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UNIT 8: BIOLOGICAL PERIODONTAL THERAPY

SUPPLEMENTARY (OPTIONAL) CONTENT FOR UNIT 8

- ☐ Read the "The American Journal of Cardiology and Journal of Periodontology Editors' Consensus: Periodontitis and Atherosclerotic Cardiovascular Disease" study by Friedewald, Kornman, Beck, Genco, Goldfine, Libby, Offenbacher, Ridker, Van Dyke, and Roberts.
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- ☐ Watch the "Bad Bugs" video by Kennedy at
  https://www.youtube.com/watch?v=kKJgwR2RScw

- ☐ Read the "Herpesviruses in periodontal diseases" article by Slots. Click here to go to pages 13-42.

- ☐ Read the "Systemic Diseases Caused by Oral Infection" review by Li, Kolltveit, Tronstad, and Olsen. Click here to go to pages 43-54.

- ☐ Read the "Identification of Pathogen and Host-Response Markers Correlated With Periodontal Disease" study by Ramseier, Kinney, Herr, Braun, Sugai, Shelburne, Rayburn, Tran, Singh, and Giannobile.
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- [ ] Read the "Periodontal disease may associate with breast cancer" study by Söder, Yakob, Meurman, Andersson, Klinge, and Söder. Click here to go to pages 77-93.

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- [ ] Read the "Periowave Photodisinfection System" IAOMT Scientific Review. Click here to go to pages 103-108.

The American Journal of Cardiology and *Journal of Periodontology* Editors' Consensus: Periodontitis and Atherosclerotic Cardiovascular Disease[†]

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Introduction

The organization of the health professions into specialties and subspecialties according to body organs and systems is often more pragmatic than scientific. The human organism is a single unit composed of a seemingly infinite number of biologic processes so intertwined that abnormalities of almost any of its parts or processes have profound effects on multiple other body areas, exemplified in this document by the common and complex theme of *inflammation*. In recent years, the immune system, once believed to be only a vital defense against infection and a promoter of healing—except in the instances of a few uncommon connective tissue disorders—is now recognized as a significant active participant in many chronic diseases, including hypertension, diabetes mellitus, arthritis, inflammatory bowel disease, psoriasis, and the 2 diseases addressed in this Editors' Consensus: atherosclerotic cardiovascular disease (CVD) and periodontitis.

This aim of this document is to provide health professionals, especially cardiologists and periodontists, a better understanding of the link between atherosclerotic CVD and periodontitis and, on the basis of current information, an approach to reducing the risk for primary and secondary atherosclerotic CVD events in patients with periodontitis.

Periodontitis, a bacterially induced, localized, chronic inflammatory disease, destroys connective tissue and bone that support the teeth. Periodontitis is common, with mild to moderate forms affecting 30% to 50% of adults and the severe generalized form affecting 5% to 15% of all adults in the United States.[1] Periodontitis has even higher prevalence in developing countries and considerable global variation, although the prevalence of the severe generalized disease appears to be similar in most populations.[2]

Patients with periodontitis are often asymptomatic. When

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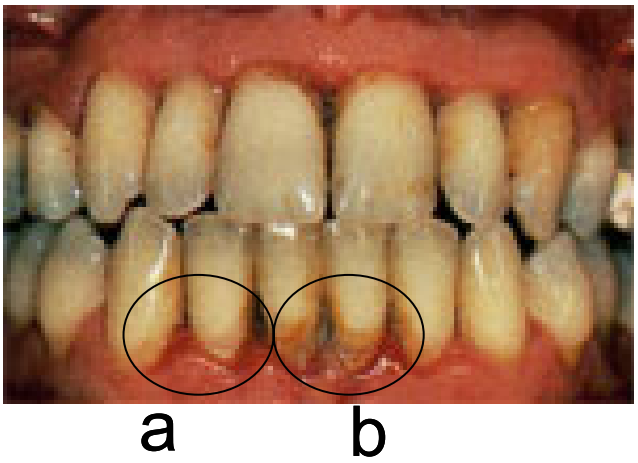

Figure 1. In this patient with untreated moderate periodontitis, changes in tissue contours and color are present *(A)*. The yellow-brown discolored areas of the teeth *(B)* are root surfaces that have been exposed with gingival recession due to destruction of the connective tissue attachment. The exposed root surfaces are covered with bacterial deposits, portions of which are calcified.

present, physical signs and symptoms are nonspecific and include (Figure 1) swollen gums that decompress, discolored gums, tender gums, bleeding gums (spontaneous or after brushing or flossing), long appearance of teeth (because of receded gums), increased spacing between teeth, pus between teeth and gums, loose teeth, change in tooth sensation when biting because of increased tooth mobility, bad taste, and halitosis (because of anaerobic infection). Patients with periodontitis who have spontaneous oral pain or pain on mastication often have complications of the disease, including abscesses and other oral mucosal and alveolar bone lesions.

The clinical diagnosis of periodontitis requires evaluation by a trained examiner and evidence of gingival inflammation, loss of connective tissue surrounding the teeth measured by clinical examination using a periodontal probe, and bone loss detected by radiography (Figure 2).

Although moderate to severe periodontitis may affect systemic inflammatory and immune markers (e.g., elevated blood levels of C-reactive protein [CRP]), such changes are either not captured by current standard laboratory test panels or are interpreted as nonspecific indicators of a chronic, low-grade, acute-phase inflammatory response. Patients with uncomplicated periodontitis have no systemic signs of infection, such as fever or leukocytosis.

Pathophysiology: Periodontitis begins with a microbial infection, followed by a host-mediated destruction of soft tissue caused by hyperactivated or primed leukocytes and the generation of cytokines, eicosanoids, and matrix metalloproteinases that cause clinically significant connective tissue and bone destruction.[3] Bacterial accumulations on the teeth are essential to the initiation and progression of periodontitis. Cells that mediate immunity, such as neutrophils, play a major role in the host response against invading periodontopathogenic microorganisms. When bacterial biofilms on the teeth are not disrupted on a regular basis, ecologic changes lead to the emergence of a small set of gram-negative anaerobic bacterial species, including *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, which consistently associate with periodontitis. These bacteria activate many host immunoinflammatory processes and disrupt host mechanisms involved in bacterial clearance and are considered pathogens in periodontitis. Environmental and genetic factors as well as acquired risk factors such as diabetes mellitus and exposure to tobacco accelerate inflammatory processes in periodontitis. Although bacteria *initiate* periodontitis, host-modifying risk factors appear to influence the severity and extent of disease.

Risk factors (nonoral): The following nonoral risk factors associate strongly with increased risk for periodontitis and disease severity: smoking, diabetes mellitus, genetics, mental anxiety, depression, obesity, and physical inactivity.

Individuals who smoke (cigarettes and pipes) have 6 to 7 times more alveolar bone loss than nonsmokers in studies in the United States and other countries.[4–7] Patients with periodontitis defined by tooth attachment loss are 3 to 5 times more likely to smoke than those without attachment loss.[8] Possible mechanisms for the smoking-periodontitis relation include increased subgingival infection by periodontal pathogens,[9] increased smoking-induced proinflammatory circulating cytokine levels such as tumor necrosis factor–α,[10] and altered collagen metabolism and wound healing.

Periodontal disease is more severe and prevalent in patients with type 1 and type 2 diabetes mellitus, on the basis of multiple domestic and global epidemiologic and clinical studies.[11] A large-scale longitudinal epidemiologic study in Pima Indians reported that the incidence of new cases of periodontitis in patients with type 2 diabetes in this ethnic population was >2.5 times greater than in nondiabetic subjects.[12] Patients with type 2 diabetes mellitus also have a faster rate of alveolar periodontal bone loss than those without diabetes with periodontitis.[13] Patients aged 10 to 18 years with type 1 diabetes mellitus have an increased prevalence of periodontitis.[14] In children and teens with diabetes, accelerated periodontal destruction relates to metabolic control.[15] Conversely, worsening periodontal disease adversely affects glycemic control.[11,13] It has been suggested that inflammation may be 1 mechanistic link between the 2 diseases.[13] Treatment of periodontal disease, especially in patients with elevated glycosylated hemoglobin, improves glycemic control.[16,17] Results from the National Health and Nutrition Examination Survey (NHANES) I and its follow-up studies suggest that nondiabetic adults with periodontal disease develop type 2 diabetes more often than those without periodontal disease.[18]

Approximately 50% of the variation in clinical severity of chronic periodontitis is explainable by genetic influences.[19] The first report of association with specific gene variants involved the interleukin (IL)–1 gene cluster,[12] but other identified genetic factors also are likely to contribute to periodontitis.[1]

Treatment: All appropriate treatment strategies for periodontitis focus on the resolution of gingival inflammation and healing of the soft and hard tissue attachment of the teeth to the alveolar process by removal of the bacterial biofilm attached to the tooth roots and reinforcement of patient oral hygiene to reduce bacterial regrowth.

