The Honors College at the University of Missouri-Kansas City

Oral Disease Bacterium Linked to Alzheimer's disease

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April 30, 2020

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A thesis submitted in partial fulfillment of the requirements to graduate as an

Honors Scholar from the University of Missouri-Kansas City

Abstract

This review proposes an association between the bacteria found in periodontal disease and the development of neural plaque contributing to Alzheimer's disease. Periodontitis is a chronic gum disease that destroys gingiva and jawbone. Many different species of bacteria are found in periodontitis such as *Porphyromonas gingivalis*, an anaerobic gram-negative bacterium. Recent studies have shown *P. gingivalis* is capable of entering the bloodstream and affecting both the brain and organ systems within a human host. In this review, I will delve into the anatomy of the human mouth, explain the cause and effects of both periodontal and Alzheimer's diseases, and attempt to explicate the relationship between periodontitis and Alzheimer's disease. I suggest a direct correlation between the bacteria present in periodontitis and the human brain impacted by Alzheimer's disease. In support of my hypothesis, I reference peer reviewed works and a published study detecting *P. gingival* in the cortex region of the brain. Additionally, in this paper, I hope to provide a clear correlation between the progression of gum disease and the onset of Alzheimer's disease. The proposed link between *P. gingival* and Alzheimer's disease suggest a new avenue of preventative treatment along with signs of early diagnosis.

Keywords: *Porphyromonas gingivalis*, gum disease, Alzheimer's disease

PERIODONTAL DISEASE

The primary mode of intake for nutrition is the oral

cavity, also known as the mouth. The human mouth is

comprised of teeth, gums, oral mucosa, an upper jaw, a

lower jaw, the tongue, salivary glands, the uvula, and the
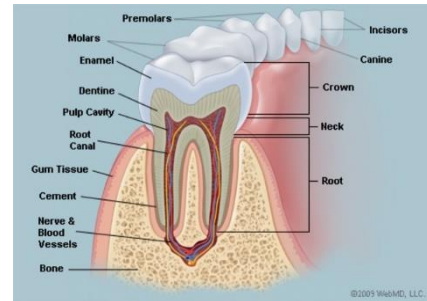
Picture of the Teeth, 2019

frenulum (Brichford, 2007). These components are constantly exposed to foreign elements such

as food. Since food particles can become lodged and left behind in many crevices within the

mouth, neglected debris can begin to infect the epithelial layers of the gums and oral mucosa.

The warm, humid nature of the mouth leads a growth of many different species of oral bacteria.

These bacteria are capable of infecting the gum line surround the teeth. Once this infection

occurs, it can quickly spread to the remaining periodontium, an area that surrounds and supports

the tooth (Posnick, 2014).

The Periodontium is made up of four separate parts: the gingiva, dental alveoli (tooth

sockets), cementum, and periodontal ligaments (Madukwe, 2014). Each of these four parts plays

a role in supporting and anchoring a tooth in a specific location. Anatomically, the root of the

tooth is placed firmly in the dental alveoli (Madukwe, 2014). This part of the periodontium is

surrounded by bone tissue and is not visible (Madukwe, 2014). Surrounding the root of the tooth

is a mineral layer known as the cementum (Madukwe, 2014). The cementum contains collagen

fibers that anchor the tooth to the dental alveoli and extend into the surrounding periodontal

ligament (Khockt, 2012). In order to withstand the masticatory force, the collagen fibers that

extend from the periodontal ligament to the cementum must form a strong bond between the

tooth and the jaw (Khockt, 2012). The exterior portion of the periodontal ligament and the region

surrounding the neck of the tooth are lined by gingiva. The gingiva, also known as the gums, is a

keratinized epithelial layer serving as a protective barrier for the underlying bone and tissue (Marquez, 2004). This protective layer is the only visible area of the periodontium (Madukwu, 2014).

Seated within the periodontium is the tooth. The tooth is divided into three regions: the root, the neck, and the crown. The crown is the visible region extending from the upper portion of the gingiva. It is composed of three parts: anatomical crown, enamel, and dentin (Santos-Longhurst, 2018). Enamel is the hardest tissue in the body and helps to protect the exposed tooth from bacteria (Santos-Longhurst, 2018). Below the crown is the neck; a transitional area between the root and the crown (Santos-Longhurst, 2018). The top of the neck and the base of the crown are surrounded by a small gingival region known as free gingiva. This area is not anchored to the tooth. The gap formed by the unanchored region between the gingiva and tooth is called the gingival crevice or gingival sulcus (Watson, 2020).

Withing this shallow, narrow sulcus is a watery fluid known as gingival crevicular fluid. Specific to the oral cavity, the gingival crevicular fluid is released into the sulcus as an inflammatory exudate from the surrounding periodontal tissue (Subbarao et al., 2019). This mass of cells contains antibodies, and immune cells such as neutrophils, leukocytes, and their complement proteins (Subbarao et al., 2019). The gingival crevicular fluid plays a crucial role in maintaining the structure and adhesive properties of the surrounding epithelium while also creating an antimicrobial defense system for the periodontium (Subbarao et al., 2019). However, without proper oral hygiene, bacteria will begin to form dental plaque biofilm in the gingival crevice (Velsko et al., 2019). These biofilms will eventually lead to a level of dysbiosis, a microbial imbalance, in the oral cavity (Yost et al., 2015).

