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TOPICAL REVIEW

From focal sepsis to periodontal medicine: a century of exploring the role of the oral microbiome in systemic disease

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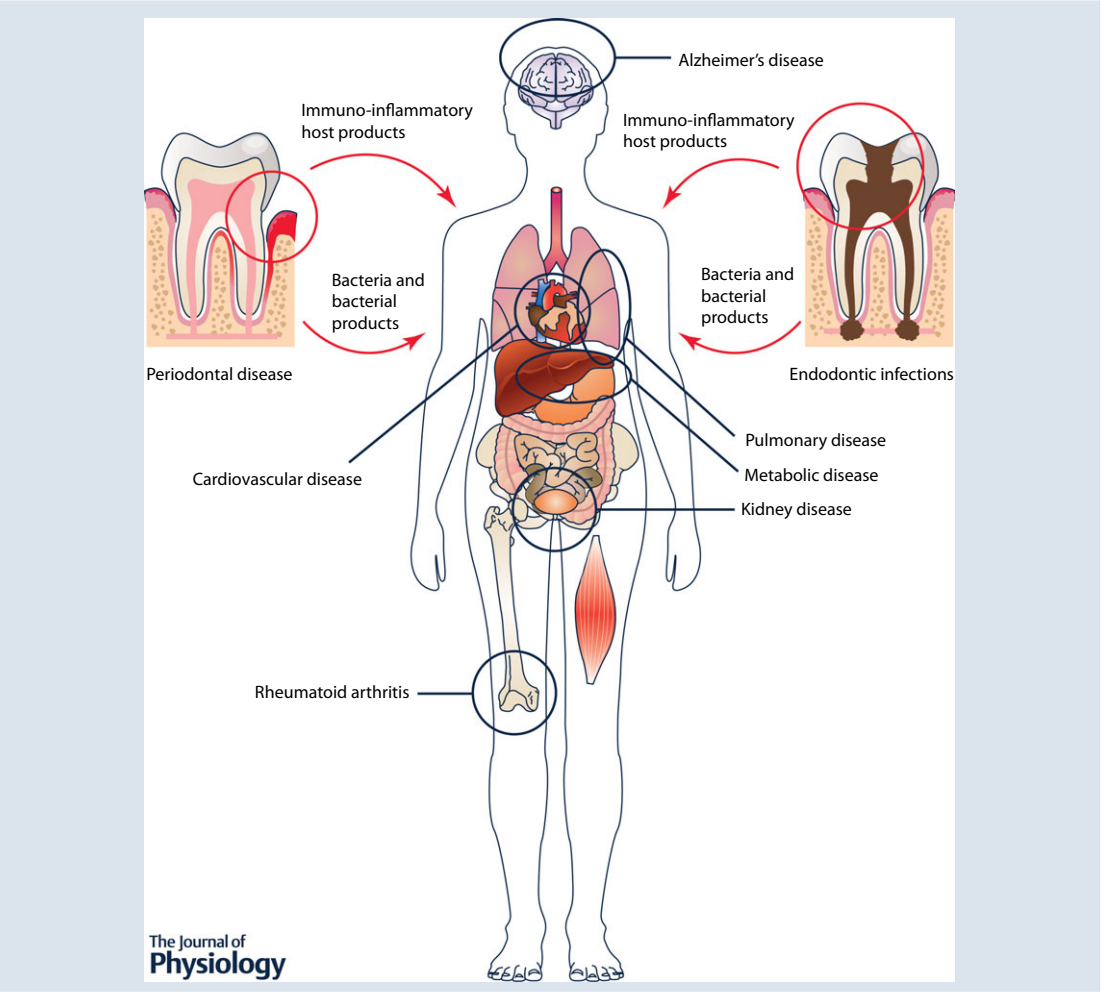

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Abstract The oral microbiome is established within a few minutes after birth and consists of stable multi-species communities that engage in a dynamic equilibrium with the host immune system. Dental caries, endodontic infections and periodontal diseases are bacterially driven diseases that are caused by dysbiotic microbiomes. Over a century ago, the focal infection theory implicated these infections in the aetiology of several systemic diseases, ranging from arthritis to neurodegenerative diseases. However, a lack of concrete evidence, combined with the urgency with which clinicians embraced this approach without regard for appropriate case selection, led to its demise within 30 years. In the last decade of the 20th century, the concept of periodontal medicine was introduced to explain the correlations that were being observed between periodontitis and cardiovascular disease, rheumatoid arthritis, Alzheimer's disease, pulmonary disease, pre-term delivery of low birth weight infants and metabolic disease. It was proposed that periodontal pathobionts played a causal role in the initiating or exacerbating certain diseases either by direct invasion or by stimulating a florid immune-inflammatory response that extended into the systemic circulation. This review will examine the strength of current evidence in establishing a causal link between oral pathobionts and systemic disease.

