**Oral microbiota and Alzheimer's disease: Do all roads lead to Rome?**

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**Abstract**

Alzheimer's disease (AD) is a progressive neurodegenerative pathology affecting milions of people worldwide associated with deposition of senile plaques. While the genetic and environmental risk factors associated with the onset and consolidation of late onset AD are heterogeneous and sporadic, growing evidence also suggests a potential link between some infectious diseases caused by oral microbiota and AD. Oral microbiota dysbiosis is purported to contribute either directly to amyloid protein production, or indirectly to neuroinflammation, occurring as a consequence of bacterial invasion. Over the last decade, the development of Human Oral Microbiome database (HOMD) has deepened our understanding of oral microbes and their different roles during the human lifetime. Oral pathogens mostly cause caries, periodontal disease, and edentulism in aged population, and, in particular, alterations of the oral microbiota causing chronic periodontal disease have been associated with the risk of AD. Here we describe how different alterations of the oral microbiota may be linked to AD, highlighting the importance of a good oral hygiene for the prevention of oral microbiota dysbiosis.

**Keywords**: Alzheimer's disease; Amyloid-β; Oral microbiome; Periodontal disease.

Chemical compounds studied in this article Atorvastin (PubChem CID: 11473066); Chlorhexidine (PubChem CID: 9552079); Doxycycline (PubChem CID: 54671203); Epigallocatechin-3-gallate (PubChem CID: 65064); Rifampicin (PubChem CID: 135398735); Simvastatin (PubChem CID: 54454).

## Introduction

Alzheimer's disease (AD) is characterized by a progressive neurodegeneration, accounting for about 60-80% of total dementia cases worldwide [1,2]. AD may either be inherited, thus revealing underlying genetic causes (familial, or genetically driven AD, accounting for about 2-5% of total AD cases), or may be sporadic in nature (late onset or sporadic AD, about 95-98% of AD cases), associated with a wide range of causes and lifestyle-related risk factors, such as advancing age, nutrition, level of physicial activity, cognitive training, level of educational attainment, sleep quality, environmental pollutants, heavy metals, metabolic syndrome and cardiovadcular risk factors [3-5]. The pathological changes of AD slowly cause cognitive impairment that ultimately leads to dementia [6]. Senile plaques constituted by beta-amyloid protein deposits (Aβ), and neurofibrillary tangles composed of the hyperphosphorylated form of the microtubule-associated protein tau represent the most common and well described AD pathological features [7]. Studies have shown a strong link between Aβ and oxidative stress and neuroinfammation in the brain, which leads to the loss of neurons and progression of the disease [8]. In this age related disease, onset of Aβ production is believed to occur decades earlier (around the age of 40, while individuals are cognitively normal), leading to cognitive impairment around 60 years of age; considering the late manifestation of clinical symptoms, treatment approaches aimed at targeting already consolidated AD pathological features (e.g., removal of plaques in patients with full blown dementia) such as acetylcholine esterase inhibitors (AChEIs) and N-methyl-D-aspartate (NMDA) receptor antagonists have little efficacy in delaying disease progression. and cognitive impairment, providing at best palliative symptomatic relief of symptoms [9].

A strong correlation has recently been found between AD and oral microbiota. Microbes in the oral cavity (microbiota) mostly cause caries, periodontal disease, and edentulism in the aged population around the world [10]. However, oral pathogens in some circumstances can approach the brain, potentially affecting memory and causing dementia [11,12]. Various researchers have found a strong correlation between alteration of the microbial profile of the oral cavity and AD, with special regard to chronic periodontal disease [13]. Similar results have been previously described by Stein and coworkers (2012) [14], in which the two oral bacteria were linked with cognitive deficit 10 years later [14]. In 2015, Shoemark and Allen [15] demonstrated that the oral microbiota includes a group of amyloid producing microbes, and that this microbiome-derived amyloid could potentially promote AD.

Here we describe how pathogens of the oral microbiota may be linked to the onset and the risk of AD.

## 1. Oral microbiota

The oral cavity is an ecosystem consisting of many habitats: the lips, the tongue, the tonsils, the palate, the gingiva and the sub-gingival spaces. The presence of food residues, epithelial debris and secretions, along with a high degree of humidity and a controlled temperature, make these spaces extremely favorable for a great variety of microorganisms. The main feature of the oral cavity is its dynamism. All the microorganisms living in the mouth, defined as a whole as the oral microbiota, are subject to constant environmental changes including daily physico-chemical disturbances from the intake of food and dietary components, antimicrobials, tobacco consumption, adoption of specific hygiene measures and fluctuations in pH.

Being influenced by many environmental factors, our microbiota has changed over the course of human evolution. From prehistory to the present day, our habits have shaped its composition. The oral microbiota shifted to a disease-associated microbial community during a transition from hunter to farming practice and which now leads to less diverse and more susceptible oral pathogenic population. Also, the cariogenic bacteria became more dominant during industrial revolution [16].

Changes in the oral environment throughout life by physiological, hormonal and behavioral modifications modify the oral microbial population [17]. The importance of the oral microbiome (i.e., all microbes that exist in the oral cavity and their collective genome) and its association with the status of health and disease has been recognized only in recent years [18]. Although the history of Microbiology begins in the 1700s with the discovery of microorganisms in oral plaque by Antonie van Leeuwenhoek, the study of oral microorganisms has been mistreated for long time. The qualitative and quantitative complexity of the oral bacterial community (it is the second most diverse microbial community in the human body after that found in the gut), its highly heterogeneous composition at different sites in the mouth [19] and the difficulties in using traditional culture-dependent techniques for the identification of many of these microorganisms, have all delayed the acquisition of detailed information regarding the oral microbiome.

Many of the microbial species living in the oral cavity are highly adapted to their oral niche environments, and these *in vivo* conditions are not completely reproducible *in vitro*. Many microorganisms require specific nutrients, temperatures and pH, along with the presence of other microorganisms, for survival. Coaggregation and metabolic cooperation are important bacterial strategies for survival in the oral cavity

[20]. More than 250 oral species have been isolated and characterized by culture in the past, but the recent adoption of increasingly sophisticated molecular biology culture-independent techniques, based on amplification and sequencing of 5S and 16S ribosomal RNA genes, has revealed an even more complex environment, enabling a more detailed investigation of the human microbiome [21]. Thanks to the advent of next-generation sequencing techniques that allow the whole-genome shotgun sequencing of microbial communities *en masse*, the knowledge about the number of species residing in the oral cavity has expanded [22,23]. Today, it is well known that approximately 770 species are present in the human oral cavity, including Bacteria, Archaea, Fungi, Protozoa and Viruses. All these microorganisms and their genomes constitute the oral microbiome [24,25]. Bacteria are the most frequent microorganism in the oral cavity and, as a consequence, the great majority of the studies on the oral microbiome are focused on bacteria, while fewer reports exist on the fungal microbiome (the so called mycobiome) [17,26-28].

The Human Oral Microbiome Database (HOMD) website (http://www.homd.org/) contains detailed information about the characteristics, genomic and phylogenetic information of oral bacteria. As stated in the homepage of the website “the *HOMD* provides comprehensive information on the prokaryote species that are present in the human oral cavity". Around 57% are formally named, 13% unnamed (but cultivated) and 30% are recognized only as uncultured phylotypes. The HOMD links the available information about genomic sequences with clinical, phenotypic, phylogenetic, and bibliographic data. Sequencing analysis shows that the oral bacterial community is composed mainly of six major phyla, Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria, and Spirochetes which represent 94% of the detected taxa. Other phyla including, Chlamydia, Chlorobi, Chloroflexi, Gracilibacteria, Saccharibacteria, SR1, Synergistetes and Tenericutes make up the remaining 6% of the taxa.