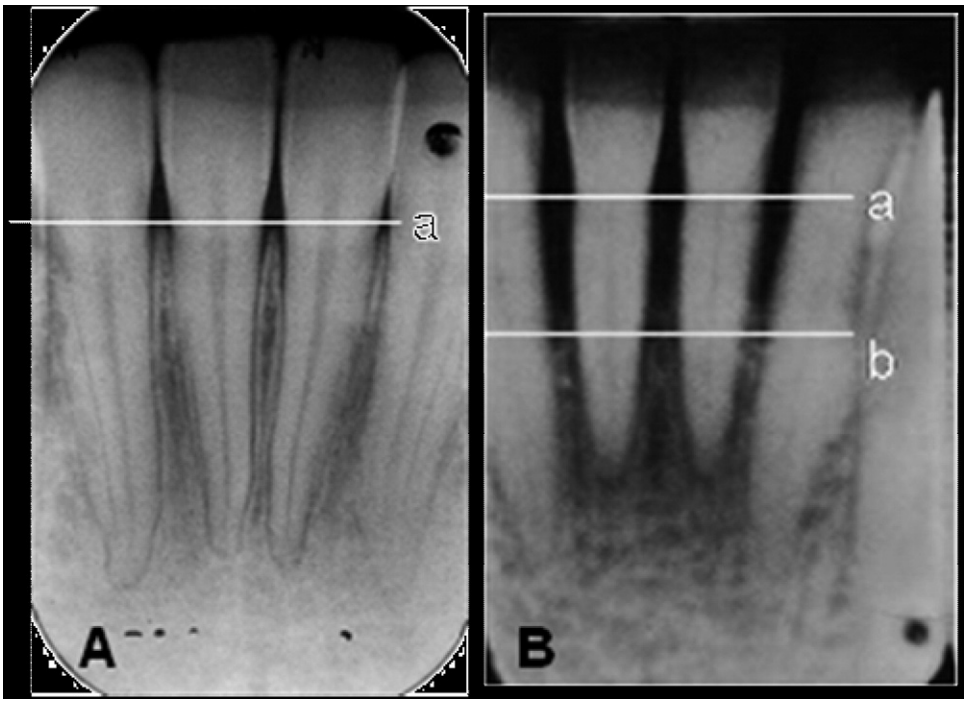

Figure 2. In periapical x-ray *A*, marginal bone levels *(line a)* are consistent with no history of periodontitis. In periapical x-ray *B*, periodontitis has caused resorption of approximately 50% to 60% of the bone supporting the mandibular anterior teeth. The approximate level of bone that would be expected in the absence of periodontitis is marked by *line a*, and the approximate level at the time of the x-ray is marked by *line b*.

Systemic antibiotics may be used as an adjunct to conventional bacterial removal in severe periodontitis and in patients with host-modifying risk factors, such as diabetes mellitus.[20] Antibiotics locally delivered into the periodontal pockets have been approved by the United States Food and Drug Administration (FDA) as an adjunct to conventional bacterial removal in the management of periodontitis. Antibiotics markedly reduce the bacterial load but taken alone do not usually eliminate periodontal pathogens in the oral cavity. Antibiotics may transiently improve localized sites of periodontitis when combined with mechanical debridement to disrupt the subgingival biofilm.

Host-modulating drugs that reduce the clinical signs and symptoms and progression of periodontitis have been evaluated, and the matrix metalloproteinase inhibitor low-dose *doxycycline* is the only FDA-approved host-modulating drug for the treatment of periodontitis. Other host-modulating agents that hold promise but are not currently approved for use in periodontal therapy include nonsteroidal anti-inflammatory drugs (systemic [flurbiprofen] and topical [ketorolac]), bisphosphonates (alendronate sodium), and resolvins.

Advanced periodontitis (moderate to severe bone loss and gingival pocket depth >5 mm) may require surgery to gain adequate access for removal of the bacterial biofilm and residual calculus on the root surfaces. In some instances, surgical approaches include bone and soft tissue regeneration to regain at least some support for the teeth and to facilitate bacterial control.

Prevention: Long-term clinical studies have clearly demonstrated that the regular and effective removal of bacterial biofilms on the teeth can prevent periodontitis.[21] Effective removal requires excellent oral hygiene, including interproximal cleaning and periodic professionally administered biofilm removal.[22,23]

Inflammation and Atherosclerotic Cardiovascular Disease

The dietary ingestion of low-density lipoprotein (LDL), mainly from animal fat, with subsequent lipid oxidation and accumulation of lipid products within the arterial vascular wall is essential for atherogenesis. Thus, the most important current strategies for preventing atherosclerotic CVD are dietary fat restriction and pharmacologic measures that lower serum levels of LDL cholesterol. A number of risk factors also relate closely to the development of atherosclerotic disease and risk for cardiovascular events (e.g., myocardial infarction and stroke), including age, gender, hypertension, diabetes mellitus, smoking, and low serum levels of high-density lipoprotein (HDL) cholesterol.[24,25]

Over the past 2 decades, inflammation has emerged as an integrative CVD factor. Inflammation can operate in "all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis."[26] Higher quantiles of CRP, measured by a high-sensitivity assay (hsCRP), predict future acute myocardial infarction and unstable angina pectoris[27,28] and the onset of systemic arterial hypertension, diabetes mellitus, and stroke,[29–31] independent of blood lipid levels.[32] CRP itself, beyond serving as a biomarker, may have a role in endothelial cell dysfunction.[32–34] The erythrocyte sedimentation rate, chemokines, and cytokines including IL-6, IL-8, IL-10, IL-18, tumor necrosis factor–α, and monocyte chemoattractant protein–1 also are frequently abnormal in patients with

acute coronary syndromes[35–37] and in many other conditions. The incidence of atherosclerotic CVD events increases in patients with chronic inflammatory diseases, in addition to periodontitis, including rheumatoid arthritis,[38] psoriasis,[39] systemic lupus erythematosus,[40,41] and some types of infections, mainly infections of the respiratory tract and urinary tract.[42] Arterial inflammation, along with arterial stiffness and remodeling, may be a factor in systemic arterial hypertension,[43–53] particularly in obese patients. Evidence supporting the role of inflammation in atherosclerotic events gained support with the findings of Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER),[54] in which treatment with rosuvastatin significantly reduced the incidence of cardiovascular events in subjects with lower levels of LDL cholesterol but with mild chronic inflammation indicated by levels of hsCRP >2 mg/L. The precise role of inflammation as a *direct*, *causative* factor in chronic atherogenesis and in the acute complications of atherosclerosis remain an area of intense current investigation.[34,55,56]

Periodontitis and Atherosclerotic Cardiovascular Disease

The association between periodontitis and atherosclerotic CVD has received considerable attention.[57–90] The findings of these studies, however, have varied greatly, ranging from determinations of no causative relation between periodontitis and CVD to strong causative connections between the 2 conditions. Reasons for the discrepancies in the results of these studies include[72] (1) variations in study populations, including differing age groups, ethnicities, and geographic locations, and (2) differing measures and definitions of periodontitis, with some studies based only on clinical measures (i.e., pocket depth, bleeding with probing, tooth attachment level) and other studies, in which the relation appeared stronger, based on nonclinical measures such as systemic antibody response[70] or radiographic evidence of alveolar bone loss. Increased carotid artery intimal medial thickness measured by ultrasound, which is associated with increased risk for acute myocardial infarction and stroke in subjects without histories of CVD,[75] often occurs in patients with periodontitis, suggesting that subclinical atherosclerosis is present in many patients with periodontitis.[76,77]