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Abstract figure legend The effects of bacteria associated with dental caries and periodontitis on various systemic diseases: a review of the currently available evidence.

The oral microbial ecosystem – real estate and habitats

In the early 1930s, Arthur Roy Clapham coined the word 'ecosystem' to describe a community that consisted of living organisms that interact with each other as a system and are linked through energy flow and nutritional and metabolic support (Blew, 1996). The oral cavity is home to arguably one of the most well studied ecosystems in the human body. This environment presents several habitats for both aerobic and anaerobic bacterial colonization: abiotic surfaces such as the tooth, dental implants and dental restorations, and biotic environments such as the subgingival crevice (space between the tooth and the gumline), keratinized mucosal surfaces on the dorsum of the tongue, hard palate and attached gingiva, and non-keratinized epithelial surfaces on the buccal mucosa, tonsils and alveolar mucosa. The subgingival crevice provides 12 cm^2 of surface area for bacterial colonization (Hartzell & Henrici, 1916), while both keratinized and non-keratinized surfaces of the oral mucosa constitute a real estate of more than 200 cm^2 (Collins & Dawes, 1987). Together with the tooth surfaces, there is 500 cm^2 of space available for bacterial colonization. Bacteria colonize these niches within a few minutes of birth and co-evolve with shifts in the host through two dentition states, concomitant changes in food habits, oral hygiene practices and lifestyle shifts.

Dysbiosis in this system underlies the aetiologies of some of the most common diseases to affect humans: caries, periodontal disease and endodontic infections. Over the past three decades, a robust body of evidence attesting to the systemic effects of these dysbiotic communities has burgeoned. However, as we examine the historical evolution of oral microbiology, it is seen that this idea had been in vogue during the turn of the 20th century as well. This review will summarize the historical as well as currently available evidence regarding the role played by the oral microbiome in causing disease in the rest of the human body.

The impact of the focal infection theory on medicine and dentistry

The focal infection theory (FIT) posits that bacteria and/or bacterial toxins and metabolic byproducts can enter the systemic circulation from a clinically asymptomatic localized lesion that contains pathogenic bacteria and translocate to distant parts, initiating disease in these organ systems. The resulting metastatic disease is chronic, but not infectious. Some examples of diseases that have been attributed to focal sepsis are gonococcal arthritis following gonorrhoeal infection, neuritis, myalgia, nephritis, osteomyelitis, emphysema, endocarditis, pneumonia, asthma, gastritis, pancreatitis, colitis, diabetes, goitre, thyroiditis and Hodgkin's disease (Roberts, 1921). The central belief of the focal infection theory is that injury occurs at a site distant from the site of infection; the focus of infection is usually unrecognizable or clinically unremarkable, as in infections of the tonsil, sinus, prostate, appendix, bladder, gall bladder and kidney; and the secondary disease occurs only in sites that

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are susceptible to the bacterial species or toxins. This distinguishes it from true 'infectious diseases' such as cholera and typhoid, where the organ damage occurs secondary to the primary systemic infection.

While this theory has been in vogue since Hippocrates reported curing arthritis by extracting a tooth (Francke, 1973), the modern form experienced a period of popularity during the early 20th century, beginning with a lecture at McGill University by Dr William Hunter, a British physician who denounced the preservation of a carious tooth by building what he called 'a veritable mausoleum of gold fillings, crowns and bridges over a mass of sepsis' as the cause of a multitude of systemic diseases (Hunter, 1900). Although some investigators believe that his words were misquoted, and that he was referring to ill-fitting crowns and dentures, influential physicians like Russell Cecil and Charles Mayo, who were significant thought leaders of the time, recommended that all teeth be extracted either to prevent or to treat any number of conditions ranging from allergy to schizophrenia, and thus earned the sobriquet 'one hundred percenters' (Dussault & Sheiham, 1982; Murray & Saunders, 2000). Together with tonsillectomy, full mouth extractions became routine treatment options for diverse conditions ranging from arthritis deformans to blindness (Billings, 1930; Bocca *et al.* 1989). Two events may have played a significant role in creating this generation of edentulous individuals. The first was an influential book by W. D. Miller in 1880 *The Micro-Organisms of the Human Mouth: The Local and General Diseases Which Are Caused by Them*, in which he introduced the term 'oral focal sepsis' and recommended dental fillings or root canal therapy to treat tooth decay, which he said was a bacterial disease (Miller, 1880). The second was the development of the dental X-ray, which revealed the presence of peri-apical radiolucencies in asymptomatic teeth and periodontal bone loss. A novel research strategy called 'reverse investigation', where the research is instigated by a conclusion and is focused on gathering evidence to support this conclusion, was used to generate evidence to support this theory (Ingle *et al.* 2008).