Different studies have found that the most abundant genera include previously characterized oral bacteria: *Actinomyces*, *Capnocytophaga*, *Corynebacterium*, *Fusobacterium*, *Leptotrichia*, *Prevotella*, *Rothia*, *Selenomonas*, *Streptococcus*, *Treponema* and *Veillonella* [29]. Concerning oral Fungi, Ghannoum et al., 2010 [26] reported the presence of a total of 101 fungal species in healthy subjects. However, fungal species found in the oral cavity of each individual was reported to be variable, ranging between nine and twenty-three. *Candida albicans* and other *Candida* species were the most common, followed by *Aspergillus, Aureobasidium, Cladosporium, Cryptococcus, Fusarium,* and *Saccharomyces.* Archea only represent a small

part of the oral microbiome. A few species have been identified in healthy subjects, namely *Thermoplasmatales, Methanobrevibacter, Methanobacterium, Methanosarcina* and *Methanosphera*. The prevalence and numbers of these Archea species increase in individuals with periodontal diseases. Most viruses present in the mouth have a pathogenic role. Multiple human herpes virus and papilloma virus serotypes are associated with oral mucosal diseases, such as oral papillomas and primary herpetic gingivostomatitis, condylomas and focal epithelial hyperplasia [30]. In addition, many oral manifestations are found in HIV infected patients, including oral candidiasis, linear gingival erythema, oral hairy leukoplakia, and necrotizing ulcerative periodontitis, or Kaposi's sarcoma [31]. Oral diseases have been found to be increasingly common as the $CD4^+$ cell count decreases. While most sites in the mouth are dominated by Streptococci, our oral microbiota varies across oral cavity habitats.

Since the cells on the surface of the oral epithelia are shed at a regular rate, adhered bacteria are also eliminated, causing the soft tissues not to host the abundant microbiota that live on the surface of the teeth. Saliva has no indigenous microbiota. The microbial population found in the saliva (up to $10^8$ microorganisms per mL) is derived from oral mucosal surfaces, with *Streptococcus*, *Veilonella* and *Prevotella* being the predominant genera found.

While the surfaces of the palate and cheek only host monolayers of bacteria, the surface of the tongue contains multiple layers of biofilm-like bacteria with crypts, which allow colonization with anaerobes. These last microorganisms are closely related to halitosis [32]. The predominant microorganisms on the dorsal surface of the tongue in healthy subjects are *Streptococcus salivarius*, *Rothia mucilaginosa*, diverse uncharacterized members of the genus *Eubacterium* (strain FTB41) and anaerobic genera such as *Prevotella* and *Veilonella* [33]. With the exception of the teeth and subgingival space, the dorsal surface of the tongue harbors a greater microbial biomass than any other site in the mouth.

Our oral microbiota is personalized, however most individuals in healthy conditions share many species. On the basis of this observation, it has been proposed that there could be a central microbiome in the oral cavity comprising the predominant species of the oral cavity [18,34-36]. Additionally, Lazarevic et al. (2010) [37] reported that salivary microbial community seemed to remain stable over time (from 5 to 29 days) and supported this concept of a core microbiome in health state. The core genera have been defined as genera present with an abundance >10% and a ubiquity >75%, and other main core genera are defined as genera

present with an abundance >1% and a ubiquity >80%. The core genera in the oral cavity are reported in Table 1. The Human Microbiome project launched by the National Institutes of Health (NIH) has shown that the oral microbiome has the largest nucleus of microorganisms commonly shared among unrelated individuals compared to other anatomical sites, such as the gut or skin [38-40]

****Insert Table 1****

## 2. Changes in the oral microbiota

Microbial colonization of the oral cavity is a dynamic process that starts just after birth and becomes increasingly complex with age. At the time of birth, the oral cavity is sterile but vertical transmission from mother and nurses to child begins immediately after. Depending on the mode of birth (vaginal or Caesarian), the first colonizers affecting the oral microbiome of the infant are different (vagina- or skin-derived) [41]. Vaginally born infants show a higher number of taxa at 3 months of age and acquire the cariogenic *Streptococcus mutans* about one year later than infants born by Caesarian section [42,43]. The system followed to feed the baby (breastfeeding or infant formula) also has an effect on the microbiome. Infants of 3 months of age who were breastfed had a higher number of oral lactobacilli with antimicrobial properties that were not found in formula-fed infants [44,45]. In addition to vertical transmission, there is also horizontal transmission of microbiota, for example, between siblings and other people who share the same environment, contributing to the diversity of oral microbiomes [46,47].

The eruption of teeth during the first years of life is a crucial phenomenon, with the tooth representing a new surface for the development of dental plaque, a dense bacterial mass (also known as biofilm) tightly adherent to the tooth, and gingival pockets providing a new environment rich in serum and poor in saliva, anoxic and acidic (anaerobic microorganisms adhered in these spaces usually produce acid and are unaffected by an acid environment). Replacement of the primary teeth with the adult dentition also modifies the oral ecosystem [48]. In the supragingival plaque, *Firmicutes* and *Actinobacteria* (genus *Corynebacterium* and *Actinomyces*) have been found to be the most prevalent. In the subgingival plaque, the predominant species comprise 9 bacterial phyla, such as *Actinobacteria, Bacteroidetes, Deferribacteres, Firmicutes, Fusobacteria, Obsidian Pool OP11, Proteobacteria, Spirochaetes* and *TM7* [49].

Among *Proteobacteria, Gammaproteobacteria* of the genus *Acinetobacter, Haemophilus* and *Moraxella* have been found to be the most prevalent in healthy gingival sulci. Regardless of this dense bacterial colonization, acute infections are infrequent in the oral mucosa, suggesting that this environment is highly tolerant [50]. Mucosal dendritic cells have been found to be key cells in mucosal tolerance. They are able to mount an effective defense against pathogens as well as to inhibit immune responses against commensal bacteria, while retaining their health benefits [51]. It has been proposed that an important factor for the successful acquisition of a normal oral microbiome is the development of fetal tolerance to the mother's microbiome during pregnancy. [52]. The comparison of the sequencing data of 320 microbiomes shows that the placental microbiome is more similar to the oral microbiome than the vaginal or gut microbiome [53]. Gomez-Arango and colleagues (2017) suggested that the microbiome present in the placental exerts a biological function, becoming an antigen collecting site during the course of pregnancy, for training the fetal immune system to become tolerant to specific antigens [54].

It is increasingly clear that occurrence of certain diseases can be related to dysbiosis of the oral microbiota. Specifically, the shift toward the most common oral diseases, caries and periodontal disease, have been related to alteration of the normal microbiota, leading to an increased proportion of pathogenic species [55]. To avoid the development and the progression of such diseases, it is important to preserve the integrity of the oral microbiota. However, the mechanisms behind the maintenance of a healthy oral microbiota are still not fully understood. Maintenance factors can be either host-derived or microbe-derived. The interactions between the microbiota and the host are bidirectional, and acute infections affecting the oral mucosa are not frequent, despite dense colonization. The human immune system (innate and adaptive immune response) shapes the mucosal microbiota and plays a major role in its maintenance; on the other hand, the microbiota is essential for the development of the immune system [56].

## 3. Mechanisms underlying oral microbiota dysbiosis and homeostasis

Microbiota contributes to the development of the human immune system through constant communication associated with the host pattern recognition receptors, specially the member of the family of Toll-like receptors (TLRs). Binding of bacterial ligands to specific TLRs can regulate the expansion of Treg and help effector T-cells overcome Treg inhibition and develop an immune response [57,58]. Resident bacteria,

through complex interactions with immune cells, may exert both pro- and anti-inflammatory actions that are essential for the maintenance of homeostasis of the oral cavity. Altered expression patterns of TLRs in the cells of the oral mucosa have been found in oral pathology [59]. In addition, the host could use chemical sensing to detect the presence of bacteria. *Pseudomonas aeruginosa* and other Gram-negative bacteria [60] produce acyl-homoserine lactone quorum sensing molecules that may directly activate the bitter taste receptor T2R38 expressed by some cells, including the epithelial cells of the oral mucosa.