Although many biological factors assist in maintaining a healthy oral microbiome, this human defense system possesses limitations. The layer of healthy bacterium forming the microbiome forms a complex heterogeneous microbial composition consisting of gram-positive and gram-negative bacteria (Berezow, 2011). The imbalance, due to either an ineffective immune response or a lack of competition from healthy bacteria, caused by dental plaque biofilms will lead to a rise of pathogenic bacteria found in the oral cavity (Berezow, 2011). When the tissue becomes infected with bacteria, the tissue becomes inflamed. In time, these infections can lead to gum disease.

Periodontal Disease, n.d.

Gum disease can be classified into seven categories depending on the degree of infection. These seven categories are gingivitis, periodontitis, chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic disease, necrotizing ulcerative gingivitis, abscesses of the periodontium, and combined periodontic-endodontic lesions (Armitage, 1999). The diagnosis of oral disease is determined on a scale beginning with gingivitis and progressing to chronic periodontitis or one of the more severe variation of gum disease.

Gingivitis, known as gum disease, is inflammation of gum tissue (Newman, 2018). Gingivitis is a type of periodontal disease, non-destructive, that does not result in any loss of bone or teeth (Newman, 2018). Symptoms may include bleeding or swollen gum, change in color of the gums, bad breath, receding gum line, and occasional pain (Newman, 2018). The most common cause of gingivitis is the hardening of dental plaque on the teeth (Newman, 2018). The formation of dental plaque damages gingival cells that surround the infected area (Newman,

2018). The gingival cells trigger an inflammatory signal causing an immune response in the

gingival region (Newman, 2018). The immune response triggers a release of high concentrating

of IgG subclass antibody-secreting cells (Kulshrestha, 2013). IgG is the main immunoglobulin

found in human blood (Kulshrestha, 2013). This protein contains long-term antibodies used to

fight a variety of infections (Kulshrestha, 2013). Although this IgG release is a necessary

immune response for the destruction of infections bacterium, healthy gingival cells are also

killed as collateral damage (Newman, 2018).

After IgG antibodies destroy both abnormal and healthy bacteria, any remaining

infectious bacteria are able to progress. As the infection advances, the gingiva begins to separate

from the neck of the tooth producing a pocket larger than the aforementioned gingival sulcus

(Kinane, 1999). Infectious bacteria will then occupy and proliferate within this pocket leading to

the formation of dental plaque below the neck of the tooth (Loesche, 1996). At this stage, dental

plaque will spread to the dental alveoli, gingiva, cementum, and periodontal ligament (Loesche,

1996). Since this region is difficult to reach and painful to clean, lack of abrasion creates an

environment that is conducive to the calcification of dental plaque (White, 1997). The

calcification of dental plaque then forms dental calculus (White, 1997). Composed primarily of

calcium phosphate, dental calculus can form both supragingival and subgingival deposits in the

gingiva (White, 1997). Supragingival calculus have been found to cause gingival recession

promoting further infection withing the periodontium. If left untreated, dental calculus will lead

to the onset of periodontitis (White, 1997).

Periodontitis is a chronic disease that affects and destroys the gum tissue and bone that

support the teeth (Mehrotra, 2019). This disease is found in both developing and developed

countries affecting nearly half the world's population (Nazir, 2017). There are two main

classifications of periodontal disease: chronic periodontitis and aggressive periodontitis (Mehrotra, 2019). These two classifications of periodontitis are classified by the level of degradation in the surrounding periodontium (Guo, 2016). More specifically, this level is measured by probing and recording at six sites around the tooth: mesio-buccal, mid-buccal, mesio-lingual/palatal, disto-buccal, mid-lingual, and disto-lingual/palatal sites. (Guo, 2016). The measurements are recorded as slight (1-2 mm attachment loss), moderate (3-4 mm attachment loss), and severe (>5 mm of attachment loss) (Vilardi, 2017). Periodontitis classification extends further to generalized and local. Generalized periodontitis affects more than 30% of the mouth, and localized affects less than 30% of the mouth (Wiebe, 2000). As the disease progresses, bacterial accumulation increases exponentially.