Soon, the infected periodontal pocket drew attention as yet another, larger nidus of infection. It was held that teeth with 'pyorrhoea' (periodontitis) 'shower bacteria into the blood stream' even during everyday activities such as chewing or tooth brushing, and that these bacteria were identifiable in the circulation close to the source (peri-apical veins) and in distal blood vessels (median basilic veins) following tooth extraction or chewing on hard candy (Fish, 1940). Rosenow proposed that oral bacteria or their toxins preferentially segregate to areas predominantly composed of mesenchymal tissues, notably joints, muscles and neuronal sheaths (Rosenow, 1930). He believed that their 'unique functions of repair, regeneration and scavenging of waste products' increased their susceptibility to bacteria and bacterial toxins. Rosenow proposed that certain pathogens demonstrated a predilection for specific target tissues (the theory of 'elective localization or dissemination') and that bacteria were capable of spontaneously changing to another species (transmutation). Transmutation was held to be the reason why results could not be replicated between researchers and labs. Thus, through case reports of diseases being identified in individuals with infected root canals or teeth with 'pyorrhoea' (periodontitis), animal experiments that demonstrated induction of lesions of the 'heart muscle and endocardium, lesions of the kidney, focal and diffuse, lesions of the adventitia of the blood vessels, and iritis' by 'organisms taken from the dental path' (Hartzell & Henrici, 1916), and selective or complete edentulation of subjects with arthritis and vascular disease, several researchers demonstrated the aetiological role of oral bacteria in systemic diseases (Klotz, 1913; Hartzell & Henrici, 1916).

However, it was soon apparent that the routine removal of teeth could not predictably cure circulatory, neuro-degenerative, or kidney diseases. Patients demonstrated a worsening of arthritic symptoms and, not surprisingly, developed digestive complications following therapeutic edentulation (Cecil & Angevine, 1938; Vaizey & Clark-Kennedy, 1939), and in many cases, were cured of their psychiatric ailments even in the absence of edentulation (Wessely, 2009; Shorter, 2011). As medical experimentation and animal model research grew more sophisticated, significant flaws were identified in Rosenow's, Hartzell and Henrici's, and Price's studies, notably the lack of controls and the massive doses of bacterial inoculum used. More importantly, a concerted effort by the clinical endodontic community to establish that root-canal treatment resulted in resolution of dental and peri-apical infection led to the demise of the focal infection theory. Thus, focal infection was disregarded as a scientific theory for several decades.

Periodontal medicine: resurrecting the focal infection theory?

The endodontic community has remained steadfast in its rejection of the infected root canal as a cause of distant, non-infectious disease (Ingle *et al.* 2008). The position of the American Association of Endodontists (AAE) has been that (i) bacteraemia occurs as part of normal daily activity such as chewing and tooth brushing; (ii) there is no evidence on the inoculum size needed to generate a metastatic disease – the only consistency between the turn of the century animal studies (Rosenow, Price, Henrici and Hartzell) was that the inoculum sizes were unrealistically large; and (iii) dental extractions produce a larger circulatory bacterial load than endodontic therapy. The AAE does, however, recognize that

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untreated peri-apical infections may cause distant disease by releasing bacteria and bacterial products into the circulation.

