering mice's brains [5]. Also, they have shown that pyramidal neurons in the hippocampus were opsonized with complement components suggesting the connection of *P. gingivalis* with inflammatory processes in Alzheimer disease [5]. Furthermore, there are several pathways by which *Porphyromonas gingivalis* can directly affect BBB causing neuroinflammation and possibly affecting the onset of Alzheimer disease [45]. These include the stimulation of peripheral nerves, entering through the blood circulation, stimulation with LPS, and excretion of pro-inflammatory cytokines [45,50]. It has been shown that LPS can increase IL-1ß and prostaglandin E2 levels in liver cells which consequently interact with nervus vagus [50]. Also, *P. gingivalis* is capable of entering the bloodstream through the periodontal pockets where gingipains induce the destruction of oral tissues [46]. Moreover, LPS or pro-inflammatory cytokines stimulate the production of TNF, therefore activating the NF-κB signaling pathway in areas of the brain that lack tight junctions and BBB [50]. Disruption of BBB starts when *Porphyromonas gingivalis* begins to activate innate immune signaling pathways through TLR-2 and TLR-4 mechanisms which initiate microglial activation [5]. Following their activation, microglia secrete IFN-γ and TNF-α cytokines which are known to have pro-inflammatory effects [5]. Alongside that, the destruction of neurons and persistent inflammation can be caused by the release of reactive oxygen and/or nitrogen species, chronic release of cytokines, complement system activation, TLR-2, and TLR-4 mechanisms [5]. Specifically, reactive oxygen and/or nitrogen species and complement system mechanisms are characteristics of inflammatory processes in Alzheimer disease and can cause the deposition of β-amyloid protein outside of the cells [5]. To further solidify this idea, a study performed by

Dominy et al. in 2019 observed *P. gingivalis* in the brains of postmortem Alzheimer's patients [51]. Furthermore, they found that the pathology and progression of the disease directly correlated with the gingipain load in the cortex [51].

6.4.2. Parkinson disease

Parkinson disease is a neurodegenerative disease that is described as a degeneration of dopaminergic neurons usually symptomaticlly visible as tremors [46]. In a couple of articles, it was shown that periodontitis and chronic periodontitis may be linked with the risk of Parkinson disease [52]. When reffering to non-motor symptoms, cognitive impairment is the biggest problem in people with Parkinson disease [46]. The exact molecular mechanism is still not known, but it has been assumed that it comes to the deposition of α-Synuclein. As for the pathology, it follows a similar principle to Alzheimer disease with neuroinflammation, and gut microbiota dysbiosis playing a central role [46]. Briefly, *P. gingivalis* from periodontal pockets may enter the bloodstream where it can induce systematic inflammation and bacteremia through the secretion of pro-inflammatory cytokines [46]. In activated macrophages, it stimulates the production of peripheral amyloid-ß which, together with LPS and gingipains, disrupts BBB and can ease the entry of those factors to the brain [46]. At the same time, *P. gingivalis* can enter the gut by swallowing which promotes gut dysbiosis leading to the accumulation of α-Synuclein throughout the vagus nerve [46].

6.4.3. Depression

Previous studies on depression were focused on the psychosocial effects of gingivitis and periodontitis, such as tooth loss and inadequate dental hygiene [26]. Even though depression is affected by social psychology, scientists are attempting to show that *P. gingivalis* can be linked with the

onset of this disease [26]. As known, *P. gingivalis* increases systemic inflammation by inducing TLR mechanisms which can be related to the fact that immunotherapy with IFN-α and IL-2 can lead to depressive symptoms in a large portion of patients [26,53]. The proposed biological mechanism behind this linkage includes astrocyte activation by *P. gingivalis* [26]. Also, *P. gingivalis* might downregulate neurotrophic factor receptor p75 which inhibits the maturation of brain-derived neurotrophic factor [26].

7. Discussion

Beside gingivitis and periodontitis, *P. gingivalis* has been linked to the onset of cancers, metabolic diseases, cardiovascular diseases, and neurological diseases. Many other diseases might be linked with *P. gingivalis* like chronic respiratory diseases, bone loss, and autoimmune and inflammatory diseases [17,26]. However, this thesis focused on diseases that are of utmost importance for the public health.

When searching through the literature, a great number of scientific articles have investigated the linkage between oral carcinoma and the infection with *P. gingivalis*. For example, one research showed that samples from gingival carcinoma had 33% more abundance of *Porphyromonas gingivalis* than in the normal gingival tissue [59]. Another meta-analysis study showed that patients infected with *Porphyromonas gingivalis* have a 1.6 times increase in the oral squamous cell carcinoma development [59]. On the other hand, the linkage between pancreatic, head, and neck cancer and *P. gingivalis* remains poorly researched.

More research is also needed on the connection between nonalcoholic fatty liver disease and *P. gingivalis*, while the linkage between diabetes and the aforementioned bacterium is well documented. Kang et al. showed that the severity of diabetes is increased because of periodontitis (which is caused by *Porphyromonas gingivalis*) [60]. Moreover, when patients get the corresponding treatment for periodontitis, their control over optimal concentrations of glucose is also improved [60].

Regardless of the recent advancements in understanding this bacterium and its impact on the host, recent studies remain unable to establish a causational link with the majority of the diseases. This has especially been proven as a challenge with complex multifactorial disorders such as Alzheimer and Parkinson disease [52]. This is why more studies are needed regarding the possible correlation between *Porphyromonas gingivalis* and the onset of these diseases which represent a rising problem in the world.