Over 700 bacterial species have been found in the oral cavity (Gao et al, 2018). The hypothesis of periodontal disease progression is described as the orange complex bacteria (Periodontitis: the Complex, n.d.). Bacteria in the mouth adhere to the pellicle, a protein film of the tooth, to avoid being flushed out by the gingival crevicular fluid (Periodontitis: the Complex, n.d.) These bacteria, also known as the early colonizers, form in the dental crevice (Periodontitis: the Complex, n.d.). Since they are moderately pathogenic, they are labeled as green complex. The green complex bacteria include: *Eikenella corrodens, Capnozytophaga sputigena, Aggregatibacter actinomycetemcomitans, Campylobacter gracilis,* and *Capnocytophaga ochracea* (Carrouel et al, 2016). The orange complex hypothesis predicts that green complex vacteria will lead to the highly pathogenic bacteria of the red complex (Periodontitis: the Complex, n.d.). The orange complex bacteria includes: *Campylobacter gracilis, Campylobacter rectus, Fusobacterium necleatum, Parvimonas micra, Prevotella intermedia,* and *Prevotello*

nigrescens (Carrouel et al, 2016). As the host's oral health condition worsens, a living condition is created for strictly anaerobic bacteria also known as the red complex (Carrouel et al, 2016).

The anaerobic bacteria classified within the red complex include: *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola* (Carrouel et al, 2016). The red complex bacteria, typically inhabiting adjacent colonies, are proposed to destroy tissue cooperatively (How et al, 2016). *Porphyromonas gingivalis* is a gram-negative anaerobic bacterium which forms black-pigmented colonies (How et al, 2016). This red complex bacterium relies on the fermentation of amino acids for energy production (How et al, 2016). Poor oral hygiene and compromised periodontium health lead to increase protein concentrations within
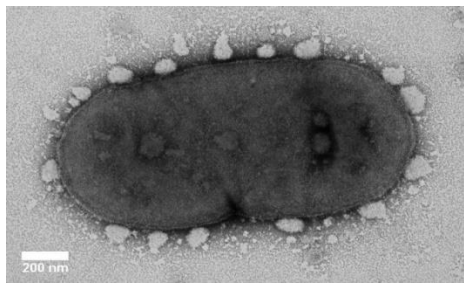
Porphyromonas gingivalis, 2016

crevices. The amino acids that make up these proteins provide *P. gingivalis* with enough energy to thrive in a deep periodontal pocket (How et al, 2016). *P. gingivalis* deploys many virulence factors such as fimbriae, lipopolysaccharides, and proteases (How et al, 2016).

These factors aid *P gingivalis* in things such as bone resorption, adhesion to host membranes, and degradation of plasma proteases inhibitors (How et al, 2016). An important virulence technique is the use of arg-gingipain and lys-gingipain cysteine proteinase (Kataoka et al, 2014). This technique causes abnormal immune and inflammatory responses within the host and further degrades tissue proteins (Kataoka et al, 2014). The lys-gingipain is encoded by a single gen *kgp* (Kataoka et al, 2014). However, arg-gingipain is capable of being encoded by two genes *rgpA* and *rgpB* (Kataoka et al, 2014). The *kgp-rgpA-rpgB* triple mutation is responsible for lesion formation and inducing abscesses of the mouth (Yoneda et al, 2003). These abrasions are worsened by normal oral functions such as chewing. These lesions provide the red complex

bacteria access to the host's bloodstream. Once in the bloodstream, these bacteria are capable of traveling throughout the body and affecting the host's organs. These bacteria have been linked to diseases such as hypertension and diabetes.

Alzheimer's disease

Alzheimer's disease is a chronic neurodegenerative disease (Weller et al, 2018). This disease is irreversible and accounts for 60-70% of all dementia cases (World Health Organization, 2019). Early symptoms of Alzheimer's may include forgetfulness, loss of time, and inability to recall familiar places (World Health Organization, 2019). Alzheimer's disease is a progressive disease with middle stage symptoms including the inability to remember recent events and people's names, recall details about familiar locations, communicate effectively, and complete familiar tasks (World Health Organization, 2019). Eventually, this disease will lead to late stage symptoms which include confusion with time, forgetting places such as home, difficulty recognizing relatives, and difficulty walking (World Health Organization, 2019). These symptoms are brought on by a physiological change in the brain that triggers neuron degeneration.

Inside the cell membrane of the brain, *APP* genes regulate the production of a protein called amyloid precursor protein (APP gene, n.d.). Located across a phospholipid bilayer, the amyloid precursor protein reaches inside and outside of the cell. This precursor protein helps repair and grow neurons in the brain. However, the amyloid precursor protein can become worn and need to be recycled (APP gene, n.d.). When the protein needs to be recycled, enzymes cleave the protein in two places (O'Brien et al, 2011). α-secretase and γ-secretase cleave the amyloid precursor protein in two locations (Chow et al, 2011). Cleaving takes place both inside

and outside of the phospholipid bilayer. This produces a soluble peptide fragment that is harmless to the human brain (Moore, 2020).