Coronary artery disease (CAD): Although some past studies have not supported a causal relation between periodontitis and CAD,[76,86] a meta-analysis[91] of data linking CAD and periodontitis concluded that periodontal disease is a risk factor or marker independent of traditional CAD risk factors, with relative risk estimates ranging from 1.24 to 1.35. Another meta-analysis[92] also found significantly increased prevalence and incidence of CAD in patients with periodontitis, again raising the possibility that periodontitis independently predicts CAD. The 2 meta-analyses concluded, however, that further studies are needed to better define the relation between the 2 diseases. Analysis of >1,200 men in the Veterans Affairs Normative Aging and Dental Longitudinal Studies[61] determined that in men aged <60 years, there was a "significant dose-dependent association" between CAD prevalence and periodontitis, with a hazard ratio of 2.12 (95% confidence interval 1.26 to 3.30) when using clinical and radiographic criteria for periodontitis. This association was independent of standard atherosclerotic CVD risk factors or socioeconomic status. In men aged >60 years, however, the dose-dependent association between CAD and periodontitis was absent in this study. Periodontitis prevalence also has been correlated with angiographic evidence of CAD.[62]

Cerebrovascular disease: Analysis of NHANES I[93,94] and the NHANES Epidemiologic Follow-Up Study (NHEFS)[95] found that periodontal disease is an important risk factor for all forms of cerebrovascular disease, especially nonhemorrhagic stroke. Data from the Health Professionals Follow-Up Study (HPFS), which involved >50,000 male health professionals, revealed that periodontal disease and fewer teeth at baseline correlated with increased risk for stroke during the subsequent 12-year follow-up period.[96] Some studies, however, have not found a relation between periodontitis and cerebrovascular disease.[2,76]

Peripheral arterial disease: A small study reported a direct link between peripheral arterial disease and periodontitis, which related the 2 conditions in association with increases in the serum cytokines IL-6 and tumor necrosis factor–α.[97] Another study of peripheral arterial disease in 212 young women (mean age 48 ± 7 years) found an independent relation between peripheral arterial disease and a history of periodontitis, unaffected by the level of hsCRP.[98]

Mechanisms for an Association Between Periodontitis and Atherosclerotic Cardiovascular Disease

A *direct* causal relation between periodontitis and atherosclerotic CVD is not established. Multiple studies, however, support 2 biologically plausible mechanisms[99–102]: (1) Moderate to severe periodontitis increases the level of systemic inflammation, a characteristic of all chronic inflammatory diseases, and periodontitis has been associated with increased systemic inflammation as measured by hsCRP and other biomarkers. Treatment of moderate to severe periodontitis sufficient to reduce clinical signs of the disease also decreases the level of systemic inflammatory mediators.[100,101] (2) In untreated periodontitis, 10^8 to 10^{12} gram-negative bacteria may be found in periodontal pockets surrounding each diseased tooth and in approximation to ulcerated epithelium, and bacterial species found predominantly in the periodontal pockets also have been found in atheroma.[102]

An *indirect* relation between periodontitis and atherosclerotic CVD is the many shared risk factors that commonly occur in the 2 diseases. Thus, many factors, especially cigarette smoking,[5–10] are confounders in determining their relative importance in this relation. There is evidence that periodontal disease is related to CVD in young (aged ≤55 years) nonsmokers.[90] In addition to tobacco use, the following risk factors are common to periodontitis and CVD: (1) Diabetes mellitus: there are no reported interventional studies designed to ascertain whether periodontal disease prevention or treatment reduces CVD prevalence or

Table 1
Confidence and evidence codes

Confidence	Description
1	Very confidant
2	Confident
3	Marginally confidant
4	Not confidant
Type of evidence	
A	Well-designed RCT conducted in patients who have reported adverse experiences
B	Single RCT with a highly statistically significant result
	Well-conducted retrospective case-control studies with adverse experiences as primary end points
	Managed care claims database analysis with a highly statistically significant result
C	Reports to regulatory agencies judged to exceed population averages and reporting bias
	Multiple case studies with nonblinded dechallenge and rechallenge
	Strong trends, not reaching statistical significance, for safety issues in large RCTs
	Well-conducted prospective cohort study, giving a result that is statistically well above population average
	Metabolic or clinical surrogate studies
D	Undocumented opinion of experienced research investigators and clinicians
	Poorly controlled or uncontrolled studies
	Nondefinitive evidence from regulatory agency reporting systems or managed care claims databases
U	Unknown, no appropriate evidence, or evidence considered subject to bias

RCT = randomized controlled trial.

mortality in patients with either type 1 or type 2 diabetes mellitus. (2) Obesity: systemic inflammation, defined by increased circulating tumor necrosis factor–α, is associated with obesity and periodontitis and has been proposed as a mechanism for the connection between these conditions.[103–106] Systemic inflammatory responses also could explain the association between periodontitis and type 2 diabetes by cytokine-induced insulin resistance. (3) Lipids: a case-controlled study showed that periodontitis is associated with elevated plasma triglycerides and total cholesterol.[107] A large epidemiologic study in the United States determined that total serum cholesterol and plasma levels of CRP and fibrinogen are elevated in patients with periodontitis.[86] Other epidemiologic studies in Japan[106] and Germany[107] also found that dyslipidemia is more common in patients with periodontitis. (4) Hypertension: an epidemiologic study in Sweden of >4,000 subjects showed an increased prevalence of hypertension in patients with periodontitis.[108] Smaller studies in the United States found that, after adjusting for confounders, hypertension was more prevalent in patients with severe alveolar bone loss,[109] and significantly more hypertension occurs in patients with periodontitis compared with populations with little or no periodontal disease.[110] Whether hypertension is a risk factor for periodontitis, however, remains uncertain. Systemic inflammation, a feature of hypertension, as evidenced by increased hsCRP plasma levels in patients with prehypertension and patients with established hypertension,[31] may link these 2 conditions.

Major depression, physical inactivity, family histories of CVD and periodontal disease, advancing age, and male gender are other risk factors for atherosclerotic CVD that are commonly found in patients with periodontitis and also may serve as confounders.

Clinical Recommendations: Patients With Periodontitis

Although the treatment of periodontitis reduces systemic markers of inflammation and endothelial dysfunction, no prospective periodontitis intervention studies have evaluated CVD outcomes. It seems reasonable, however, on the basis of current data, to acknowledge that because untreated or inadequately controlled moderate to severe periodontitis increases the systemic inflammatory burden, periodontitis *may* independently increase the risk for CVD. (See Table 1 for confidence and evidence level codes.)

I. Patient Information

Recommendation A: **Patients with moderate to severe periodontitis should be informed that there may be an increased risk for atherosclerotic CVD associated with periodontitis.**

Confidence and evidence level: 2C

Recommendation B: **Patients with moderate to severe periodontitis who have 1 known major atherosclerotic CVD risk factor, such as smoking, immediate family history of CVD, or history of dyslipidemia, should consider a medical evaluation if they have not done so in the past 12 months.**

Confidence and evidence level: 3D

Recommendation C: **Patients with periodontitis who have ≥2 known atherosclerotic CVD major risk factors should be referred for medical evaluation if they have not done so in the past 12 months**.

Confidence and evidence level: 2D

II. Medical and Dental Evaluations

In concert with the following recommendations, it is recommended that patients with periodontitis assess their risk for future (next 10 years) CVD events (e.g., stroke, myocardial infarction) by completing either the Reynolds Risk Score[111] (http://www.reynoldsriskscore.org) or, for risk assessment for CAD events only, the National Cholesterol Education Program Risk Calculator (http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=prof), based on the Framingham Heart Study.

Recommendation A: **Medical evaluation of patients with periodontitis should include assessment of atherosclerotic CVD risk, including past CVD events, and family histories of premature atherosclerotic CVD disease or sudden coronary death, diabetes mellitus, systemic hypertension, or dyslipidemia.**

Confidence and evidence level: 2D

Recommendation B: **Medical evaluation of patients with periodontitis should include a complete physical examination and annual measurement of blood pressure**

at rest (seated for 5 minutes with the feet on the floor and attention to appropriate blood pressure cuff size).