Lee and colleagues (2014) [60] suggested the existence of a direct relationship between secreted bacterial compounds and chemosensory activation mechanisms for mucosal clearance. It has been shown that genetic differences in the T2R38 receptor, which confer a greater capability to perceive bitter-tasting phenyl-thiocarbamide, influence the ability to signal the presence and subsequent depuration of the biofilm of *P. aeruginosa* by respiratory epithelial cells [61].

Other host-derived factors contributing to the maintenance of the oral microbiota are the secretory immunoglobulin A (S-IgA) found in the saliva and gingival crevicular fluid, and the salivary proteins. S-IgA interferes with microbial adhesion and hinders colonization [62]. However, commensal oral microorganisms circumvent the action of these antibodies by the production of IgA proteases [63], a mechanism also used by important human pathogens like *Streptococcus pneumoniae* and *Neisseria meningitidis.*

Salivary glycoproteins contain glycans that can act as decoys to prevent pathogens from attaching to epithelial cells, which influences healthy microbial homeostasis. Other proteins such as agglutinins, defensins, mucins, histatins, lactoferrin, lysozyme, peroxidase, and statherin [64], can have microbiocidal activities or may be used as nutritional substrates by microorganisms. Salivary flow rate and its composition play a crucial role in the maintenance of healthy oral microbiome.

The importance of the immune system in oral health is evident when it undergoes alterations, as in patients with hematopoietic stem cell transplantation who have received immunosuppressive therapy. In these patients, the mucous barrier is usually damaged, causing severe mucositis and favoring viral and fungal infections that endanger life [65], in addition to oral infections by non-oral species [66,67]. Microbe-derived microbiome maintenance factors are attributed to the mechanisms of interspecies and interkingdom collaborations that regulate the microbial community.

The microorganisms that constitute our microbiota are structurally and functionally organized into biofilms. The homeostasis of the biofilm is regulated through specific inter-microbial adhesion (coaggregation), cell signalling via cell-to-cell contact, metabolic exchanges in terms of collaboration and antagonism, and quorum sensing [68-70]. Thanks to recent advances in technology, we have begun to unravel the complexity of the oral microbiome, but the relationship between humans and their colonizing microorganisms are far from being fully elucidated. It is evident that we are not randomly colonised, but the resident microbes have co-evolved with us, and this co-evolution over a period of 1.5 billion years has led to mutual adaptation and functional integration generating a symbiotic beneficial relationship. As perturbations to the composition of the microbiota and its functions can lead to significant consequences for human health, much more research is needed in the near future to develop strategies to maintain the integrity of the oral microbiota.

## 4. Associations between oral health status and AD

Growing evidence suggests that patients with AD experience more oral disease than healthy people [71]. AD and oral health are associated, since the cognitive impairment typically found in patients with AD leads to difficulty in oral motor skills (i.e., impaired lip movements and reduced swallowing function) and difficulties in performing normal daily activities, including the ability to perform oral care, which in turn increases the risk of developing periodontitis and caries. The major risk factors for development of oral disease are poor oral hygiene, leading to recurrent and chronic infections, and diet, which interferes with cariogenic pathogen activity. As far as diet is concerned, the high and frequent consumption of sugar and sugar-rich foods and beverages, and low consumption of foods and beverages exerting antibacterial activity and reducing oral pathogen virulence factors (i.e tea, wine, coffee, nutmeg, apple, cranberry), contribute to formation of biofilms, adhesion of cariogenic Streptococci to teeth, and acidogenicity responsible for tooth enamel demineralisation, thus leading to caries formation and gingivitis [72].

To assess the status of oral health, a comprehensive intra-oral examination including the evaluation of teeth, oral mucosal tissue and periodontal tissue is required. To determine oral status, several dental indices are used. Among these, the sum of teeth cavitated, extracted, and filled with amalgam, resin or prosthetic crowns, is taken as the Decayed, Missing and Filled Teeth (DMFT) index, calculated through the examination of teeth and, if present, dentures [73]. Another index is the Oral Hygiene Index (OHI), which

considers the levels and position of biofilm and calculi on both teeth and prosthesis [74]. For periodontal disease, Basic Periodontal Examination (BPE) is commonly used to assess periodontal status. In addition, the gingival index (GI), plaque index (PI), probing depth (PD), clinical attachment level (CAL), and percentage of bleeding sites (%BOP) are commonly used to evaluate the perodontal status. To carry out a full clinical assessment of the oral health of the patient, an additional examination of the head and neck is performed. This examination consists of the evaluation of skin, lymph nodes, facial bones and temporomandibular joint. Moreover, the Oral Health Impact Profile (OHIP) was designed to determine the self-reported dysfunction, discomfort and disability attributed to oral conditions [75].

Since the first report of the oral conditions of AD patients, published in 1985 on the Journal of the American Dental Association, several investigations have been performed to evaluate oral health in patients with AD. The most interesting data are summarized and critically discussed below [76].

In 1990 Ship and colleagues observed that the salivary flow was diminished in patients affected by AD, suggesting this phenomenon as a potential causative agent for oral diseases [77]. Two years later, this finding was partially confirmed by the same reaserch group in a study that included 41 AD patients and 19 healthy subjects. Although the results confirmed that only the AD group presents salivary gland dysfunctions, authors did not find any significant difference between both groups about oral health [78]. Conversely, in 2013, a study on 158 patients (57 males and 101 females, with a mean age of 74 ± 5 years) affected by AD was performed to evaluate the relationship between the most common oral diseases (i.e., caries, periodontal disease) on the quality of life in AD patients (assessed by an Oral Health Impact Profile (OHIP-14) questionnaire). The oral diseases were described using the DMFT index, OHIP-14, the number of cavities, the extent of periodontitis, gingival bleeding, biofilm index and degree of tooth mobility. The results showed that AD is correlated with the quality of life linked to oral health [79]. Comparable results were obtained in a 2014 study by de Souza Rolim et al., in which orofacial pain and periodontitis were more frequently observed in AD patients (n = 29) than in healthy subjects (n = 30). They suggested that oral exams should be routinely performed to improve the quality of life of the patients, treating their oral diseases at an early stage [80]. In the same year, François et al. observed that the buccal mucosal cells from subjects affected by AD and mild cognitive impairment (MCI) were characterized by an alteration of the cytological conformation,

which included an increase in the DNA content and an abnormal nuclear form, compared to healthy individuals [81].

In 2018, Watanabe et al. described a correlation between mild cognitive impairment and oral status in a cross-sectional study that involved 3599 subjects (930 patients with MCI and 2669 subjects without MCI, with mean age of 71 years). Evaluation of cognitive impairment was done using a standardized personal interview, mini-mental state examination (MMSE), and a Geriatrics and Gerontology-Functional Assessment Tool. The oral status assessment included a comprehensive intra-oral examination, the evaluation of difference in masseter muscle thickness, occlusal force, and oral diadochokinesis (ODK). The results showed that oral status was better in healthy people than in MCI patients. Moreover, an association was found between MCI and impaired oral function (as assessed by ODK)[71]. The association between the AD and impaired oral function was also confirmed by two observational studies published in 2019 by Tiisanoja et al. and Choi et al., considering 170 and 262,349 individuals, respectively [82,83]. Tiisanoja and colleagues suggest three main possible mechanisms for this association: direct invasion of the brain by periodontopathogens, augmented pro-inflammatory markers, and induction of atherosclerotic plaques [82]. In conclusion, the literature data show that oral health status is associated with cognitive performance and the risk of AD.