When β-secretase replaces α-secretase, a non-soluble monomer, known as amyloid beta, is formed (Moore, 2020). As these monomers are produced, they clump together forming amyloid beta plaque (Moore, 2020). This plaque is comprised of amyloid beta proteins, glial debris, and neuritic debris (Sheng et al, 1997). This plaque formation interrupts the crucial function of astrocytes and microglia promote neuron development by clearing cellular debris in the central nervous systems (Fakhoury, 2018). Astrocytes and microglia promote neuron development by clearing cellular debris in the central nervous system (Fakhoury, 2018). In response to the invasive plaque, the microglial cells activate an abnormally large neuroimmune response (Lenz, 2018). In an attempt to destroy the amyloid beta plaque, the neuroimmune response causes neuroinflammation resulting in the dysfunction and degeneration of the microglia (Clayton, 2017).
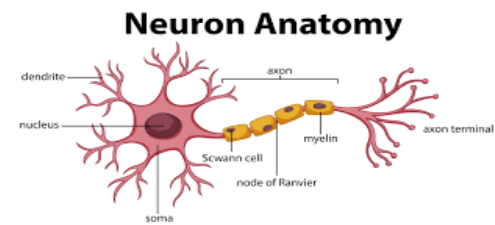
Neuron Anatomy, n.d.

The accumulation of beta amyloid plaque between neurons generates a disruption of chemical signaling impairing communication between the synapses (What Happens to, n.d.). Unable to transmit signal, the pathway between the soma, the area of the where neuro-signals join, of the neuron and synapse begin to degrade (Clayton et al, 2017). The degradation of these pathways directly affects the dendrite; the dendrite is the internal components of the neuron located between the soma and synapse which receives impulses from other cells (Dharani, 2015). Within the neuron, microtubules provide structure and transport substances throughout the cell (Alberts et al, 2002). These microtubules are composed of thirteen filamentous strands composed

of chain proteins called tubulin (Alberts et al, 2002). These filamentous strands deliver neurotransmissions from the soma to the synapsis (Alberts et al, 2002).

Another component of the microtubule is the Tau protein. Tau proteins line the microtubules of the neuron to stabilize and maintain the structure of the microtubule filaments (Mandelkow et al, 2012). As the neuron degrades, the microtubule begins to dephosphorylate causing a release of tau proteins throughout the neuron (Mandelkow et al, 2012). These free-floating proteins begin to form filaments throughout the neuron (Guo et al, 2014). The buildup of tau proteins produces neurofibrillary tangles (Lewis et al, 2000). As neurofibrillary tangles begin to aggregate, they be begin degrading the neuron. Eventually, the neuron loses all synaptic communication and dies (Johnson et al, 2004).

Neurofibrillary tangles begin by forming in neurons of the hippocampus (Furcila et al, 2019). The hippocampus is important in the formation of memories, learning abilities, and emotions (Tyng et al, 2017). As neurofibrillary tangles develop in the hippocampus, early stages of Alzheimer's disease such as difficulty forming memories begin to show (Wint et al, 2014). As neurofibrillary tangle concentrations increase within the hippocampus, they begin spreading to other regions of the brain. Similar to results within the hippocampus, the neurofibrillary tangles cause atrophy throughout the entire brain (Wint et al, 2014). This gives rise to brain abnormalities resulting in loss of cognitive function. Recent studies suggest that this neuroinflammatory. Recent studies suggest that this neuroinflammatory response began by infiltration of periodontal bacteria entering the brain via the bloodstream (Teixeira et al, 2017).

Periodontal Disease Linked to Alzheimer's Disease

Periodontitis is a chronic disease that causes inflammation and destruction of gingiva and tooth-supporting bone in the mouth. Cases of periodontitis are very common among the American population with nearly 50% of adults suffering from periodontitis (CDC: Half of, 2012). The likelihood of developing periodontitis increases with age. Individuals over the age of 65 and suffering from Alzheimer's were believed to have suffered from periodontitis due to the inability to perform adequate oral health upkeep. However, recent studies suggest periodontitis is the causal root of both dementia and Alzheimer's disease (Cerajewska et al, 2016). Since periodontitis is a chronic disease, periodontal bacteria exposure is far greater in elderly individuals. Once present in above threshold concentrations, periodontal bacteria will continue to persist, even after regular brushing (Loesche et al, 2001). When left untreated, these infectious periodontal bacteria enter the bloodstream. Recent studies suggest these bacteria traveled to organs such as the pancreas, heart, and brain in individuals affected by periodontitis (Iwai, 2009).

Due to the destructive nature of the bacterium, *Porphyromonas gingivalis*, a red complex pathogen in chronic periodontitis, has been labeled as a risk factor for developing amyloid beta plaque, dementia, and Alzheimer's disease (Ide et al, 2016). The possible mechanism of periodontal disease being an underlying factor in the development of Alzheimer's disease was suggested after the completion of an observational study. The study graded Alzheimer's disease patients on a scale of cognition, Alzheimer's disease assessment scale-cognitive, and mini mental state examination scale (Carotenuto et al, 2018). Over a 6-month study, Alzheimer's disease patients who currently suffered from chronic periodontitis reported a decline in basic function of cognition (Carotenuto et al, 2018). The results prompted the hypothesis that periodontal bacteria are directly linked to the development of Alzheimer's disease.