On the other hand, untreated periodontal disease has continued to be examined as a source of circulatory bacteria. This became especially important when the American Heart Association released a position paper on the role of oral streptococci in bacterial endocoarditis (Rammelkamp *et al.* 1957). The last decades of the 20th century saw the emergence of new techniques for bacterial identification and classification, especially oral microorganisms. Non-targeted molecular assays such as 16S sequencing revealed the presence of novel and hitherto unsuspected organisms in the oral cavity (Paster *et al.* 2001; Kumar *et al.* 2003), while innovations in culturing and microscopical approaches allowed the identification of uncommon phenotypes in known species (Beighton *et al.* 1991; Kell *et al.* 2015). Several systemic pathogens, ranging from respiratory pathobionts such as *Hemophilus influenzae*, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* to gut pathogens such as *Trophyrema whipplei* (Liljemark *et al.* 1984; Zinkernagel *et al.* 2003; Persson *et al.* 2008; Kumar *et al.* 2011; Mason *et al.* 2014), have been identified in significant numbers in the periodontal pocket. Explorations of atheromatous plaques, knee implants, placenta, amniotic sac, the tracheobronchial tree, joint cavities and the pancreas have revealed the presence of periodontal pathogens, for example *Porphyromonas gingivalis*, *Treponema denticola*, *Fusobacterium nucleatum* and *Campylobacter rectus*, in these areas, especially in regions that were previously considered sterile (Bearfield *et al.* 2002; Leon *et al.* 2007; DiGiulio *et al.* 2008; Aagaard *et al.* 2014). These advances in microbiological methodologies and clinical techniques produced data that suggested that the oral cavity could indeed act as a reservoir of bacteria that might metastasize to distant sites in the body and cause disease in susceptible individuals. The World Workshop in Periodontics introduced the term 'periodontal medicine' in 1996 to describe the role played by periodontitis in exacerbating or initiating systemic diseases (Offenbacher, 1996). Thus, the last two decades have seen what may be considered a resurrection of the focal infection theory; however, investigators are using an abundance of caution in advocating therapy based on these links.

While several lines of evidence are emerging to suggest that periodontitis may be linked to osteoporosis, diabetes, atherosclerotic circulatory disease, rheumatoid arthritis, pregnancy-related complications, pulmonary disorders, pancreatic cancer, chronic renal disease, obesity and Alzheimer's disease, there is little evidence at this point in time that oral bacteria or bacterially driven pathways play a role in all of these linkages. Therefore, in this review, we will focus only on studies that have examined the contributions of oral bacteria to periodontal-systemic disease.

The microbial ecosystem in periodontal disease

The oral cavity is an open microbial ecosystem in that, at any given time, it is home to several allochthonous species (transient visitors) in addition to autochthonous members (stable colonizers). Together, over 20 billion organisms can be found in this environment (Loesche, 1982), representing nearly 700 different species (Aas *et al.* 2005). These organisms live in a state of dynamic equilibrium with the host immune system, a situation that is reflected as clinical health. When the micro-environment changes, as a function of systemic antibiotics that negate the protective influence of commensals, reduced oxygen tension due to increase in biofilm thickness, altered host defences, or nutritional, metabolic and structural stresses within the ecosystem, a dysbiosis occurs in the indigenous microbiome, reducing the abundance of the commensal population and creating a pathogen-rich ecosystem (Socransky & Haffajee, 2005). The florid immune-inflammatory response to this pathogenic colonization leads to destruction of the attachment between the tooth and the gingiva, and loss of structures that anchor the tooth to the jawbone. Together, these two events result in a deepening of the gingival sulcus, which is the space between the tooth and the gingiva (Listgarten, 1986). This inflamed sulcus, now called a periodontal pocket, provides an anaerobic, protein- and haem-rich, oxidant-rich niche that promotes colonization by anaerobes, many of which are pathogenic to humans. At a conservative estimate, there are over 10 billion bacteria in 1 mg of dental plaque (Gibbons *et al.* 1964). Since these bacteria are packed into the space between the tooth and the sulcular epithelium, the breakdown of epithelial integrity caused by inflammation results in seeding of the systemic circulation with these pathogens when the biofilm is disrupted. The diseased periodontal pocket also contains significant levels of inflammatory mediators, especially those that mediate chronic inflammation. Tumour necrosis factor α, interleukins 1, 2 and 8, and prostaglandins can be released into the circulation from the diseased periodontium (Offenbacher *et al.* 1993; Hernichel-Gorbach *et al.* 1994), and may contribute to systemic inflammation.

As was demonstrated during the turn of the 20th century, newer studies have confirmed that simple oral hygiene procedures can translocate bacteria, bacterial products, toxins and inflammatory products to other sites in the body (Carroll & Sebor, 1980; Baltch *et al.* 1988), especially in individuals with oral infections. For example, in children with extensive dental decay, the frequency of bacteraemia following tooth brushing has been reported to range from 17 to 40% (Roberts *et al.* 1997), 100% following dental extraction (Heimdahl *et al.* 1990), 70% after professional dental cleaning (Lofthus *et al.* 1991), 97% following injection of dental anaesthetics and 20%

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following root canal treatment (Debelian *et al.* 1995). In immunocompetent individuals, the transient bacteraemia is eliminated from the circulation. However, individuals with a compromised immune system, e.g. diabetics and people with upper respiratory disease, may not exhibit similar abilities to clear the systemic bacteraemia, rendering them more susceptible to disease. The disrupted immune-inflammatory axes in these individuals may also result in profuse amounts of bacterial products (e.g. lipopolysaccharide and endotoxin) as well as host response mediators to be released into the circulation, triggering inflammatory responses in the target organs. Thus, the underlying pathophysiology of systemic diseases caused by periodontal infections may be metastatic infection, metastatic injury or metastatic inflammation.