### 4.1. Dental caries

Adequate dental hygiene is essential for the prevention of oral problems such as dental caries, gum disease and halitosis. Caries can appear throughout life, both in primary and permanent dentitions, leading to damage of the tooth crown and, if not treated properly, exposed root surfaces. Dental caries is a highly prevalent, complex, chronic, and multifactorial disease. Specifically, dental caries is the most prevalent human infectious oral disease and is directly associated with lifestyle, socioeconomic situation and genetic factors, but also with the individual characteristics of the oral environment [84,85]. In recent decades, the prevalence and severity of caries have declined in all ages, though mainly in children, especially in high-income countries; however, there is limited epidemiological evidence from systematic reviews on the global prevalence of caries [86]. The main factors that favor the occurrence of dental caries are regular exposure to simple carbohydrates, low saliva flow and the presence of acid-producing bacterial cells, which can ferment

these carbohydrates to small chain organic acids decreasing oral pH [87]. On the contrary, protective factors include normal salivary production with antibacterial agents and adequate buffering capacity, position and surface features of teeth and the use of fluoride toothpaste or other antibacterial substances.

Dental caries is a disease that derives from a bacterial infection associated with the formation of biofilms that lead to a decrease in pH in the mouth. If this low pH is maintained for a prolonged period it causes demineralization and tooth destruction [88]. Under normal conditions, human teeth present symbiotic microbial communities, principally composed of Gram-positive saprophytic bacteria that are, in principle, harmless to the teeth. However, when this microbial community is exposed to an environment rich in simple carbohydrates, acidogenic bacterial populations become predominant, producing and releasing weak acids that gradually demineralize the enamel. If this situation is combined with a lack of hygiene favouring the accumulation of food debris on the surface of the tooth and a deficient saliva function, demineralization of the teeth and infection can occur [89]. When this condition is not properly handled, it can lead to the appearance of a cavity through the enamel layer. At this stage, the cariogenic bacteria proliferate and release additional acids in the cavities that progressively deepen the lesion [90]. When the enamel barrier breaks, dentin continues to be degraded by Gram-positive bacteria, mainly streptococci, lactobacilli, and actinomyces, leading to damage in deeper layers of the tooth, affecting the nerves and ultimately causing tooth loss [91].

The relation between dental caries and AD has been reported by different researchers. In a first approach, Jones et al., 1993 evidenced that the number of dental caries at baseline was significantly higher in subjects with moderate and advanced AD and also the mean annual increments of coronal and root caries respect the control group [92]. In another study, the number of coronal and root caries was significantly higher in subjects with a diagnosis of AD when compared with other diagnoses of dementia and those without dementia [93]. The same research group also showed that cases of recently diagnosed AD with multiple surfaces of active coronal caries have a greater probability of presenting additional surfaces of active root decay [94]. A cross-sectional study reported that patients with AD were more likely to have carious teeth, compared to people without dementia [95]. However, in this case, the researchers did not observe differences between various types of dementia with respect to oral hygiene. In a case-control study, patients with AD had higher incidence of periodontal disease and caries, altered saliva quantity and quality and more mucosal

lesions [96]. Moreover, in a cross-sectional study, individuals exposed to mercury amalgam for filling prepared cavities after removing caries had higher risk of AD than non-exposed subjects, especially women [97]. A recent study, evidenced that dental caries and the associated inflammatory burden was related to a higher likelihood of having AD with respect to periodontal disease and stomatitis [82]. It is important to note than diverse authors indicate that patients with mild dementia had difficulties to perform oral care by themselves leading to poorer gingival health and oral hygiene, but, on the contrary, these patients report better self-perceived mouth health [98,99].

### 4.2. Associations between periodontal diseases and AD

Periodontitis is an oral infection caused by anaerobic, Gram-negative bacteria in the subgingival biofilm (i.e, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Tannerella forsythensis*, *Eikenella corrodens*, and *Treponema denticola*), which produce local and systemic infections, releasing pro-inflammatory cytokines into systemic circulation (i.e., interleukin (IL)-1α, -1β, -6, tumor necrosis factor alpha (TNF–α), prostanoids, matrix metalloproteinases (MMP)). The main hallmarks of periodontitis are gingival retraction and bone and connective tissue resorption, leading first to destruction of the tissues and the bone surrounding and supporting the teeth, and then to tooth loss.

The correlation between periodontitis and systemic disorders has been under study since the 1980s [100].

One of the first scientific studies suggesting associations between periodontitis and AD was a demonstration that inherited diversity in the genes of the family of IL-1 proteins are associated with a variation in inflammatory response and the development of chronic diseases, such as coronary artery disease, AD, gastric cancer, and periodontitis [101]. More recently, the hypothesis that systemic inflammation could lead to neuroinflammation, strongly implicated in AD, has been suggested by many other researchers [102]. Kubota and colleagues observed that the components of the AD pathway were significantly raised in inflamed gingival tissues, obtained from patients affected by chronic periodontitis. In particular, the synthesis of amyloid beta (A4) precursor protein (APP), complement component 1 (q subcomponent, A chain) (C1QA), and IL-1β, was upregulated [103]. The results from a case-control study performed on 80 AD patients, carried out by Farhad et al., confirmed that TNF-α is significant higher in AD patients with chronic periodontitis, compared to AD patients without periodontitis, suggesting serum levels of TNF-α as a

diagnostic marker of periodontal disease in patients affected by AD [104]. In 2016, Ide and collegues published a six-month observational cohort study aimed to determine if periodontitis is associated with both increased dementia severity and cognitive decline, and an increased systemic pro-inflammatory condition in AD. Sixty AD patients were cognitively assessed and a blood sample taken for systemic inflammatory markers, C reactive protein (CRP), TNFα and IL-10. The results showed that periodontitis at baseline was associated with a relative increase in the pro-inflammatory state over the six months follow up period. Authors conclude that periodontitis is associated with an increase in cognitive decline in AD, independent to baseline cognitive state, which may be mediated through effects on systemic inflammation [105].

One of the first investigations that associated AD with periodontitis was published in 2007 by Kim et al., showing the relation between the number of teeth and use of dentures, and the onset of dementia and AD in a study that involved 686 dementia-free subjects over the age of 65, with a follow-up of 2.4 years [106].

Focusing on more recent research from the past five years, Martande et al., in 2014, published the results of a study on 60 healthy participants and 58 patients with AD, which were subdivided into patients with mild, moderate, and severe cognitive impairment, assessed using a MMSE (age ranging from 50 to 80 years). Periodontal disease was described using gingival and plaque indexes, probing depth, clinical attachment stage and percentage of bleeding sites. The obtained data showed that all indices were higher in AD patients, and increased with an increasing degree of cognitive impairment [107].

Sochocka et al. studied the association between periodontitis, AD, and cytokine levels in a study that involved 128 subjects using a mathematical function. The degree of cognitive impairment was measured using MMSE; the periodontal status was determined using Bleeding on Probing (BoP), number of teeth, and measures of probing depth in mm; as far as systemic pro- and anti-inflammatory cytokine levels are concerned, IL-6, IL-1β, IL-10, TNF-α were used as predictory factors for the inflammatory state. To avoid confounding factors, patients with a history of street drug or oral steroid use, weight loss of 25%, or current alcohol abuse were excluded from the study. The results showed that systemic inflammation significantly aggravated the degree of periodontitis and cognitive impairment [108].