Literature Review

Cortexyme Incroporate, a biopharmaceutical company, states they are altering the effects of degenerative diseases in the human body. Due to factors such as aging, Cortexyme hypothesizes that our body progressively loses the ability to protect itself from pathogens such *Porphyromonas gingivalis*. Cortexyme's hypothesis states that the blood brain barrier becomes more permeable with age (Cortexyme initiates new, 2019). Increased permeability allows bacteria such as *Porphyromonas gingivalis* to flow from the mouth to the brain (Cortexyme initiates new, 2019). Once in the brain bacteria begin to attack brain cells. During this time, *Porphyromonas gingivalis* begins to secrete gingipains to increase the bacterium's virility.
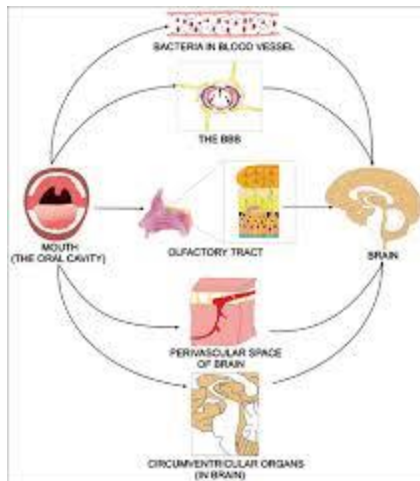

Olsen et al, 2015

Although the brain retaliates with a neuroimmune response, the gingipains are capable of protecting *Porphyromonas gingivalis* (Cortexyme initiates new, 2019). The gingipains respond by degrading cytokines and triggering the formation of amyloid beta plaques (Cortexyme initiates new, 2019). As the plaque continues to buildup, chemical signaling between neurons is stopped and microtubules along the skeleton of the neuron breakdown (Cortexyme initiates new, 2019). This degradation leads to an increased concentration of neurofibrillary tangles and results in the death of neuron involved. Cortexyme believes the use of a competitive inhibitor can stop the gingipain, virtually starving *Porphyromonas gingivalis* (Olsen et al, 2014).

Coxtexyme began a series of preclinical trials to determine the association between *Porphyromonas gingivalis* and Alzheimer's disease. Aiming to find the presence of gingipains and *P. gingivalis*, the study examined the post-mortem DNA cortex tissues in the brains of Alzheimer's patients (Cortexyme Presents Data, 2020). Cortexyme's post-mortem study revealed

that over 90% of the brains affected by Alzheimer's disease also tested positive for the presence of *P. gingivalis* (Cortexyme Presents Data, 2020). Cortexyme used qPCR technology to detect *Porphyromonas gingivalis* in human brain tissue and cerebral spinal fluid (Specific methods, 2019). Using advanced sequencing techniques, they were able to determine the positive presence of both *Porphyromonas gingivalis* and gingipains in brain samples using bacteria specific primers: *hmuY, 16s,* and *Kgp* (Specific methods, 2019). Next, *Porphyromonas gingivalis* specific primers were selected using DNA fragments: forward sequence ACCTTTAAACCCAATAAATC, reverse sequence ACGAGTATTGCATTGAATG, and fluorescent probe CGCTCGCATCCTCCGTATTAC (Specific methods, 2019). After the qPCR, the data was able to definitively confirm the presence of
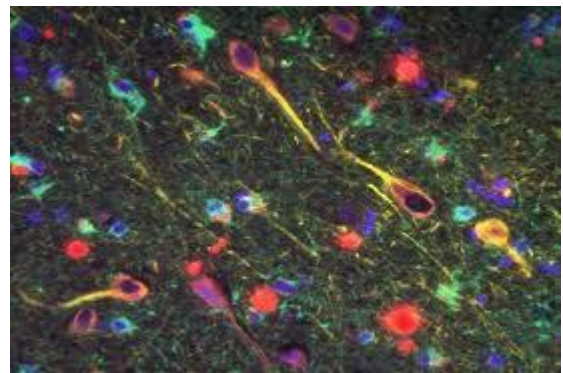
Stoye, 2019

Porphyromonas gingivalis and gingipains in a brain suffering from Alzheimer's disease (Specific methods, 2019). After the positive identification of gingipains and the bacterium *Porphyromonas gingivalis* in the human brain and spinal fluid, Cortexyme is hoping to find an inhibitor to block gingipain function and induce a successful neuroimmune response (Wang, 2019) Although the inhibitor is still in the preclinical trial phase, Cortexyme's study and resulting hypothesis may stop the progression of Alzheimer's in patients exhibiting periodontitis.