Periodontal disease and pulmonary diseases

While pneumonia can be caused by infection with a bacterium, virus, fungus or parasite, the most common type is bacterial pneumonia. Typically, the lower respiratory tract is protected from microorganisms by the cough reflex, ciliary movement of the lining cells, and innate immune mediators (Levison, 1994), which are capable of dispersing salivary bacteria aspirated during sleep or from accidental swallowing. However, impairment of these defences (as in long-term smoking, diabetes, chronic obstructive pulmonary disease or immunosuppression, and during intubation or prolonged post-operative hospital stay) can result in nosocomial pneumonia (Toews, 1986; Sinclair & Evans, 1994). Cross-sectional studies have demonstrated that in dentate patients, poor oral hygiene and non-compliance with dental hygiene visits increase the risk for developing pneumonia, indicating that oral pathobionts may be a potential link between oral and lung diseases (Terpenning *et al.* 1993). Hospitalized subjects suffering from pneumonia have been shown to harbour the respiratory pathogens *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae* and *H. parainfluenzae* (Scannapieco *et al.* 1992; Russell *et al.* 1999; Scannapieco, 2006), while periodontal pathogens, for example, *P. gingivalis*, *F. nucleatum*, *Prevotella oralis*, *Campylobacter gracilis*, *Fusobacterium necrophorum* and *Aggregatibacter actinomycetemcomitans*, have been identified in lung aspirates of subjects with pneumonia (Yuan *et al.* 1992; Zijlstra *et al.* 1992; Lorenz & Weiss, 1994; Shinzato & Saito, 1995). Furthermore, periodontal treatment and improved oral hygiene decreased the incidence of pneumonia in children and hospitalized adults (Yoneyama *et al.* 1996; Scannapieco & Binkley, 2012).

The studies reviewed above and several others have suggested that oral bacteria may cause respiratory diseases when (i) oral bacteria or respiratory pathogens from oral reservoirs are aspirated into the lower respiratory tract, (ii) salivary enzymes released during chronic periodontal disease or smoking modify the oral mucosa and lead to increased adhesion by respiratory pathogens, and/or (iii) circulating pro-inflammatory cytokines released as a consequence of periodontal inflammation modify the respiratory mucosa (Li *et al.* 2000; Paju & Scannapieco, 2007).

The consensus report of the Joint European Federation of Periodontology/American Academy of Periodontology Workshop on Periodontitis and Systemic Diseases states that while there is insufficient evidence to date to infer causal relationships in most systemic diseases due to a paucity of prospective studies, it is highly likely that organisms originating in the oral microbiome can cause lung infections (Linden *et al.* 2013). A recent finding from the Women's Health Initiative Observational Study was that while periodontal disease was not independently associated with lung cancer in non-smoking post-menopausal women, in smokers this risk was increased beyond what could be expected from the sum of the each effect separately (Mai *et al.* 2014).

Periodontal disease and cardiovascular diseases

Cardiovascular disease is an umbrella term that encompasses a range of conditions, from high blood pressure to acute myocardial infarction, angina and stroke. The central pathophysiology of these diverse diseases is the atheromatous plaque. The infection hypothesis of atherosclerosis sprang up in the early 19th century, when Gilbert and Lion identified that systemic inoculation of the bacterium *Bacillus typhosus* could induce fatty sclerosis in the aortal wall in rabbits (Nieto, 1998). Osler, who is credited with the infection hypothesis of atherosclerosis, listed major wear and tear, acute infections, 'intoxications' (smoking, diabetes mellitus, obesity) and high blood pressure as the four major causes (reviewed by Nieto, 1998). In the early 1970s, several studies demonstrated that viral infections could induce damage to the endothelium and activate acute inflammatory mediators. Together with formation of foam cells, these events could lead to creation of thrombi and unstable atheromatous plaques (Fabricant *et al.* 1978; Hajjar *et al.* 1986; Etingin *et al.* 1990; Laitinen *et al.* 1997). It has also been demonstrated that cross reactivity between bacterial heat-shock proteins and human Hsp60 could initiate an autoimmune response that culminates in atherosclerosis (Wick *et al.* 1997; Kol *et al.* 1998).