In the same year, Chen et al. performed a retrospective cohort study to determine the influence of chronic periodontitis on the incidence of increased cognitive impairment in AD patients. In more details, this retrospective cohort study made use of the National Health Insurance Research Database of Taiwan to select

9291 participants with a diagnosis of chronic periodontitis between 1997 and 2004. In addition, 18,672 patients without chronic periodontitis were studied. At the final follow-up, 115 patients (corresponding to 1.24%) and 208 subjects (corresponding to 1.11%) in the groups of participants with and without chronic periodontitis had developed AD, respectively, confirming that chronic periodontitis is a risk factor of developing AD [109].

The most recent research article found on this topic was published in 2018 [96]; a case-control study was performed to evaluate the oral health status (measured using the DMFT index, namely the sum of the decayed, missed and filled tooth surfaces), Community Periodontal Index and prosthetic status (fixed, implant-supported, removable partial, or complete prostheses) of 70 AD patients and 36 control subjects. The results confirmed that oral health (caries and periodontal disease) of AD patients was worse than healthy subjects.

In conclusion, the association between AD and periodontitis is clearly confirmed in the literature. Nevertheless, the studies performed to date have not yet demonstrated a causative relationship between these pathologies.

## 5. Role of oral microbiota in Alzheimer's disease

It has been found that alterations of the oral microbiota play a role in the progression of AD (Figure 1).

**Insert Figure 1**

Each individual carries an average of 200 different oral microbes [24,110], which change with oral diseases, commonly including gingivitis, periodontitis, plaque and mucositis [111-115]. Among these microbes, *P. gingivalis*, *T. forsythia* and *T. denticola* are considered the main responsible pathogens in periodontitis, and several inflammatory responses, and have been associated with the development of AD [116-119]. For instance, several different types of oral Treponema species have been found in AD brain samples, which include *T. amylovorum. T. denticola*, *T. maltophilum, T. medium*, *T. pectinovorum* and *T. socranskii* [116]. The lipopolysaccharides (LPS) produced by these microbes (in particular by *P. gingivalis*) were found in 12 hour-postmortem AD brain tissue (table 2) [117]. In elderly patients, periodontitis was found to be associated with cognitive impairment and immunoglobulin response against *P. gingivalis* [120]. Also, the antibody levels against *P. gingivalis* infections are increased in AD patients [121].

**Insert table 2**

The oral microbiota activates pro-inflammatory mediators such as IL-1P, IL-6, TNF-α, CRP and TLR signaling [122]. Chronic inflammation triggers an immune response, free radical production, apoptosis, and Aβ deposition [123]. The herpes simplex virus (HSV) including Epstein-Barr virus and Cytomegalovirus, are also responsible for AD pathology [124] mostly *via* Aβ accumulation and hyperphosphorylation of tau proteins in the brain [125]. HSV has been found to inhibit autophagy and thus prevent the degradation of these proteins [126].

HSV-1 was found in higher levels in AD patients when compared to controls [127]. This HSV-1 virus is absent in young brains, but enters the brain due to lowered immune responses in old age [128], and it may also be responsible for lowering the immune response itself [129]. Fungal infections, including yeast infections in periodontal pockets, the root canal or on the mucosae and underneath dentures [130-133] are responsible for systematic mycosis and AD conditions [134].

Oral microbiota could be transported from the mouth to the brain through the blood stream during brushing, flossing, chewing, or tooth pick use in patients with periodontitis, causing bacteremia [135]. Also, increase of pro-inflammatory responses can weaken the blood brain barrier (BBB) [15], promoting the penetration of bacteria, LPS or other toxic products. Serum antibodies for periodontal disease bacteria have been found at an elevated level in AD patients, compared to control, and these antibodies play a crucial role in the progression of AD. The serum IgG levels of seven oral bacteria including *A. actinomycetemcomitans, Campylobacter rectus, Fusobacterium nucleatum, P. gingivalis, P. intermedia, Tannerella forsythia* and *T. denticola* were shown to be elevated in AD patients, respect to normal control [14].

Periodontal disease produces LPS and toxic products resulting in an increase in brain pro-inflammatory mediators including IL-1β, IL-6, TNF-α and CRP, which ultimately increase the production of Aβ and hyperphosphorylation of tau, leading to neurodegeneration, dementia and AD [136]. In certain experiments, molecular and immunological techniques have shown that oral *Treponema* was found to infect the brain, leading to AD. It was found that *T. pectinovorum* and *T. socranskii* antigens were detected in trigeminal ganglia [116]. In another study, the levels of TNF-α and IgG antibody to periodontal bacteria were elevated in AD patients respect to normal control [121]. A similar study has been conducted on AD patients, and

serum IgG levels to periodontal microbiota, including *P. gingivalis*, *T. forsythia*, *A. actinomycetemcomitans*, *T. dentivola*, *C. rectus*, *E. nodatum*, and *A. naeslundii*, were found to be higher than in control subjects [137]. Elderly patients are prone to periodontitis because of a reduction in the capability to take care of oral hygiene. Oral bacteria are associated with increased pro-inflammatory molecules, particularly cytokines, which can also lead to dementia and AD. A six-month study conducted on 60 community dwelling participants suffering from mild to moderate AD, found that periodontitis was related to an increase in the rate of cognitive decline. Periodontitis was also associated with an increase in pro-inflammatory markers [105].

Periodontal disease is characterized by the decay of tooth supporting tissue due to immune-inflammatory responses. Lipopolysaccharide (LPS) participates in the TLRs, CD14 and nuclear factor κB (NF-κB) signaling pathway [138,139]. In some experiments, it was reported that *E. coli* LPS inoculated in APPS transgenic mice showed upregulation of APP expression and Aβ release [140]. Similarly, LPS from *P. gingivalis* was found to activate cathepsin B, which plays a role in neuroinflammation [141] and causes memory defects. The LPS from *S. abortus* equii were found to cause neuroinflammation and tau protein phosphorylation [142].

## 6. Oral pathogens involved in the production of AD functional amyloid

A large number of microbes essential for physiological function can be found in the body, referred to as microbiota. Microbes contribute to metabolic health [143], and to the proper development and regulation of the immune system [144]. On the other hand, nutrition affects microbiota composition [145]. Over the last decade, a growing body of scientific evidence suggests that through neural, endocrine and immune signalling, alterations in microbiota can lead to neurologic pathologies, such as anxiety, depression, autism, multiple sclerosis, Parkinson's disease and AD [146]. Since the mouth is the primary access site for microbes, the oral microbiota has a key role in modulating the activities of the other body microbiomes, including the pharynx, nasal cavity, esophagus and gut. In this context, Krishnan and colleagues summarized the importance of oral microbiota and its potential impact on health and diseases [147]. The authors provided an overview of (i) the Human Oral Microbiome Database (HOMD; www.homd.org), which includes the principal bacterial species present in the human oral cavity; (ii) the main approaches in defining oral

microbiota such as culture dependent and independent methodologies, DNA microarray and 16S rDNA sequencing; and (iii) oral and non-oral bacterial diseases [147]. During AD there is an imbalance between Aβ peptide generation and elimination, which leads to the accumulation of these peptides in the human central nervous system (CNS) [148,149].

Oral microbiota includes a group of microbes that produce Aβ, referred to as microbiome-derived amyloid, which can access the rest of the body and which may be able to promote chronic CNS pathologies with amyloidogenic features, such as AD. In this regard, Shoemark and Allen summarized the epidemiological and experimental evidence linking oral bacteria with the risk of developing AD [15]. A large number of microbes are able to produce a self-developed protective matrix known as biofilm. Microbial surface-related structures, such as amyloid proteins are the main components of this biofilm (**table 3**) [150]. Mature insoluble amyloid fibrils are produced when soluble monomers promote aggregation into oligomeric intermediates. Some oral microbes eliciting protein aggregation and amyloid formation include *Enterobacteriaceaes, Pseudomonads, Mycobacteriums, Streptococcus, Staphylococcus, Listerias and Bacillus.* The main characterisstics of these bacterial species are summarized below.