Confidence and evidence level: 2D

Recommendation C: **Medical evaluation of patients with periodontitis should include a blood lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, and fasting triglycerides) and blood glucose measurement. A plasma hsCRP determination is optional but should be considered, because recent studies have suggested that elevated plasma hsCRP may have added value by helping determine how aggressively standard risk factors should be treated, especially lifestyle changes.**[56,112,113]

Confidence and evidence level: 2D

III. Risk Factor Treatment: Abnormal Lipids

Recommendation A: **Patients with periodontitis and ≥1 abnormal serum lipid and/or elevated plasma hsCRP are recommended to follow a multifaceted lifestyle approach to reduce atherosclerotic CVD risk according to the National Cholesterol Education Program Adult Treatment Panel III guidelines.**[114]

Confidence and evidence level: 1C

According to Adult Treatment Panel III guidelines, emphasis on weight loss and physical activity to enhance weight reduction in subjects with elevated serum LDL cholesterol should be undertaken. Goals for LDL cholesterol levels are based on CVD risk assessment: (1) 1 atherosclerotic CVD risk factor and LDL cholesterol >160 mg/dl: target LDL cholesterol <160 mg/dl; (2) ≥2 atherosclerotic CVD risk factors and LDL cholesterol >130 mg/dl: target LDL cholesterol <130 mg/dl; an optional target is LDL cholesterol <100 mg/dl if factors such as age, metabolic syndrome, abnormal plasma hsCRP, or abnormal coronary calcium score (75th percentile) are present; (3) atherosclerotic CVD disease is present or there are CAD risk equivalents, such as diabetes mellitus: target LDL cholesterol <100 mg/dl or an optional target of <70 mg/dl if atherosclerotic CVD is present and there are high-risk features, such as diabetes mellitus, metabolic syndrome, heavy cigarette smoking, or acute coronary syndromes.

Lifestyle changes that should be undertaken are reduced intake of saturated fats (<7% of total calories) and low levels of trans fats and dietary cholesterol (<200 mg/day); enhancement of LDL lowering with optional dietary strategies, such as ingesting plant stanols or sterols (2 g/day) and increased viscous (soluble) fiber (10 to 25 g/day); weight reduction; increased physical activity; and limited alcohol ingestion ("*Moderation* is defined as the consumption of up to 1 drink per day for women and up to 2 drinks per day for men. Twelve fluid ounces of regular beer, 5 fluid ounces of wine, or 1.5 fluid ounces of 80-proof distilled spirits count as one drink. This definition of moderation is not intended as an average over several days but rather as the amount consumed on any single day."[114]) However, alcohol does not add to atherosclerotic CVD risk and may convey some protective effect against future CVD events. Patients who need to lose weight should be cautioned, however, that alcohol is high in caloric content. Subjects who do not drink alcohol should not be advised to begin drinking alcohol for the purpose of CVD risk modification, because other risks of alcohol consumption, such as higher frequencies of accidents and medical illnesses, outweigh the possible CVD-preventive benefits of alcohol.

Recommendation B: **Drug therapy for elevated LDL cholesterol should be prescribed in patients with periodontitis in whom target LDL cholesterol levels are not achieved with lifestyle changes.**

Confidence and evidence level: 2D

IV. Risk Factor Treatment: Cigarette Smoking

Recommendation: **All patients with periodontitis who smoke tobacco should discontinue this habit because this is a major risk factor for atherosclerotic CVD and periodontitis.**

Confidence and evidence level: 1C

V. Risk Factor Treatment: Hypertension

Recommendation A: **All patients with periodontitis and elevated blood pressure should be treated to target levels as defined by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7).**[24]

Confidence and evidence level: 1C

JNC-7 defines hypertension as follows: (1) *prehypertension*: systolic blood pressure 120 to 139 mm Hg or diastolic blood pressure 80 to 89 mm Hg; (2) *stage 1 hypertension*: systolic blood pressure 140 to 159 mm Hg or diastolic blood pressure 90 to 99 mm Hg; and (3) *stage 2 hypertension*: systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg. Using JNC-7 recommendations, the target blood pressures in patients with periodontitis are (1) <140/90 mm Hg in all patients with periodontitis and ≤2 major risk factors for CAD and (2) <130/80 mm Hg in patients with previous atherosclerotic CVD, diabetes mellitus, chronic renal disease, or ≥3 major risk factors.

Recommendation B: **All patients with periodontitis and elevated blood pressure should undertake lifestyle changes**.

Confidence and evidence level: 1A

Elevated blood pressure can be significantly decreased by lifestyle changes, including (pressures in parentheses indicate changes that can be anticipated with adequate patient compliance) weight reduction in subjects who are overweight (systolic blood pressure reduction 5 to 20 mm Hg), a diet high in potassium and calcium (the American Heart Association DASH diet[115]; systolic blood pressure reduction 4 to 8 mm Hg), a diet low in sodium (systolic blood pressure reduction 2 to 8 mm Hg), physical activity (systolic blood pressure reduction 4 to 9 mm Hg), and moderation of alcohol intake (systolic blood pressure reduction 2 to 4 mm Hg).

In addition to lowering blood pressure, lifestyle modifications also increase the efficacy of antihypertensive drug therapy and decrease the risk for atherosclerotic CVD.

Recommendation C: **All patients with periodontitis and elevated blood pressure not controlled to target levels with lifestyle changes should be treated with pharmacologic therapy.**

Confidence and evidence level: 2D

The following drug classes are approved for the initial treatment of hypertension: thiazide-type diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, direct renin inhibitors, β blockers, and calcium channel blockers (see recommendation D).

Recommendation D: **Patients with periodontitis prescribed calcium channel blockers for hypertension or any other indication should be monitored for worsening of periodontitis in association with gum hyperplasia.**

Confidence and evidence level: 1D

Gingival hyperplasia has been reported with all 3 classes of calcium channel blockers.[116] This effect is reported most often with nifedipine, occurring in up to 6% of patients,[117] and less often with diltiazem, amlodipine,[118,119] and verapamil.[120,121] The mechanism is unknown but may be due to increased gingival collagen production by fibroblasts.[122] However, there are no specific reports of the effect of calcium channel blockers on the severity of periodontitis.

VI. Risk Factor Treatment: Metabolic Syndrome

Metabolic syndrome is diagnosed when ≥3 of the following features are present: (1) increased waist circumference (men ≥40 in [≥102 cm], women ≥35 in [≥88 cm]), (2) increased serum triglyceride level (150 mg/dl [1.7 mmol/L]) and/or drug treatment for elevated triglycerides (most commonly fibrates and nicotinic acid), (3) decreased serum HDL cholesterol level (men <40 mg/dl [1.03 mmol/L], women <50 mg/dl [1.3 mmol/L]) and/or drug treatment for decreased serum HDL cholesterol, (4) elevated blood pressure (≥130 mm Hg systolic and/or ≥85 mm Hg diastolic or antihypertensive drug treatment of patients with histories of hypertension, and (5) elevated fasting glucose (blood glucose ≥100 mg/dl and/or drug treatment for hyperglycemia).

Recommendation: **Patients with periodontitis meeting criteria for metabolic syndrome should be identified, and all risk factors for atherosclerotic CVD should be treated, beginning with lifestyle changes aimed at weight reduction.**

Confidence and evidence level: 1D

Metabolic syndrome is closely linked to insulin resistance and is a secondary target of lipid therapy because the risk factors for metabolic syndrome are highly concordant and, in aggregate, enhance the risk for atherosclerotic CVD at any serum level of LDL cholesterol.[123] *Many patients with periodontitis meet criteria for the metabolic syndrome*.[104] Because measures of systemic inflammation are a common feature of periodontitis and metabolic syndrome, it may be particularly important to identify patients who meet these criteria for CVD prevention strategies.

VII. Special Considerations in the Treatment of Atherosclerotic CVD in Patients With Periodontitis

No reported studies present evidence that patients with periodontitis and atherosclerotic CVD should receive different treatment from other patients with CVD, with the possible exception of the use of calcium channel blockers. Recent studies suggest that standard treatments of periodontitis in patients with CVD are effective.[124] The panel did make special note that additional studies are needed regarding the effect of other drugs used in cardiovascular medicine on periodontitis. There is, however, no conceptual basis for concern that any current standard treatment for periodontitis should be altered in patients with concurrent atherosclerotic CVD.