While the role of oral haemolytic streptococci in the aetiology of subacute bacterial endocarditis was well established, the role of periodontal disease in its aetiopathogenesis was not as well known until the late 1990s, when correlations were observed between tooth loss and cardiovascular disease (Mattila *et al.* 1989;

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Mattila, 1993; Mattila *et al.* 1998). Since two infectious diseases, caries and periodontitis, can result in tooth loss, the relative contributions of these two oral infections were examined by Grau *et al.* (2004), who found that in subjects with periodontitis the risk for stroke was 400% greater than in those with caries. Desvarieux *et al.* (2003) found a correlation between intimal thickening of the carotid artery (a metric of atherosclerosis) and periodontal pathogens but not health-compatible organisms in over 600 individuals. DNA from the oral pathogens *Tannerella forsythia*, *F. nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis* and *A. actinomycetemcomitans* has been identified in carotid atheromas (Cairo *et al.* 2004), providing further evidence of bacterial translocation. Systemic antibody levels to periodontal pathogens correlated with the incidence of coronary heart disease and subclinical atherosclerosis (Beck *et al.* 2005; Pussinen *et al.* 2006) Animal studies on apolipoprotein E-knockout ($ApoE^{-/-}$) mice have demonstrated a role for dendritic cells in translocation of oral bacteria to the vasculature. Furthermore, treatment of chronic periodontitis was shown to decrease systemic markers of inflammation and improve endothelial dysfunction (Tonetti *et al.* 2007). The joint workshop of the European Federation of Periodontology and American Academy of Periodontology (EFP/AAP) concluded that while there is evidence to support the hypothesis that translocated oral microbiota may induce systemic inflammation that influences atherothrombogenesis, and that this biological mechanism is supported by *in vitro* experiments, animal models and clinical studies, 'intervention trials to date are not adequate to draw further conclusions' (Tonetti *et al.* 2013). The present consensus thus appears to support a temporal relationship between periodontal and cardiovascular diseases, with oral bacteria playing either a direct or an indirect role in disease causation.

Periodontal disease and pregnancy outcomes

The infection hypothesis of adverse pregnancy outcomes postulates that preterm delivery of a low birth-weight infant may occur as a result of either a local or systemic maternal infection. In the early 1990s, bacterial vaginosis was shown to contribute to preterm delivery of a low birth-weight infant (Kurki *et al.* 1992; Hillier *et al.* 1995). The dental community was already aware that the abundances of certain bacteria, notably the group of organisms collectively known as black pigmented bacteria, are higher in subgingival crevice during pregnancy, possibly because of the increased availability of oestradiol (Kornman & Loesche, 1980). Together, these two findings opened up several lines of research to examine the role of periodontal pathogens on neonatal health. The results have extremely equivocal (Mitchell-Lewis *et al.* 2001; Sanchez *et al.* 2004; Dasanayake *et al.* 2005; Noack *et al.* 2005; Michalowicz *et al.* 2006; Offenbacher *et al.* 2006; Seymour *et al.* 2007; Novak *et al.* 2008). Pre-term birth and low birth weight have been associated with high levels of *Tannerella forsythia*, *Campylobacter rectus*, *Prevotella intermedia*, *Prevotella nigrescens* and *Porphyromonas gingivalis* in maternal subgingival plaque (Mitchell-Lewis *et al.* 2001; Sanchez *et al.* 2004; Offenbacher *et al.* 2006). *Porphyromonas gingivalis* has been detected in both amniotic fluid and subgingival plaque of 31% of women with threatened pre-term labour (Leon *et al.* 2007). Recent evidence has identified *F. nucleatum* in the placental microbiome (Aagaard *et al.* 2014) and has implicated this organism in the aetiopathogenesis of pre-term deliveries (Han *et al.* 2004). Two mechanisms have been proposed and tested to explain the correlation between adverse pregnancy outcomes and periodontal infection.

(i) Intrauterine inflammation. It has been shown that periodontal bacteria elicit high levels of prostaglandin E2 (PGE2) and cytokines in circulation and in the placenta (Madianos *et al.* 2001; Liu *et al.* 2007). Taken together with the fact that periodontal therapy reduces this pathogen load and is accompanied by a 3.8-fold decrease in pre-term births (Offenbacher *et al.* 2006), these results suggest that circulating periodontal pathogens may trigger an inflammatory response in the uterus, which could contribute to pre-term birth.