*Enterobacter:* Enterobacteriaceae is a common family of nonpathogenic or pathogenic enteric microorganism Gram-negative bacteria. The most important species in this family able to express amyloid proteins are *E. coli* and *Klebsiella pnumoniae.* For biofilm formation and bacteria survival, *E. coli* produces a bacterial functional amyloid known as curli, the main component of which is Curliprotein subunit A (CsgA) [151,152]. The potential link between Gram-negative bacterial molecules and AD neuropathology has been reported by Zhan and colleagues, who found that the levels of two Gram-negative bacterial proteins (*E. coli* K99 and *E. coli* LPS) were significantly higher in the AD brain with repect to controls [153]. Other bacterial molecules able to produce amyloids include MicrocinE492 (MccE492), a bacteriocin secreted by *Klebsiella pneumonia.* The soluble form of MccE492 has antibacterial activity, but after conformational and posttranslational modification, insoluble MccE492 decreases in antibacterial activity and is able to produce amyloid-like fibrils [154,155].

*Pseudomonads*: *Pseudomonadaceae* is a family of pathogenic Gram-negative bacteria with the capacity to form biofilms. Fap operon is a functional amyloid-like fibril produced by *Pseudomonads*, which is essential for biofilm development, as amyloid protein FapC is the most important component of biofilm [156,157].

Recently, Rouse and colleagues suggested a new mechanism by which FapC is secreted across the outer membrane, in which FapE and FapB are relevant in induction of Fap release, whereas catalytic action of C39 protease FapD could promote the activation of the FapF translocator complex for secretion of FapC [158].

*Mycobacteriums: Mycobacteriaceae* is a family of nonpathogenic or pathogenic bacteria generally considered to be Gram-positive and able to form biofilm. An important species in this family is *Mycobacterium tuberculosis*, which can express amyloid proteins termed *Mycobacterium tuberculosis* pilus (MTP) [159]. However, it has been recently reported that the contribution of MTP to *in vitro* pellicle biofilm formation is strain dependent [160]. Rifampicin, a well-known antibiotic for treating mycobacterium infections including *M. tuberculosis*, has been proven effective in decreasing the accumulation and toxicity of intracellular amyloid-β oligomers in murine models of AD [161], and to exert promissing effects in preventing the pathological process in elderly patients with AD-type hypometabolism [162].

*Streptococcus: Streptococcaceae* is a family of Gram-positive bacteria. *Streptococcus mutans* is a major dental pathogen that produces polysaccharide-encased biofilm called dental plaque [163]. The cell-surface-localized adhesin P1 (antigen I/II, AgI/II, Pac or P1) produced by *S. mutans* is responsible for amyloid fibrillization and biofilm formation [164]. Moreover, it has recently been reported that wall-associated protein A (WapA) and heretofore-uncharacterized secreted protein SMU_63c are also amyloid-forming proteins associated with biofilm [165].

*Staphylococcus*: *Staphylococcaceae* is a family of Gram-positive anaerobic bacteria that includes *Staphylococcus aureus*, which is able to form biofilms [166]. The main protein facilitating this biofilm development is phenol-soluble modulin (PSM), which can also produce amyloid fibrils [157,167,168].

*Bacillus: Bacillaceae* is a well categorized family of Gram-positive bacteria, which includes *Bacillus subtilis,* a model organism for the analysis of biofilm formation [169]. So far, three proteins have been reported to contribute to biofilm formation by *B. subtilis,* i.e., TasA a as a main component, TapA as a minor component, and SipW, a bifunctional signal peptidase that controls secretion of TapA and TasA [170]. Interestingly, *B. subtilis* is able to produce scyllo-inositol (SI), a potential modified inositol metabolite (currently in phase III development of clinical trials) that through interaction with Aβ peptide can block amyloid aggregation [171].

*Listerias: Listeriaceae* is a family of Gram-positive bacteria that includes *Listeria monocytogenes,* an important contaminant in the food processing environment with the ability to form biofilms [172]. Listeriolysin O (LLO) is a soluble momomer produced by *L. monocytogenes*, essential for the intracellular life cycle of bacteria, the formation of the pore complex, escape from endocytic vacuoles and virulence [173,174]. The activity of LLO depends on pH (more active at acidic pH) and oxidation (inactive when oxidized). In fact, changes in pH lead to an insoluble form of LLO, which promotes protein aggregation and amyloid formation [174-176].

**Insert table 3**

## 8. Control of oral microbiota for prevention and early management of AD

It has been confirmed that recent lifestyle changes have adversely affected the oral microbiome, reducing its diversity and increasing its pathogenicity, therefore perturbing the symbiosis between the microbiome and the host [177]. It has been suggested that in AD patients, immunosenescence favors the overgrowth of certain resident oral anaerobic bacteria, eliciting an innate pro-inflammatory immune response that disrupts the BBB, allowing opportunistic pathogens to spread and silently stimulate the pathogenesis of AD [15,178]. Conventional therapies have attempted to regulate the metabolic activities and *dysbiosis* of predominant oral cavity bacteria such as *Streptococcus, Veillonella, Haemophilus, Neisseria, Prevotella* and *Fusobacterium* species, as well as opportunistic strains. There has been recent confirmation that several hundred species of commensal microorganisms are involved in the initiation and progression of dysbacteriosis (*dysbiosis*) within plaque biofilms. Therefore, restoration of the oral microbiota composition with antibiotic treatments could be a helpful approach. Undoubtedly, there is an urgent need to introduce novel oral microbiota-based prediction models, develop new personalized medicine approaches accounting also for oral microbiota analysis, as a possible alternative and/or complementary therapeutic intervention strategy for AD [179,180].

### 8.1. Direct targeting of the oral microbiota

Formerly, the control of infectious diseases has been focused on direct non-specific removal of whole sets of pathogens using broad-spectrum antibiotics. The abuse of antibiotic treatments has led to several adverse effects as observed in current “post-antibiotic era” [181], such as antibiotic resistance and antibiotic-induced

diseases. Despite this fact, the routine treatment option for oral *dysbiosis*-related disorders is still antibiotic therapy. Many bacterial species present in the human oral microbiota have the ability to colonize gingival crevices and create biofilms (dental plaques) on the outside of the tooth, and in these conditions some antibiotics are used empirically for treatment or as prophylaxis, such astetracyclines, macrolides, lincosamides, ß-lactams, and nitroimidazoles [182]. In this sense, the indiscriminate elimination of the oral population through the use of broad-spectrum antibiotics can favor the subsequent colonization of the pathogen and make it dominant, reducing normal and healthy microbial diversity [183]. Current studies have indicated that while narrow-spectrum antimicrobial therapy is the first line treatment for *P. gingivalis* (in periodontitis) and *S. mutans* (in dental caries), this can eventually lead to the establishment of dysbiotic situations in the oral cavity [see the review 184]. All this makes necessary the use of new therapeutic targets that allow targeting a specific therapeutic target and restore oral microbiota balance. Among these new targets one could act against genes essential for the survival of the pathogen in the host together with new delivery methods can improve specificity [184]. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat Spacers) has evidenced the capability to specifically distinguish between different strains of the same species in a complex population [185]. Interestingly, it has been indicated that 65% of neonatal oral microbiota have a maternal origin, and maternal intrapartum antibiotic treatment could impact the initial neonatal oral microbiome [186]. Although there are no studies that relate the effects of antibiotics on the oral microbiota and the progression of AD, it has been observed that the systemic intake of antibiotics reduces cognitive decline. In this sense, in a randomized, triple-blind, clinical trial, oral daily doses of doxycycline (200 mg) and rifampin (300 mg) antibiotics during 3 months significantly ameliorated the cognitive decline in AD patients after 6 months follow-up and reduced dysfunctional behavior at 3 months, but not at 6 or 12 months [187]. The authors suggest that the mechanism of action could be associated with anti-inflammatory effects and the interference with the expansion of neurofibrillary tangles. However, in another multicentre clinical trial, the intake of the same two antibiotics alone or in combination in the same doses as in the previous study did not exert beneficial effects on cognition or function in patients with moderate AD [188]. In addition, a delay in neurodegeneration and a prevention in microgliosis was found in AD11 mice model which develops impaired memory function, Aβ and hyperphosphorylated tau lesions, when are raised in sterile conditions [189]. The authors suggest that the inflammatory state of the brain

derived from infections during early life and adulthood can favour cognitive decline and AD progression in elderly people. Similarly, in an AD10 mice model which also develops a complex neurodegenerative phenotype, the stimulation of the immune system when animals were maintained in a microbially unprotected environment contributed to more severe neurodegenerative phenotype with respect to animals housed in pathogen-free conditions [190].