Clinical Recommendations: Patients With Atherosclerotic Cardiovascular Disease With or Without a Previous Diagnosis of Periodontitis

I. Patients With Atherosclerotic CVD and *Previous* Diagnosis of Periodontitis

Recommendation: **Periodontists and physicians managing patients with CVD should closely collaborate to optimize CVD risk reduction and periodontal care.**

Confidence and evidence level: 1D

II. Patients With Atherosclerotic CVD and *No Previous* Diagnosis of Periodontitis

Recommendation A: **Periodontal evaluation should be considered in patients with atherosclerotic CVD who have signs or symptoms of gingival disease, significant tooth loss, and unexplained elevations of hsCRP or other inflammatory biomarkers.**

Confidence land evidence level: 2D

Recommendation B: **Periodontal evaluation of patients with atherosclerotic CVD should include a comprehensive examination of periodontal tissues, as assessed by visual signs of inflammation and bleeding on probing, loss of connective tissue attachment detected by periodontal probing measurements, and bone loss assessed radiographically. If patients have untreated or uncontrolled periodontitis, they should be treated with a focus on reducing and controlling the bacterial accumulations and eliminating inflammation.**

Confidence and evidence level: 2D

Recommendation C: **When periodontitis is newly diagnosed in patients with atherosclerotic CVD, periodontists and physicians managing patients' CVD should closely collaborate to optimize CVD risk reduction and periodontal care.**

Confidence and evidence level: 1D

Recommendations for Future Research

Although the inflammation hypothesis provides a plausible and attractive explanation for the periodontitis-atherosclerosis relation, further research is needed to define the mechanisms linking the 2 diseases and how patients with periodontitis should best be managed to reduce their risk for CVD. Specific questions that the consensus panel believes should be addressed in future research include the following: (1) Is periodontitis an independent risk factor for atherosclerotic CVD? (2) If periodontitis is an independent risk factor for atherosclerotic CVD, what is the mechanism of the relation, and at what stage(s) of atherogenesis is it important? (3) Regardless of whether periodontitis is an independent risk factor for atherosclerotic CVD, should risk factors for atherosclerotic CVD be treated more aggressively in patients with periodontitis than current guidelines recommend for the general population? (4) Do periodontal therapeutic interventions, such as infection and inflammation control, directly reduce the rate of atherosclerotic plaque development and its complications, especially acute myocardial infarction and stroke? (5) Because periodontitis in the general population is greatly underdiagnosed and undertreated, what measures can improve its detection and management in persons at increased risk for primary and secondary atherosclerotic CVD events? (6) Are there

specific oral microbial pathogens that add to CVD risk and therefore should be targeted for antibiotic treatment? (7) In addition to the possible role of periodontal inflammation caused by infection, does secondary endotoxemia play a causative role in the relation between periodontitis and atherosclerotic CVD? (8) Are acute events such as acute myocardial infarction and stroke more likely to occur during periods of worsening periodontitis? (9) Do calcium channel blockers have any adverse effect on periodontitis other than causing gingival hyperplasia in some persons, and if so, what is the magnitude of this effect? (10) In addition to calcium channel blockers, are there other cardiovascular medications that may adversely affect periodontitis?

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Herpesviruses in periodontal diseases

JØRGEN SLOTS

> '*…If, as is sometimes supposed, science consisted in nothing but the laborious accumulation of facts, it would soon come to a standstill, crushed, as it were, under its own weight…The suggestion of a new idea, or the detection of a law, supersedes much that has previously been a burden on the memory, and by introducing order and coherence facilitates the retention of the remainder in an available form…*' *Lord Rayleigh, University of Cambridge, 1884.*

Periodontitis is a disease attributable to multiple infectious agents and interconnected cellular and humoral host immune responses (60, 226, 238). However, it has been difficult to unravel the precise role of various putative pathogens and host responses in the pathogenesis of periodontitis. It is not understood why, in hosts with comparable levels of risk factors, some periodontal infections result in loss of periodontal attachment and alveolar bone while other infections are limited to inflammation of the gingiva with little or no discernible clinical consequences. Also, many periodontitis patients do not show a remarkable level of classical risk factors. Detection and quantification of periodontopathic bacterial species are useful for identifying subjects at elevated risk of periodontitis, but do not consistently predict clinical outcome. These uncertainties have galvanized efforts to find additional etiologic factors for periodontitis.

Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease. Since the mid 1990s, herpesviruses have emerged as putative pathogens in various types of periodontal disease (43). In particular, human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) seem to play important roles in the etiopathogenesis of severe types of periodontitis. Genomes of the two herpesviruses occur at high frequency in progressive periodontitis in adults, localized and generalized aggressive (juvenile) periodontitis, HIV-associated periodontitis, acute necrotizing ulcerative gingivitis, periodontal abscesses, and some rare types of advanced periodontitis associated with medical disorders (212, 224). HCMV infects periodontal monocytes/macrophages and T-lymphocytes, and EBV infects periodontal B-lymphocytes (45). Herpesvirus-infected inflammatory cells elicit tissue-destroying cytokines and may exert diminished ability to defend against bacterial challenge. Herpesvirus-associated periodontal sites also tend to harbor elevated levels of periodontopathic bacteria, including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Dialister pneumosintes/Dialister invisus*, *Prevotella intermedia*, *Prevotella nigrescens*, *Treponema denticola*, *Campylobacter rectus* and *Actinobacillus actinomycetemcomitans* (210, 224). Transcripts of HCMV and EBV have been identified in the great majority of symptomatic periapical lesions as well (231, 232). In the light of the close statistical relationship between herpesviruses and periodontitis, it is reasonable to surmise that some cases of the disease have a herpesviral component.

This chapter summarizes evidence that links herpesviruses, especially HCMV and EBV, to the development of severe types of periodontitis, and outlines potential mechanisms by which herpesviruses may contribute to periodontal tissue breakdown. It is suggested that the coexistence of periodontal HCMV, EBV and possibly other viruses, periodontopathic bacteria, and local host immune responses should be viewed as a precarious balance that has the potential to lead to periodontal destruction. Understanding the pathobiology of periodontal herpesviruses may help delineate molecular determinants that cause gingivitis to progress to periodontitis or stable periodontitis to convert to progressive disease. Evidence of a causal role of herpesviruses in periodontitis may

form the basis for new strategies to diagnose, prevent, and treat the disease.

Mammalian viruses

Viruses cause many acute and chronic diseases in humans. New viruses are continually being discovered and already known viruses are being implicated in clinical conditions with previously unknown etiologies.

Viruses occupy a unique position in biology. They are obligate intracellular agents, which are metabolically and pathogenically inert outside the host cell. Even though viruses possess some properties of living systems such as having a genome and the capability of replicating, they are in fact nonliving infectious entities and should not be considered microorganisms. The complete virus particle, called a virion, generally has a diameter of only 30–150 nm. Most mammalian viruses are also small in the genetic sense, having genomes from 7 to 20 kb in length, and a correspondingly small complement of virion proteins. Members of the herpesvirus family are larger, with virion diameters of 150–200 nm and with genome lengths of 125–235 kb. HCMV is the largest of the human herpesviruses. Reflecting their large genomic size, herpesviruses possess a high protein coding capacity, with estimates ranging from 160 to more than 200 open reading frames. The sequence of the HCMV genome has been known for over a decade.