(ii) Fetal response to maternal pathogens. Aagaard *et al.* (2014) using a deep-sequencing strategy, have demonstrated that the placenta is not a sterile environment, as was previously believed, but in fact plays host to several oral organisms. Pre-term babies, but not full-term infants, demonstrated higher levels of circulating antibodies to *C. rectus* (an oral pathobiont that has been demonstrated to cross the placental barrier) (Madianos *et al.* 2001), suggesting another possible mechanism by which certain oral bacteria contribute to pre-term birth.

However, studies using targeted assays to examine the presence of selected oral organisms, including a large-scale investigation on 823 pregnant women, found no association between presence or levels of subgingival periodontal pathogens and adverse pregnancy outcomes (Noack *et al.* 2005; Michalowicz *et al.* 2006; Novak *et al.* 2008). Furthermore, periodontal therapy did not result in improvement in pregnancy outcomes. However, in light of evidence that the placental microbiome is already established in early pregnancy, it would be surprising indeed if periodontal therapy during the second trimester did change pregnancy outcomes. It is important to examine this temporal relationship using prospective studies that provide periodontal therapy before the beginning of pregnancy.

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Periodontitis and rheumatoid arthritis

Rheumatoid arthritis (RA) is a disease that occurs when normal immune function becomes dysregulated, leading to the production of self-reactive antibodies (autoantibodies) which ultimately result in a chronic autoimmune inflammatory disease that is characterized by inflammation of synovial cavities, and progressive degeneration of cartilage and bone.

This debilitating disease affects more than 30% of individuals over 65 years of age. Infectious agents, such as the Epstein–Barr virus, cytomegalovirus, certain species of Proteus, and *Escherichia coli* have been implicated in the pathogenesis of this disease for a long time, and molecular mimicry (especially between bacterial heat-shock proteins and human Hsp60) has been suggested as a mechanism for creating the autoantibodies (Auger & Roudier, 1997; Kamphuis *et al.* 2005). Two lines of evidence have emerged to support a role for the gut microbiome in the etiology of this disease:

(i) Animal studies using germ-free and gnotobiotic animals (those that are colonized by selected, specific species) have revealed that a dysbiosis in the gut microbiome resulting in an increase in the levels of pathobionts and a decrease in commensals, predisposes arthritis-prone mice to inflammatory joint disease (Wu *et al.* 2010; Scher *et al.* 2016), This finding lends support to the molecular mimicry hypothesis which implicates the microbial production of cross-reactive epitopes in the creation of a pathogenic immune response against self-antigens.

(ii) There is evidence for a gut–joint axis from human studies, which demonstrated that the presence of organisms like *Tropheryma whipplei* in the intestine is sufficient to cause joint disease in susceptible individuals (Moos & Schneider, 2011). Further, bacterial sequences that mimic key motifs in the RA-related human antigens have been identified at significantly higher levels in both gut and oral microbiomes of individuals with RA.

It has been known for some time that an autoimmune response to citrullinated proteins underlies the aetiology of RA. Citrullination is a physiological process that is important for neuronal development and chromatin remodelling; however, it is also upregulated during apoptosis, intracellular stress and inflammation, events that are typically seen during a response to a bacterial infection. Peptidyl deiminase is an enzyme that is involved in deimination of arginine residues (citrullination). *P. gingivalis*, an oral pathogen, has been implicated in the aetiology of this disease for over two decades, since it is the only organism known to produce peptidyl deiminase. This organism citrullinates fibrinogen, enolase, vimentin and collagen II (Marotte *et al.* 2006; Lundberg *et al.* 2010; Gilliam *et al.* 2011; Kinloch *et al.* 2011). It has been shown that infection with *P. gingivalis* precedes onset of RA and that autoantibodies to citrullinated protein (ACPA) titres are higher in aggressive periodontitis (Hendler *et al.* 2010). Moreover, patients with both RA and periodontitis are more likely to be ACPA positive (Dissick *et al.* 2010).

While there is an emerging body of evidence to suggest an association between RA and periodontal pathogens, non-causal confounding factors cannot be ignored. Both diseases have several aspects in common, notably an inflammatory phenotype that is characterized by high levels of cytokines, matrix-metalloproteinases, neutrophil-derived mediators and oxidative stress. Also, several contributory factors, especially smoking and lower socio-economic status, are common to both diseases. Polymorphisms in interleukin genes and Fc-γ receptor, as well as over-expression of the MHC class II *HLA-DRB1* allele, are implicated in the aetiopathogenesis of both diseases (Marotte *et al.* 2006; Song *et al.* 2013; Mikuls *et al.* 2014).