### 8.2. Indirect targeting of oral microbiota

The human oral mucosal surface contains a very complex microbial habitat, constantly in symbiosis with the host environment and modulated by the specific local factors. Under healthy conditions, oral biomass exists in a mechanical and chemical homeostasis with the host immune system. In such a multifaceted ecosystem, the elimination of only one species can result into a dramatic change in the entire ecosystem, facilitating the invasion of undesirable external infection agents [191].

It has been demonstrated that indirect control of oral microbial flora through modification of microbial biomass (control without killing) may reduce the incidence of some serious periodontal and systemic human disorders. Such preventive strategies are currently regarded as possibly effective for the management of oral cavity dysbiosis-induced human diseases. In this ecological approach, oral microbiota transplantation has been hypothetically proposed by Pozhitkov (2015) and Mira (2017), but so far, neither microbial evaluations nor clinical outcomes have been reported [192,193]. In this sense, the results showed that the oral microbiome in subjects with periodontitis had a greater bacterial diversity compared to the subjects who had established caries, edentulism and oral health (Pozhitkov et al., 2015). Safety concerns linked to the efficiency of oral microbiota transplantation have been raised, parallel to those for oral probiotics. Both probiotics and microbiota-transplanted biofilms should not cause disease and should have a high degree of genetic constancy [180]. Increasing evidence has demonstrated that probiotics, such as *L. reuteri*, *L. acidophilus* (LA-5), *L. casei* (LC-11), *L. paracasei*, *L. rhamnosus* (GG), *S. salivarius* (K12 and M18), *Bifidobacterium sp*, and *Animalis subsp*. *Lacis* have a notable therapeutic and/or preventive function in the development of oral cavity diseases [194-201].

Data from a randomized clinical trial have revealed that four weeks of daily consumption of fermented milk containing probiotics (*L. rhamnosus* SD11) may have beneficial effects on oral health by reducing salivary levels of *S. mutans* and total bacterial counts, whereas increased lactobacilli counts [202]. It seems that *L. rhamnosus* SD11 can colonize in the human mouth since this probiotic strain could be detected up to 4 weeks after concluding the treatment. Similar results were obtained by the same group of researchers when supplying milk-powder with *Lactobacillus paracasei* SD1 once daily during 6 months to healthy volunteers [203]. In addition, salivary IgA was increased in the probiotic group respect to the control group suggesting an immune-stimulatory effect. Terai and colleagues showed that *L. crispatus* (YIT 12319), *L. fermentum* (YIT 12320), *L.gasseri* (YIT 12321) and *S. mitis* (YIT 12322) exhibited minor risks of experimental infective endocarditis in a rat model, and did not have cariogenic potential when assayed in an artificial mouth system [204]. Moreover, these strains are characterized by not generating volatile sulfur compounds and exerting a remarkable antibacterial activity against periodontal bacteria. In another randomized control trial, 12-week consumption of *L. reuteri* did not change species abundance, but influenced a shift in oral microbiota composition, although the effects disappeared 1 month after supplementation [205]. Also, it is important to consider that the test strains could not be detected in about 30% of the participants. In another clinical trial, individuals with presence of *L. reuteri* before supplementation during 3 weeks daily with lozenges containing two strains of *L. reuteri* presented higher concentrations of salivary IgA at the end of the intervention compared to the group without presence of *L. reuteri* [206]. A clinical trial reported that probiotic supplementation (200 ml/day containing *L. acidophilus, B. bifidum*, and *B. longum* 2 x $10^9$ CFU/day each) during 12 weeks significantly improves cognitive decline in AD with respect to the control group measured with the MMSE, and exerted favourable effects on malondialdehyde and hs-CRP levels, markers of insulin metabolism, and serum levels of triglycerides and VLDL [207]. However, the possible effects of the probiotics on oral microbiota were not analysed. Similar effects in improving cognitive function were found by the same group after 12 weeks of co-supplementation with probiotic and selenium (200 µg/day) in AD patients [208]. In addition, the co-supplementation decreased the expression of TNF-α and increased peroxisome proliferator-activated receptor gamma (PPAR-γ) and low-density lipoprotein receptor (LDLR) in peripheral blood mononuclear cells, but without altering IL-8 and transforming growth factor beta (TGF-β).

### 8.3. Therapeutic perspectives regarding oral microbiota

It has been demonstrated that selective targeting of *S. mutans* by 3F1, a 2-aminoimidazole small molecule derivative, effectively disperses biofilms independently of glucosyltransferases and antigen I/II (biofilm-related factors), reducing dental caries *in vivo* without affecting oral microbiome [209]. Dysbiosis of the oral microbiome can be attributed to daily intake of sugar and not the abundance of *S. mutans*, since the reduction of *S. mutans* by the 3F1 compound did not significantly modify the microbiome in the rat caries model. Hydroxychalcones with the capability to inhibit *S. mutans* glucosyl transferases can selectively inhibit cariogenic biofilms but without affecting two commensal species (*Streptococcus sanguinis* and *S. gordonii*) up to 200 μM [210]. Similarly, it was found that the antimicrobial peptide GH12 (8 mg/L) selectively suppresses *S. mutans* in multispecies biofilm and reduced glucan synthesis, and lactic acid production, without targeting *S. gordonii* and *S. sanguinis* species [211]. A randomized controlled study presented that, while dental plaque is quickly colonized by multidrug-resistant bacteria, the incidence of methicillin-resistant *S. aureus* colonisation could be dramatically decreased by using 2% chlorhexidine after patient admission to the intensive care unit [212]. Another randomized clinical study confirmed that toothpastes supplemented with proteins and enzymes considerably induced a shift in the species levels of the oral microbiome, resulting in a community with a superior link to oral health [213]. The toothpaste enhanced the natural salivary defenses by increasing the bacteria associated with gum health (including *Neisseria* spp.) and decreasing those associated with periodontal disease (including *Treponema* spp.). It has been demonstrated using a toothpaste with ethanolic extract of Polish *propolis* and Australian *Melaleuca alternifolia* oil in its composition significantly reduces approximal plaque index, sulcus bleeding index and also oral hygiene index in patients suffering from varying conditions of the gingiva, by maintaining balance in the oral microbiome [214].

Likewise, a gel containing 3% of ethanolic extract of Brazilian green propolis was also effective in maintaining oral hygiene in patients with postoperative oral mucosal wounds reducing pathogenic and opportunistic character microorganisms, but with no detrimental influence on normal physiological flora composition [215]. On the other hand, arginine-containing dentifrice could effectively normalize the oral microbiome of individuals with active caries, and equally toothpastes containing arginine and fluoride

significantly delay the demineralizing ability of saliva-derived oral biofilm via induction of *S. sanguinis* and suppression of *S. mutans* enrichment [216].