More than 30,000 different viruses are known to infect vertebrates, invertebrates, plants or bacteria, encompassing all three domains of life – Eukaryotes, Archaea and Bacteria. Viruses are grouped into 3600 species, 71 families, and 164 genera. Fewer than 40 viral families and genera are identified to be of medical importance in humans (Table 1). Viruses are the cause of a large array of life-threatening infectious diseases and have been implicated in 15–20% of malignant neoplasms in humans. Viruses that have

Table 1. Examples of medically important virus families

DNA, double-stranded, enveloped viruses	
Herpesviridae	Herpes simplex virus 1 and 2, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, herpesvirus 8 (Kaposi's sarcoma virus)
Hepadnaviridae	Hepatitis B virus
Poxviridae	Smallpox virus (variola)
DNA, double-stranded, naked viruses	
Papovaviridae	Papillomaviruses (warts)
RNA, double-stranded, enveloped viruses	
Retroviridae	Human immunodeficiency virus (HIV), human T-cell lymphotropic virus
Orthomyxoviridae	Influenza virus type A, B and C
Paramyxoviridae	Mumps virus, measles virus
Coronaviridae	Severe acute respiratory syndrome (SARS)
Flaviviridae	Hepatitis C virus, yellow fever virus
Togaviridae	Rubella virus
Rhabdoviridae	Rabies virus
Filoviridae	Ebola virus
RNA, double-stranded, naked viruses	
Reoviridae	Rotavirus gastroenteritis (infantile diarrhea)
RNA, single-stranded, naked viruses	
Picornaviridae	Polioviruses, Coxsackie viruses, hepatitis A virus
Caliciviridae	Hepatitis E virus, Norwalk group of gastroenteritis viruses

been convincingly linked to various types of human cancer include human papillomaviruses (cervical carcinoma), human polyomaviruses (mesotheliomas, brain tumors), EBV (B-cell lymphoproliferative diseases and nasopharyngeal carcinoma), Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma), and human T-cell leukemia virus-1 (T-cell leukemias) (113). Most cancers associated with HIV infections are related to oncogenic virus infections, such as Kaposi's sarcoma herpesvirus, human papillomavirus and EBV. Viral gene functions that prevent apoptosis, enhance cellular proliferation, or help counteract the immune attack are likely to be important determinants of malignant transformations. Undoubtedly, future research will link an increasing number of known and yet unidentified viruses to human cancer.

All viruses consist of two basic components, nucleic acid (either DNA or RNA but not both) and a protective, virus-coded protein coat termed a capsid. The genome with its protein cover is referred to as the nucleocapsid. Some viruses have additional covering in the form of an envelope that consists of a lipid–protein bilayer derived from the cell membrane of the host. Viral glycoproteins, which extend from the surface of the virus envelope, act as viral attachment proteins for target mammalian cells and are major antigens for protective immunity. The viral envelope cannot survive the intestinal tract and can be disrupted by drying, detergents, solvents, and other harsh conditions, resulting in inactivation of the virus. To ensure infectivity, enveloped viruses must remain wet and are generally transmitted in fluids, respiratory droplets, blood or tissue. In contrast, nonenveloped (naked) viruses can survive the adverse conditions of the intestinal tract and may dry out while still retaining infectivity. Naked viruses can be transmitted easily, on fomites, hand-to-hand, dust, and small droplets. Table 2 lists important characteristics of enveloped and naked viruses.

Classification of viruses is based on the type of the nucleic acid genome (DNA or RNA), the strandedness of the viral nucleic acid (single-stranded or double-stranded genome), the presence or absence of an envelope (enveloped or naked), and other characteristics, such as the virion morphology, chemical composition, and mode of genomic replication (Table 1). Viral names may describe their characteristics, the diseases with which they are associated, or locations where they were first identified. The names picornavirus (pico, meaning small; rna, RNA) and togavirus (Greek for mantle, referring to the membrane envelope surrounding the virus) relate to the structure of the virus. The retrovirus name (retro, meaning reverse) conveys the virus-directed synthesis of DNA from an RNA template. Papovavirus is an acronym for members of the family (*pa*pilloma, *po*lyoma and *va*cuolating viruses). Reoviruses (*r*espiratory *e*nteric *o*rphan) are named for their first sites of isolation, but were not related to other classified viruses and were therefore designated orphans. Coxsackievirus is named after the town of Coxsackie in the state of New York, where the virus was first isolated. Herpesvirus (herpes, 'creeping') describes the nature of the pathologic lesion. 'Cytomegalovirus' refers to the increased cellular size of viral inclusion-bearing cells. The Epstein-Barr virus is named after the two individuals who first described the virus about 40 years ago.

Since viruses have no capacity to produce energy, reproduce their genomes or make their own structural proteins, their replication depends on their hosts to provide energy, substrates and machinery for replication of the viral genome and synthesis of viral proteins. Viruses acquire many of their functions for replication through piggybacking on cellular genes, thereby getting access to basic cellular machinery. Processes not provided by host cells must be encoded

Table 2. Characteristics of enveloped and naked viruses

Property	Enveloped viruses	Naked viruses
Surface structure	Lipid-protein membrane	Proteins
Virion stability	Environmentally labile	Environmentally stable
Virion release	Budding or cell lysis	Cell lysis
Virion transmissibility	Must stay wet	Readily
Predominant immunity	Cell-mediated response	Antibody response
Vaccine development	Complicated	Relatively easy

in the genome of the virus (e.g. the reverse transcriptase enzyme of the retroviruses).

Viral infection can lead either to a rapid replication of the agent and destruction of the infected cell, or to a prolonged period of latency. DNA viruses (except poxviruses) replicate in the nucleus and are more likely to persist in the host, whereas RNA viruses (except retroviruses) replicate in the cytoplasm. Viral replication starts with the virion particle recognizing and attaching to surface receptors of the mammalian cell. These events are followed by viral penetration into the cell, transcription of viral mRNA, viral protein synthesis, and replication of the viral genome. Viral receptor–ligand interactions and viral entry excite cellular responses, cytoskeletal rearrangement, and the induction of transcription factors, prostaglandins and cytokines. After assembling the viral genome and structural proteins, the virions are released from the cell by exocytosis or by cell lysis.

Key to an effective antiviral host response is the ability to recruit appropriate types and numbers of inflammatory cells and mediators to the site of infection. Suboptimal recruitment can lead to an inadequate inflammatory response, whereas overexuberant cell recruitment may result in damage to host tissues. Both cellular and humoral immunity responses are recruited in viral infections, but the pathogenic importance of the two arms of the immune system varies in different viral diseases. Enveloped viruses typically initiate cell-mediated inflammatory responses and delayed type hypersensitivity, which affect viral replication by killing mammalian cells that express viral proteins. Disease is often the result of inappropriate immune responses. Naked viruses are controlled mainly by antibody, and vaccines are generally effective. The role of humoral immunity is to produce antibodies against proteinaceous surface structures and thereby cause inactivation or clearance of the virus. Conversely, viruses have developed important means of escaping from immune detection, and have redirected or modified a normally protective host response to their advantage (256).

Viral diagnostics is a rapidly changing field in terms of assay principles and available diagnostic kits. Identification of viruses has traditionally been based on cell culture to detect characteristic cytopathic effects, morphologic determination of intracytoplasmic and intranuclear inclusion bodies, immunohistochemical techniques, immunoassays to identify viral antigens in clinical specimens, or the measurement of total or class-specific antibodies against specific viral antigens. In some viral infections, IgM antibodies are useful for determining primary infection, and IgG antibodies for assessing the susceptibility to primary infection and viral reactivation. Oral fluid collection may constitute a convenient and noninvasive method for serological surveillance of immunity to common viral infections (159).

Recently developed molecular technologies for detecting viral DNA or RNA in clinical specimens are now routinely used in virology laboratories. Viral nucleic acid can be measured directly by hybridization, or be detected after amplification by nucleic acid amplification methods (54). Polymerase chain reaction (PCR) offers a rapid and relatively inexpensive method of identifying viral nucleic acids in clinical specimens. Recent advances in quantitative real-time PCR techniques can provide additional insights into the natural history and disease associations of viral infections. Real-time PCR detection systems generally have a broad dynamic range and display high sensitivity, reproducibility and specificity. The use of PCR to monitor herpesvirus DNA load provides particularly high specificity (14). However, in order to evaluate the diagnostic utility of ultrasensitive PCR assays, correlations with clinical outcome are essential. The microarray-based detection assay provides a single-format diagnostic tool for the identification of multiple viral infections and will most likely become increasingly important in clinical virology. In the periodontal studies discussed below, PCR-based techniques were used to identify herpesviruses and bacterial species.

Herpesviruses

For a general introduction to herpesviruses, the reader is referred to a number of authoritative reviews (179, 195, 198). Because of the lack of effective therapeutics and vaccines, herpesvirus diseases continue to constitute a significant problem for public health. Herpesviral characteristics of potential importance in the pathogenesis of periodontitis are outlined below. Emphasis is placed on a description of HCMV and EBV because of these viruses' major suspected etiopathogenic role in human periodontitis (225).