Discussion

Gum disease is one of the most common diseases throughout America. If left untreated, gum disease can progress from gingivitis to chronic periodontitis. Periodontitis is a disease that infects and damages the bone and tissue that supports the tooth. The bacterium known to cause periodontitis is *Porphyromonas gingivalis*. This bacterium has been linked to coronary artery

disease, rheumatoid arthritis, and complications in diabetes (Periodontitis, 2020). Recent research has been able to positively identify *Porphyromonas gingivalis* and gingipains in the brains of those suffering from Alzheimer's disease. After entering the bloodstream through the mouth, the bacterium travels throughout the host's body infecting the organs. Companies, such as Cortexyme Inc, are developing drugs to combat *Porphyromonas gingivalis*'s defense. They hope this would starve out the bacterium causing these diseases.

I propose a bacterial vaccine to eradicate the body of the bacterium *Porphyromonas gingivalis.* After exposure, the body builds up defenses against the bacterium in the form of antibodies. However, *Porphyromonas gingivalis* is capable of evading the host's defenses by releasing gingipains. A bacterial vaccine composed of deactivated *Porphyromonas gingivalis* and gingipains would help the host build antibodies. This vaccine could be administered by a dentist after the identification of *Porphyromonas gingivalis*. This method could be used as a preventive measure against periodontitis and linked diseases.

Reference

American Academy of Periodontology. (2012, September 4). CDC: Half of American Adults Have Periodontal Disease. https://www.perio.org/consumer/cdc-study.htm

APP gene - Genetics Home Reference - NIH. (n.d.). https://ghr.nlm.nih.gov/gene/APP

Armitage, Gary C. "Development of a Classification System for Periodontal Diseases and Conditions." *American Academy of Periodontology*, American Academy of Periodontolgy, 1 Dec. 1999. http://aap.onlinelibrary.wiley.com/doi/pdf/10.1902/annalss.1999.4.1.1.

Berezow, A. B., & Darveau, R. P. (2011). Microbial shift and periodontitis. *Periodontology 2000*, *55*(1), 36–47. https://doi.org/10.1111/j.1600-0757.2010.00350.x

Brichford, C. (2007, October 16). *Beyond Teeth: What's Inside Your Mouth*. Everyday Health. https://www.everydayhealth.com/dental-health/101/beyond-your-teeth.aspx

Carotenuto, A., Rea, R., Traini, E., Ricci, G., Fasanaro, A. M., & Amenta, F. (2018). Cognitive Assessment of Patients With Alzheimer's Disease by Telemedicine: Pilot Study. *JMIR mental health*, *5*(2), e31. https://doi.org/10.2196/mental.8097

Carrouel, F., Viennot, S., Santamaria, J., Veber, P., & Bourgeois, D. (2016). Quantitative Molecular Detection of 19 Major Pathogens in the Interdental Biofilm of Periodontally Healthy Young Adults. *Frontiers in microbiology*, *7*, 840. https://doi.org/10.3389/fmicb.2016.00840

Cerajewska, T., Davies, M., West, N., (2016, April 01)., Periodontitis: A Potential Risk Factor for Alzheimer's disease. *Nature*, http://www.nature.com/articles/bdjteam201662

Chow, V. W., Mattson, M. P., Wong, P. C., & Gleichmann, M. (2010). An overview of APP processing enzymes and products. *Neuromolecular medicine*, *12*(1), 1–12. https://doi.org/10.1007/s12017-009-8104-z

Clayton, K. A., Van Enoo, A. A., & Ikezu, T. (2017). Alzheimer's Disease: The Role of Microglia in Brain Homeostasis and Proteopathy. *Frontiers in neuroscience*, *11*, 680. https://doi.org/10.3389/fnins.2017.00680

Cortexyme initiates new trial of Alzheimer's medicine. (2019, April 17) *Clinical Trials Arena*, http://www.clinicaltrialsarena.com/news/cortetxyme-alzheimers-trial/

Cortexyme Presents Data Supporting Role of P. Gingivalis in Alzheimer's Pathology at Advances in Alzheimer's and Parkinson's Therapies Virtual Focus Meeting 2020. (2020, April 2). https://www.businesswire.com/news/home/20200402005245/en/

"Dementia." (2019) *World Health Organization*, World Health Organization. http://www.who.int/news-room/fact-sheets/detail/dementia

Dharani, K. (2015). Physiology of the Nueron. *The Biology of Thought*. http://www.sciencedirect.com/topics/psychology/dendrites

Fakhoury M. (2018). Microglia and Astrocytes in Alzheimer's Disease: Implications for Therapy. *Current neuropharmacology*, *16*(5), 508–518. https://doi.org/10.2174/1570159X15666170720095240

Furcila, D., Domínguez-Álvaro, M., DeFelipe, J., & Alonso-Nanclares, L. (2019). Subregional Density of Neurons, Neurofibrillary Tangles and Amyloid Plaques in the Hippocampus of

Patients With Alzheimer's Disease. *Frontiers in neuroanatomy*, *13*, 99.

https://doi.org/10.3389/fnana.2019.00099

Gao, L., Xu, T., Huang, G., Jiang, S., Gu, Y., & Chen, F. (2018). Oral microbiomes: more and

more importance in oral cavity and whole body. *Protein & cell*, *9*(5), 488–500.