Available treatments for the eradication of *Porphyromonas gingivalis* include antibiotics metronidazole and amoxicillin [61]. However, excessive antibiotic usage represents a challenge for public health because it leads to the development of bacterial resistance. Some new therapies for combating bacterial infection include smart bacteria-responsive drug delivery systems [62]. These involve hydrogels, nanoparticles, nanospheres, micelles, multiple-layered films, titanium nanotubes, and scaffolds [62]. Some other ideas for combating the infection with *Porphyromonas gingivalis* is the replacement of more pathogenic strains with less pathogenic ones [52]. This can be done using gums and/or toothpaste containing less pathogenic strains of *P. gingivalis* and ionic toothbrushes which are more effective than manual ones in reducing plaque [63].

Moreover, new potential drugs can be based on attenuation or increasing the expression of some genes with miRNA. As said earlier, *Porphyromonas gingivalis* is, among other things, linked with diabetes through the inhibition of GSK3ß [36,37]. Potential miRNA can be designed in a way that it activates the transcription of this gene, and, therefore, increases insulin sensitivity. Alongside that, there are some mechanisms such as the secretion of pro-inflammatory cytokines which occurs after the infection with *P. gingivalis*. Along with that, the activation of the NF-κB pathway is common in described diseases. All of this may contribute to the development of new drugs to combat the onset of diseases potentially linked with *P. gingivalis*.

8. Conclusion

To conclude, *Porphyromonas gingivalis* is a Gram-negative, rod-shaped oral bacterium that has recently emerged as a plausible candidate potentially associated with the onset and severity of cancers, metabolic diseases, cardiovascular diseases, and neurological diseases, beside gingivitis and periodontitis. Alongside its virulence factors, the ability of *P. gingivalis* to induce inflammation through the secretion of pro-inflammatory cytokines by the host's immune system may have an impact on the onset of diseases. Nevertheless, it is crucial to acknowledge that these diseases may also manifest independently of the bacterium presence, with the causal link still unproven for the majority of diseases.

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Curriculum vitae

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Home: Marčeneško Polje 27, 52420 Buzet (Croatia)

EDUCATION AND TRAINING

High School Diploma
High School Buzet, General Gymnasium [09/2016 – 06/2020]

City: Buzet
Country: Croatia
Website: http://ss-buzet.skole.hr/

Digital marketing basics
Google digital garage [2018 – 2018]

From GLOBE to Erasmus+
Erasmus+ project [2019 – 2019]

City: Rotterdam
Country: Netherlands

Drug commercialization
Coursera; online course [2023]

City: San Diego
Country: United States

Laboratory demonstrator on the course of Microbiology
University of Rijeka, Department of Biotechnology [11/2022 – 12/2022]

University Undergraduate Study - Biotechnology and drug research
University of Rijeka, Department of Biotechnology [2020 – Current]

Address: 51000 Rijeka (Croatia)
Website: https://www.biotech.uniri.hr/hr/

INTERNSHIP

Hidrolab d.o.o
[07/2022 – 07/2022]

II was working at the analysis laboratory for waste, underground and surface water, waste, sediment, sludge, soil, and ecotoxicology.

International Center for Genetic Engineering and Biotechnology
[05/06/2023 – 31/08/2023]

I work in the laboratory for Functional Cell Biology under the supervision of Dr. Luca Braga. Upon coming to the lab, I joined a project focused on researching Idiopathic Pulmonary Fibrosis. The main interest of this project are ATI and ATII epithelial cells.

HONOURS AND AWARDS

Praise card

[11/09/2020]

Praise for dedicated work and remarkable results in regular education and extracurricular activities given by Buzet City Council.

Praise card

[27/07/2023]

The praise card is given for exceptional success and achievements in the academic year 2021/2022 by the Department of Biotechnology at the University of Rijeka.

CONFERENCES AND SEMINARS

1st symposium of Biotechnology students "PosteRi"

[2021]

International Darwin Conference

[2021]

Mosa Conference

[2022]
Link: http://www.mosa-conference.info/

2nd symposium of Biotechnology students "PosteRi"

[2022]

Joint ICGEB – ALS Society of Canada Symposium on Inflammation and Proteinopathy in ALS/FTD Spectrum Disorder

[2022]

Brain gut axis conference

[2022]

VOLUNTEERING

Croatian Red Cross

[Buzet, 2016 – Current]

I volunteered actions of voluntary blood donation and collection of needs for earthquake-stricken areas (December 2020). Also, I volunteered to help the pupils of Elementary school "Vazmoslav Gržalja" Buzet to master the school curriculum. Along with that, I participated in the inter-county Red Cross competition.

Association of Biotechnology students of the University of Rijeka (Udruga studenata Biotehnologije, USBRI)

[Rijeka, 2020 – Current]

- "Biotech" magazine
- one of the managers of the "Students-mentors" project
- PR team
- Conference "Future and Perspective"
- International conference "Darwin", India
- symposium "PosteRi"

LANGUAGE SKILLS

Mother tongue(s): **Croatian**

Other language(s):

English	Italian
LISTENING B2 **READING** B2 **WRITING** B2	**LISTENING** A2 **READING** A2 **WRITING** A2
SPOKEN PRODUCTION B2 **SPOKEN INTERACTION** B2	**SPOKEN PRODUCTION** A2 **SPOKEN INTERACTION** A2

Levels: A1 and A2: Basic user; B1 and B2: Independent user; C1 and C2: Proficient user

PUBLICATIONS

Mali rječnik buzetskih govora, "Small dictionary of Buzet speeches"
[2019]

One of the co-authors of the book.

DIGITAL SKILLS

MS Office (Word Excel PowerPoint) / Komunikacijski programi (Skype Zoom TeamViewer)

DRIVING LICENCE

Driving Licence: AM
Driving Licence: B