Membership in the family *Herpesviridae* is based on a four-layered structure of the virion (Fig. 1). Herpesviruses have (i) a core containing a large double-stranded DNA genome encased within (ii) an isosapentahedral capsid containing 162 capsomers, (iii) an amorphous proteinaceous tegument and, surrounding the capsid and tegument, (iv) a lipid bilayer envelope derived from host cell membranes. The viral

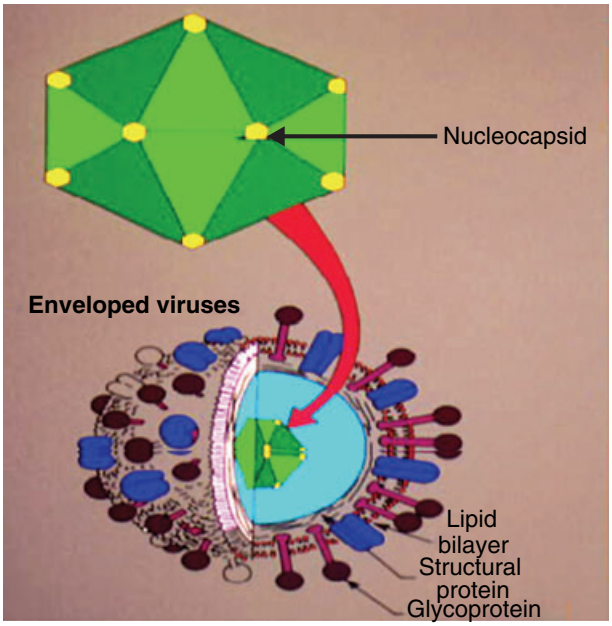

Fig. 1. Herpesvirus virion.

envelope contains viral-induced glycoproteins, which are ligands for cellular attachment and important targets for host immune reactions. Several herpesvirus proteins of the capsid, tegument, glycoprotein, replication, and immunomodulatory protein families have been identified and characterized.

Of the approximately 120 identified different herpesviruses, eight major types are known to infect humans, namely, herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, EBV, HCMV, human herpesvirus (HHV)-6, HHV-7, and HHV-8 (Kaposi's sarcoma virus). Research has identified more than 5000 different strains of herpesviruses. Humans are the only source of infection for these eight herpesviruses. Human herpesviruses are classified into three groups (α, β, γ) based upon details of tissue tropism, pathogenicity, and behavior under conditions of culture in the laboratory (Table 3). Alpha-herpesviruses are neurotropic, have a rapid replication cycle, and display a broad host and cell range. The β- and γ-herpesviruses differ in genomic size and structure, but replicate relatively slowly and in a restricted range of cells, mainly of lymphatic or glandular origin.

Herpesviruses can occur in a latent or a productive (lytic) state of replication. During latency, the herpesvirus DNA is integrated into and seems to behave like the host chromosomal DNA. In the viral productive cycle, the herpesvirus genome is amplified 100- to 1000-fold by the viral replication machinery. Figure 2 outlines the mode of the productive replication of herpesviruses. Herpesvirus transcription, genome replication, and capsid assembly occur in the host cell nucleus. The tegument and the envelope are acquired as the virion buds through the nuclear membrane. Herpesvirus virion genes are replicated in a specific order:

i) immediate-early genes, which encode regulatory proteins;
ii) early genes, which encode enzymes for replicating viral DNA;

Table 3. Human herpesviruses

Herpesviruses	Abbreviation	Herpes group	Major diseases
Herpes simplex virus type 1	HSV-1	α	Acute herpetic gingivostomatitis, keratitis, conjunctivitis, encephalitis, dermal Whitlow
Herpes simplex virus type 2	HSV-2	α	Herpes genitalis
Varicella-zoster virus	VZV	α	Varicella (chickenpox), zoster (shingles)
Epstein-Barr virus	EBV	γ	Classic infectious mononucleosis, Burkitt's lymphoma (Africa and New Guinea), Hodgkin's lymphoma, nasopharyngeal carcinoma, squamous carcinoma (Southern China), oral hairy leukoplakia, chronic fatigue syndrome (?)
Human cytomegalovirus	HCMV	β	Congenital symptomatic cytomegalovirus infection (growth retardation, jaundice, hearing defects, etc.), retinitis, encephalitis, mononucleosis-like syndrome, organ transplant rejection
Human herpesvirus 6	HHV-6	β	Exanthem subitum (roseola infantum) in young children and undifferentiated febrile illness
Human herpesvirus 7	HHV-7	β	Exanthem subitum (roseola)-like illness in young children
Human herpesvirus 8	HHV-8	γ	Kaposi's sarcoma in AIDS patients and intra-abdominal solid tumors

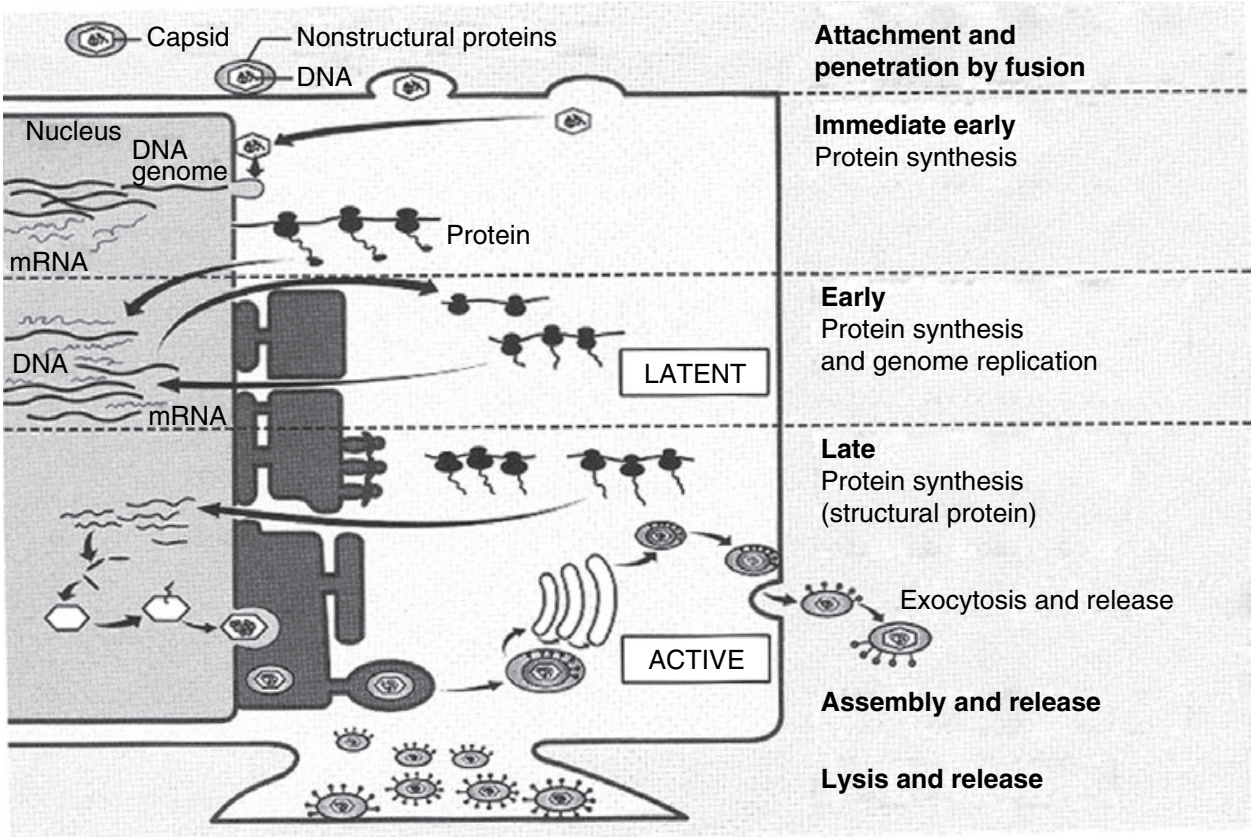

Fig. 2. Herpesvirus replication. Virion initiates infection by fusion of the viral envelope with plasma membrane following attachment to the cell surface. Capsid is transported to the nuclear pore where viral DNA is released into the nucleus. Viral transcription and translation occur in three phases: immediate early, early, and late. Immediate early proteins shut off cell protein synthesis. Early proteins facilitate viral DNA replication. Late proteins are structural proteins of the virus that form empty capsids. Viral DNA is packaged into preformed capsids in the nucleus. Virions are transported via endoplasmic reticulum and released by exocytosis or cell lysis.

iii) late genes, which encode structural proteins of the capsid of the virion.