https://doi.org/10.1007/s13238-018-0548-1

Guo, J. L., & Lee, V. M. (2014). Cell-to-cell transmission of pathogenic proteins in

neurodegenerative diseases. *Nature medicine*, *20*(2), 130–138. https://doi.org/10.1038/nm.3457

Guo, Y. J., Ge, Z. P., Ma, R. H., Hou, J. X., & Li, G. (2016). A six-site method for the evaluation

of periodontal bone loss in cone-beam CT images. *Dento maxillo facial radiology*, *45*(1),

20150265. https://doi.org/10.1259/dmfr.20150265

Hoffman, M., (n.d.)., Picture of the Teeth. *WebmMB*. http://webmd.com/oral-health-of-the-

teeth#1

How, K. Y., Song, K. P., & Chan, K. G. (2016). Porphyromonas gingivalis: An Overview of

Periodontopathic Pathogen below the Gum Line. *Frontiers in microbiology*, *7*, 53.

https://doi.org/10.3389/fmicb.2016.00053

Ide, M., Harris, M., Stevens, A., Sussams, R., Hopkins, V., Culliford, D., … Holmes, C. (2016,

March 10). Periodontitis and Cognitive Decline in Alzheimer's Disease.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4786266/

Iwai, T. (2009, October 23). Periodontal bacteremia and various vascular diseases.

https://onlinelibrary.wiley.com/doi/10.1111/j.1600-0765.2008.01165.x

Kataoka, S., Baba, A., Suda, Y., Takii, R., Hashimoto, M., Kawakubo, T., … Yamamoto, K. (2014). A novel, potent dual inhibitor of Arg-gingipains and Lys-gingipain as a promising agent for periodontal disease therapy. *The FASEB Journal*, *28*(8), 3564–3578.

Khocht A. (2012) The periodontium. *MedEdPORTAL. 8*. 90-91. https://doi.org/10.15766/mep-2374-8265.9091

Kinane, D. F., Lappin, D. F., Koulouri, O., & Buckley, A. (1999). Humoral immune responses in periodontal disease may have mucosal and systemic immune features. *Clinical and experimental immunology*, *115*(3), 534–541. https://doi.org/10.1046/j.1365-2249.1999.00819.x

Kulshrestha, R., Biswas, J., & Srinivasa, T., (2013, April). *Role of Immunoglobulin G and A in Periodontitis: A Review*. Journal of Pure and Applied Microbiology, 70(1):673-676

Johnson, G. V. W., & Stoothoff, W. H. (2004, November 15). Tau phosphorylation in neuronal cell function and dysfunction. https://www.ncbi.nlm.nih.gov/pubmed/15537830

Lenz, K. M., & Nelson, L. H. (2018). Microglia and Beyond: Innate Immune Cells As Regulators of Brain Development and Behavioral Function. *Frontiers in immunology*, *9*, 698. https://doi.org/10.3389/fimmu.2018.00698

Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Slegtenhorst, M. V., … Hutton, M. (n.d.). Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. https://www.nature.com/articles/ng0800_402

Loesche WJ. Microbiology of Dental Decay and Periodontal Disease. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 99. https://www.ncbi.nlm.nih.gov/books/NBK8259/

Loesche, W. J., & Grossman, N. S. (2001). Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. *Clinical microbiology reviews*, *14*(4), 727–752. https://doi.org/10.1128/CMR.14.4.727-752.2001

Madukwe, I. (2014). Anatomy of the periodontium: A biological basis for radiographic evaluation of periradicular pathology. *Journal of Dentistry and Oral Hygiene*, *6.* 71-75.

Marquez, I. (2004). *The role of keratinized tissue and attached gingiva in maintaining periodontal/peri-implant health.* NCBI. https://www.ncbi.nlm.nih.gov/pubmed/15055675

Moore, E., (2020). *Alzheimers Disease And Infectious Causes the theory and evidence*. JEFFERSON: MCFARLAND.

Nazir M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. *International journal of health sciences*, *11*(2), 72–80.

Neuron Anatomy., (n.d.). *Vecteezy.*, http://www.istockphoto.com/vector/diagram-of-neuron-anatomy

Newman, Tim. "Gingivitis: Causes, Symptoms, and Treatment." *Medical News Today,* MediLexicon International, 5 Jan. 2018, http://www.medicalnewstoday.com/articles/241721.php

O'Brien, R. J., & Wong, P. C. (2011). Amyloid precursor protein processing and Alzheimer's disease. *Annual review of neuroscience*, *34*, 185–204. https://doi.org/10.1146/annurev-neuro-061010-113613

Olsen & Singhrao et al., (2015) *Neurology India.,*

http://www.neurologyindia.com/viewimage.asp?img=ni_2018_66_2

Olsen, I., & Potempa, J. (2014). Strategies for the inhibition of gingipains for the potential treatment of periodontitis and associated systemic diseases. *Journal of oral microbiology*, *6*, 10.3402/jom.v6.24800. https://doi.org/10.3402/jom.v6.24800