Periodontitis and diabetes

Studies on the inter-relationship between diabetes and periodontitis began over half a century ago, when it was seen that Pima Indians with Type 2 diabetes had more widespread periodontitis, which was also more severe when compared to normoglycemic individuals. Periodontitis became known as the sixth complication of diabetes (Loe, 1993), and several lines of evidence demonstrated that the advanced glycation end products (AGEs) influence immune-inflammatory homeostasis in the periodontium. AGEs are formed when lipids and proteins combine with reducing sugars (all monosaccharides and some di- and oligosaccharides), and undergo a series of irreversible molecular rearrangements. The gingival epithelium, endothelium, immune cells and fibroblasts all carry receptors for AGE, known as RAGE. The AGE–RAGE interactions lead to impaired barrier function, among other defects. It is held that these AGE–RAGE interactions are responsible for lowered immunity, higher cellular oxidant stress, lowered wound healing potential, and pro-inflammatory phenotypes that increase the risk for periodontitis. Treatment of periodontal disease was shown to reduce glycaemic levels, improve glycaemic control and decrease the amount of hypoglycaemic medication required to titrate blood glucose levels (reviewed by Lalla & Papapanou, 2011; Taylor *et al.* 2013).

While all of these investigations were unanimous about the changes in subgingival microenvironment wrought by hyperglycaemia, the studies that explored the effect of this glucose-rich, pro-oxidant, protein-rich and anaerobic environment on the periodontal microbiome were not as conclusive. While some early studies found an increase

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in selected bacterial species in diabetics, they either did not report the periodontal status of the individuals, or did not have a control group, or did not report the statistical test used to validate their results (reviewed by Ohlrich *et al.* 2010; Taylor *et al.* 2013). Therefore, it was assumed that the periodontal destruction in diabetics was largely due to its effects on the host, rather than a greater-than-ordinarily virulent microbiome. However, evidence is emerging to indicate that the periodontal microbiome in diabetics is distinct from that of normoglycaemics (Casarin *et al.* 2013; Zhou *et al.* 2013). While there is convincing evidence to support the effect of periodontal disease on glycaemic control, the mechanisms underlying this are not well studied. Furthermore, while the effect of AGE–RAGE interactions on the subgingival pathophysiology has been investigated in depth, the effect of diabetes on the oral microbiome definitely warrants further investigation.

Periodontitis and Alzheimer's disease

Alzheimer's disease is a chronic neurodegenerative disorder that leads to progressive cognitive deterioration, and is the leading cause of dementia in individuals over 65 years of age. It has been known for several decades that infections by viruses, notably, human herpes simplex virus 1 (HSV-1), and bacteria such as *Helicobacter pylori*, *Chlamydophila pneumoniae* and *Borrelia burgdorferi* may affect the neuronal axis through central nervous system (CNS) infection, inflammation or by creating auto-immune antibodies that target the brain. The effect of these organisms on initiation or exacerbation of Alzheimer's disease has also been investigated, and there is a robust body of evidence from both human and animal studies to support the infectious aetiology of Alzheimer's disease (Jamieson *et al.* 1992; Balin *et al.* 1998; Malaguarnera *et al.* 2004; Letenneur *et al.* 2008).

Periodontal pathogens such as *Treponema denticola* and *Porphyromonas gingivalis* have been identified in the cerebrospinal fluid and neuronal ganglia (Riviere *et al.* 2002; Poole *et al.* 2013). Animal studies have demonstrated that in susceptible hosts, *P. gingivalis* crosses the blood–brain barrier, and leads to complement C3 activation with bystander neuronal injury (Poole *et al.* 2013). This has been proposed as a mechanism by which periodontal disease may contribute to initiation or progression of Alzheimer's disease.

In summary, a century of research and clinical correlations have identified a role for periodontal diseases in influencing systemic disease. While the very nature of multifactorial, chronic diseases has made it difficult to establish a definitive causal role for periodontal pathobionts in systemic infection, the body of literature supporting an aetiopathological role for these organisms is too substantial to be ignored as merely coincidental. Therefore, well-controlled, large-scale prospective study designs or highly representative animal-model studies are much needed to explore the relationship between a dysbiotic oral microbiome and systemic disease at a mechanistic level.

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Additional information

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No competing interests declared.

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