A recent study using planktonic monocultures and multi-bacterial biofilms has shown that simvastatin efficiently inhibited the growth of periodontitis bacteria *Porphyromonas gingivalis* more than 1,300-fold relative to the control. This considerable inhibitory effect of simvastatin as well as its safety against commensal oral microbiota, suggests it may be a potential candidate for adjunctive management of chronic periodontitis [217]. Another study evidenced that atorvastatin administration using a chitosan formulation delivery system, with bioadhesive and penetration enhancing properties, significantly increased the anti-inflammatory effects of atorvastatin and bone and tissue healing in periodontitis induced rat model [218]. Bhaskaran and colleagues have shown that short-chain fatty acids derived from resident bacteria control the pathology induced by oropharyngeal candidiasis [219].

These fatty acids are capable to regulate T cell cytokines during mucosal infections both *in vitro* and *in vitro*. Another study showed that 8-week ingestion of lactoferrin and lactoperoxidase (saliva antimicrobial proteins)-containing tablets stimulate a shift from a greatly diverse and Gram-negative-dominated community to a Gram-positive-dominated in the oral cavity correlating with improvements in oral health [220]. The same group of researchers also found a reduction in the number of *P. gingivalis* and *F. nucleatum* after 8 weeks of lactoferrin and lactoperoxidase supplementation in tongue coating and a reduction in total bacteria and *P. gingivalis* in the supragingival plaque [221]. It has been also indicated that epigallocatechin-3-gallate (EGCG), the major polyphenolic compound of *Camellia sinensis* (Japanese green tea), can inhibit the growth and biofilm formation of canine *S. mutans*, without interacting with streptococcal lipoteichoic acid [222]. Another study reported that the treatment with 250 μg/ml EGCG significantly reduced the biomass and acid production in *S. mutants* biofilms, whereas a concentration of 500 μg/ml is required if *S. mutants* grows together with the probiotic *L. casei* [223]. The results suggest that the presence of a probiotic could interfere the inhibitory effects of EGCG against *S. mutans*.

Vaccine-based approaches aimed at preventing dysbiotic shifts would provide a suitable therapeutic strategy against periodontal diseases. Since formation of dental plaque is a multilayered and polymicrobial process, multivalent or polyvalent vaccines targeting numerous bacterial species could be effective in clinical applications. It has been shown that intranasal immunization with a divalent mucosal vaccine containing a

mixture of FlaB-tFomA (targeting virulence factor of *F. nucleatum*) and Hgp44-FlaB (targeting virulence factor of *Porphyromonas gingivalis*) fusion proteins induced protective immune responses inhibiting alveolar bone loss stimulated by *F. nucleatum* and *P. gingivalis* infections [224]. In addition, the immunization of mice with recombinant arginine-gingipain (arginine-specific protease common to all *P. gingivalis* strains) after being orally infected with *P. gingivalis* prevented alveolar bone loss by 50% and shifted the humoral response towards and anti-inflammatory profile [225].

Phages play a key role in the natural balance of the human microbiome, and thus have the potential to be efficient anti- biofilm agents. Therefore, as in many other therapeutic fields, phage therapy offers novel horizons in controlling oral cavity infections [226]. To this end, *Actinomyces naeslundii*, *A. actinomycetemcomitans*, *Enterococcus faecalis*, *F. nucleatum*, *Lactobacillus* spp., *Neisseria* spp., *Streptococcus* spp., and *Veillonella* spp. lytic phages have been isolated and characterized and their possible therapeutic applicability should be further explored [227]. Also, Castillo-Ruiz et al. [228] isolated a bacteriophage specific for *A. actinomycetemcomitans* capable to kill 99% of the bacteria within a biofilm.

Finally, it is interesting to note that dietary patterns can influence oral microbiota composition, and specific dietary interventions may help maintain microbiota homeostasis and protect against AD. Regular consumption through the diet of compounds with antibacterial activity has been linked to a reduction in the risk of AD. In this sense, foods such as garlic, olive oil, curcumin or cinnamon have significant antibacterial effects that could exert some protection against AD [229-232]. These products if ingested regularly, may affect the structure and composition of the oral microbiota. Moreover, the Mediterranean diet pattern has also been proposed as a preventive strategy for AD. In a 4-year follow-up trial, a beneficial association was shown between a higher adherence to the Mediterranean diet and a lower risk of AD [233]. Similarly, in another study better adherence to the Mediterranean diet was not only related to a lower risk of AD but also a gradual reduction in mortality risk [234]. However, to date, there are no specific studies that analyze the relationship between dietary patterns, oral health and risk of AD.

## 9. Conclusions and future prospects

Oral cavity microbiome can exert a pivotal role in the prevalence of AD in the elderly population around the world, which in turn correlates with nutrition and life style of the community. Available literature strongly

indirectly suggests the important contribution of the oral microbiome in the development of AD. In fact, oral microbiota produces inflammatory mediators able to migrate into the bloodstream and affect distant tissues and organs, thus representing a source of systemic infection and inflammation, causing neuroinflammation and possibly acting as primary agents for AD. Oral health care is a key factor for the prevention or delay of conditions associated with oral microbiota dysbiosis, such as AD. Proper schooling of aged people regarding oral health care and its long term impact on health can help in the reduction/delay of AD. Nutritionists could also utilise their role to increase awareness of the importance of the diet and prevention of neuroinflammation. In this regard, regular training workshops for health care professionals and the aged community could also be effective. Similarly, antibiotic treatment, used with discernment, could be a therapeutic strategy to control oral microbiome infections as well as the antivilurence therapy, including the inhibition of virulence factors, such as disruption of quorum sensing, inhibition of biofilm formation, bacterial motility, and synthesis of enzymes, toxins, and surfactants used as bacterial defense. Such treatment approaches may be beneficial as complementary therapies for the management of AD and/or to diminish the risk of cognitive decline.

Considering that perturbations of the composition of the oral microbiota and its functions can lead to significant health consequences, further studies aimed at developing strategies to maintain the integrity of the oral microbiota are highly warranted.

## 10. Take-home message

The oral microbiota is an integral part of human physiology and metabolism. However, recent studies have also reported the possible association between an unbalanced oral microbiota with many diseases, including AD.

With the development of the Human Oral Microbiome database launched in 2010, there has been a greater advancement in the understanding of oral microbes and their roles. However, their role in the progression of AD has not been fully elucidated. Based on the findings up to now, it can be recommended that strategies aimed at balancing the symbiotic oral microbiota along with oral hygiene should be considered as important aspects of human healthcare for all age groups. Further large cohort studies are warranted to increase our understanding of the mechanisms underlying oral microbiota effects on mental health and AD risk.

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**Legends:**

**Table 1.** The core bacterial taxa found in the oral cavities of over 200 healthy individuals participating in HMP. Parenthesis () shows core family. High abundance core genera in >75% samples at >10% abundance and other major core genera in >80% samples at >1% abundance (modified from Li et al., [39]).

**Table 2.** Oral microbioma pathogens involved in development of AD.

**Table 3.** Oral microbiome producing amyloids proteins.

**Figure 1**. Oral bacteria pathogenesis in the development of AD. The entering of bacteria through BBB into the brain tissue causes and also the secreted outer membrane vesicles (OMVs) as well as lipopolysaccharides (LPSs) causes (A) glial activation and neuroinflammation that contributes to the neuron degeneration and also tau hyperphosphorylation; (B) Bacteria have also the capacity to release porin-like proteins that increase the permeability of the neuronal membrane and also calcium leakage with direct effect upon neuron synapses and viability; (C) the antibacterial role of Aβ determines a feedback mechanisms that indirectly contributes to the accumulation of Aβ plaques through increased secretion due to bacterial presence.