Periodontal Disease., (n.d.). *Greater Pittsburg Dental Implants & Periodontics*. http://pittsburghimplantsandperio.com/blog/periodontal-disease/

Periodontitis., (2020, February 14). *Mayo Clinic*. https://www.mayoclinic.org/diseases-conditions/periodontitis/symptoms-causes/syc-20354473

Periodontitis: the complex theory according to Socransky. (n.d.). https://www.micro-ident.de/en/dentists/periodontitis/risk-factor-bacteria/bacterial-complexes/

Porphyromonas Gingivalis – The Bacteria of Periodontitis II (microorganisms, treatment)., (2016, January 25)., *perio.prostho.cc-passion in dentistry*. http://www.perioprosthocc.wordpress.com/2016/01/25/porphyromonas-gingivalis-the-bacteria-of-periodontitis-ii-microorganisms-treatment/

Posnick, J. (2013, October 10). *Periodontal Considerations in the Evaluation and Treatment of Dentofacial Deformities*. ScienceDirect. https://www.sciencedirect.com/science/article/pii/B978145572698100006X

Santos-Longhurst, A. (2018, May 15). *Tooth Anatomy*. Healthline. https://www.healthline.com/health/tooth-anatomy

Scotti, E., Boué, S., Sasso, G. L., Zanetti, F., Belcastro, V., Poussin, C., … Hoeng, J. (2017). Exploring the microbiome in health and disease. *Toxicology Research and Application*, *1*, 1-7

Sheng, J., Mrak, R. & Griffin, W. Neuritic plaque evolution in Alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms. *Acta Neuropathol* **94,** 1–5 (1997). https://doi.org/10.1007/s004010050664

Stoye, E., (2019, January 24)., Alzheimer's linked to infection of the brain by gum disease bacterium. *Royal Society of Chemistry*. https://www.chemistryworld.com/news/alzheimers-linked-to-infection-of-the-brain-by-gum-disease-bacterium/3010031.article

Teixeira, F. B., Saito, M. T., Matheus, F. C., Prediger, R. D., Yamada, E. S., Maia, C., & Lima, R. R. (2017). Periodontitis and Alzheimer's Disease: A Possible Comorbidity between Oral Chronic Inflammatory Condition and Neuroinflammation. *Frontiers in aging neuroscience*, *9*, 327. https://doi.org/10.3389/fnagi.2017.00327

Tyng, C. M., Amin, H. U., Saad, M., & Malik, A. S. (2017). The Influences of Emotion on Learning and Memory. *Frontiers in psychology*, *8*, 1454.
https://doi.org/10.3389/fpsyg.2017.01454

Velsko, I.M., Fellows Yates, J.A., Aron, F. *et al.* Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. *Microbiome* **7,** 102 (2019). https://doi.org/10.1186/s40168-019-0717-3

Vilardi, M., (2017, May 01). *Understanding Periodontal Pockets*. Dear Doctor Dentistry & Oral Health. https://www.deardoctor.com/articles/undertanding-periodontal-pockets/

Wang, B. (2019, January 24). Chronic Gum Disease Bacteria Porphyromonas gingivalis (Pg) Drives Alzheimer's Disease (AD) Pathology. *Next Big Future*.
https://www.nextbigfuture.com/2019/01/chronic-gum-disease-bacteria-porphyromonas-gingivalis-pg-drives-alzheimers-disease-ad-pathology.html

Watson, S. (2020, January 07). *Gingival Sulcus and Keeping Teeth Healthy*. VerywellHealth.

https://www.verywellhealth.com/what-is-gingival-sulcus-1059436

Weller, J., & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research*, *7*, F1000 Faculty Rev-1161.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6073093/

What Happens to the Brain in Alzheimer's Disease? (n.d.) https://www.nia.nih.gov/health/what-happens-brain-alzheimers-disease

White, D. J. (1997). Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *European Journal of Oral Sciences*, *105*(5), 508–522.

Wiebe, C. B., & Putnins, E. E. (2000, December). The periodontal disease classification system of the American Academy of Periodontology--an update.

https://www.ncbi.nlm.nih.gov/pubmed/11253351

Wint, D., Tavee, J., Sweeney P., (2014, April). Alzheimer's Disease. *Cleveland Clinic Center for Continuing Education*.

http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/neurology/alzheimers-disease/

Yoneda, M., Hirofuji, T., Motooka, N., Anan, H., Hamachi, T., Miura, M., … Maeda, K. (2003). Antibody responses to Porphyromonas gingivalis infection in a murine abscess model - involvement of gingipains and responses to re-infection. *Journal of Periodontal Research*, *38*(6), 551–556.

Yost, S., Duran-Pinedo, A.E., Teles, R. *et al.* Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. *Genome Med* **7,** 27 (2015). https://doi.org/10.1186/s13073-015-0153-3