Transcription of late genes can be used diagnostically to indicate active infection. Virions are transported to the cell membrane via the Golgi complex. The host cell dies with the release of mature virions or, alternatively, specific cell types may maintain herpesviruses in a latent state.

To survive, herpesviruses need to exploit macrophages, lymphocytes or other host cells for replication, while minimizing antiviral inflammatory responses of the host. Herpesviruses encode proteins that are specifically committed to subvert the immune defense of the host in order to evade virus elimination. To overcome viral immunoevasive proteins, the host in turn has evolved countermeasures to confine virus replication to below a harmful level. Herpesvirus diseases are generally limited to immunologically immature or immunocompromised individuals unable to mount an adequate host defense (189).

During their life cycles, herpesviruses execute an intricate chain of events geared towards optimizing their replication. The initial productive phase of infection is followed by a latent phase during which the viral genome integrates within the host cell's genome. Latency ensures survival of the herpesviral genome throughout the lifetime of the infected individual. From time to time, latent herpesviruses may undergo reactivation and re-enter the productive phase as a consequence of declining herpesvirus-specific cellular immunity. The balance between herpesvirus latency and activation involves the regulation of herpesvirus gene expression, but the genetic and biochemical mechanisms governing a herpesvirus latent infection and reactivation from latency are not fully understood. In general, the herpesvirus latent phase shows little tendency to transcription, whereas reactivation from latency results in a general viral gene expression (112). Nonetheless, expression of EBV-latency-associated genes has potent cell cycle-promoting activity of naive B-lymphocytes, which probably accounts for the growing panel of human cancers associated with the virus (67). During the active replication phase,

herpesvirus genomic transcription may induce changes in host cell expression of genes that encode proteins involved in immunity and host defense, cell growth, signaling, and transcriptional regulation (222). Psychosocial and physical stress, hormonal changes, infections, immunosuppressive medication, and other events impairing cellular immunity can trigger herpesviral reactivation. Transforming growth factor (TGF)-β1 in saliva seems also to have the potential to reactivate herpesviruses (164).

Herpesviruses are typically highly selective in regard to the specific tissues or organs they infect, reflecting their strong tendency to tissue tropism. Several herpesviruses reside in and may functionally alter cells of central importance for regulating the immune system (45, 156). HCMV infects monocytes/macrophages, T-lymphocytes, ductal epithelial cells of salivary glands, endothelial cells, fibroblasts and polymorphonuclear leukocytes, and establishes latent infection mainly in cells of the myeloid lineage. HCMV infection causes cytopathological effects that involve intranuclear and cytoplasmic inclusions ('owl's-eye' cells; large cells with enlarged nuclei containing violaceous intranuclear inclusions surrounded by a clear halo) in a characteristic enlargement of the host cells (cytomegaly). EBV infects relatively long-lived B-lymphocytes during primary infection and during latency, and can also infect the oropharyngeal epithelium. The molecular mechanism of tissue tropism of herpesviruses remains largely unknown.

Most herpesviruses are ubiquitous agents that often are acquired early in life and infect individuals from diverse geographic areas and economic backgrounds. An important exception is HHV-8, which is uncommon in the general population in the United States (less than 5% of the U.S. population is serologically positive for HHV-8) but is detected consistently in patients with AIDS-associated Kaposi's sarcoma and frequently in the eastern Mediterranean and sub-Saharan Africa, where Kaposi's sarcoma is endemic (34). Over the lifetime of the infected host, herpesvirus reactivation will lead to low-level infections that can be spread to acquaintances. The shedding of herpesvirus virions may take place without any detectable signs or symptoms of disease. Transmission of herpesviruses can happen vertically, either prenatally (HCMV) or perinatally, from mother to infant, or horizontally in children or adults by direct or indirect person-to-person contact. Infectious herpesviruses may be found in oropharyngeal secretions, urine, cervical and vaginal secretions, semen, maternal milk, tears, feces, and blood. Saliva of many immunocompetent and immunocompromised subjects contains several herpesvirus species and may frequently serve as a vehicle for viral transmission (72, 109). It is estimated that asymptomatic shedding of HCMV into saliva, cervical secretions, semen, and breast milk occurs in 10–30% of infected individuals (27). HCMV seroconversion, which is indicative of a recent active infection, can take place in all age groups between 18 and 60 years and, in Germany, occurs with elevated frequency in 30–35-year-old individuals (89).

Herpesvirus infections may be latent, subclinical or clinical. Herpesvirus colonization in most individuals is clinically unnoticeable, and activation of latent herpesviruses may cause both symptomatic and asymptomatic infection. Most serious clinical illness happens when primary infection occurs in adolescence or beyond. Clinical cases of herpesvirus infection are frequently the result of a reactivation of a latent infection, which is linked to the immune status of the patient. In immunocompetent hosts exhibiting protective antiviral immune responses, primary infection or reactivation of latent herpesvirus genomes is usually asymptomatic despite active virus replication and systemic dissemination. In immunocompromised patients, herpesvirus infection can produce a wide spectrum of outcomes, ranging from subclinical infection to disseminated fulminant disease having high mortality rates. Herpesvirus infections with associated immune impairment may also increase the risk or the severity of bacterial, fungal or other viral infections (24).

Herpesvirus infections are kept under control by various innate and immune responses that, although vigorous, are not capable of eliminating the viruses. The innate host response consists of a complex multilayered system of mechanical and secreted defenses, immediate chemokine and interferon responses, and rapidly recruited cellular defenses. Innate responses are the first line of defense during both primary and recurrent infection, and are essential during acute infection to limit initial viral replication and to facilitate appropriate adaptive immune responses. The humoral acquired immune response aims mainly at neutralizing and preventing initial herpesvirus infections. Gingiva of mice shows high resistance to infection by HSV, which may suggest the existence of a particularly efficacious antiherpesvirus defense in the murine periodontium (158).

The cellular immune response attempts to eliminate virus-infected cells by means of lymphocytes (86, 256). Cytotoxic T-lymphocytes and natural killer (NK) cells are the most important effector cells in immune suppression of herpesvirus replication and

in the maintenance of latency (160). Evidence for the importance of the cellular immunity in the control of herpesvirus infections comes from the observation that severe herpesvirus disease occurs almost exclusively in subjects with depressed cell-mediated immunity. Also, impaired cellular immunity leads to less efficient elimination of herpesvirus-infected host cells and to increased herpesvirus DNA replication. The T-lymphocyte response to herpesviruses changes over time from a predominantly $CD4^+$ response early in infection to a $CD8^+$ response during latent infection. $CD4^+$ cells contribute to expansion of cytotoxic $CD8^+$ T-lymphocytes. The antiviral cytotoxic T-lymphocyte response against herpesvirus is limited to a few proteins, with the predominant anti-HCMV response directed against the pp65 tegumental protein, which therefore represents a main target for cellular immunotherapy (19).

In response to antiviral host defenses, herpesviruses have devised a number of elaborate immunosubversive mechanisms to ensure persistent infections (241, 264). Herpesviruses can trigger dysregulation of macrophages and lymphocytes for the purpose of down-regulating the antiviral host immune response (24). HCMV can interfere with the immune functions of antigen-presenting monocyte-derived dendritic cells by impairing their maturation, antigen presentation and allostimulatory capacity (19). HCMV and other herpesviruses have also the ability to inhibit the expression of major histocompatibility complex (MHC) class I and II on the surface of macrophages (265), to evade cytotoxic T-cell recognition and attenuate induction of antiviral immunity (256), and to encode proteins that interfere with the presentation of viral peptide antigens to cytotoxic T-cells (256). The presence of genes that encode proteins that interfere with HCMV antigen presentation helps herpesvirus-infected cells escape $CD8^+$ and $CD4^+$ T-cell immunosurveillance. Cells that lack MHC class I molecules are normally recognized and eliminated by NK cells, but herpesvirus-infected cells have developed strategies to circumvent NK cell-mediated lysis (26, 265). The destruction of components of MHC class I and class II pathways within macrophages, which markedly impair their principal role in antigen presentation, together with the silencing of NK cells, help ensure the permanence of herpesvirus infections (152). HCMV has also the ability to inhibit the expression of macrophage surface receptors for lipopolysaccharide and thereby the responsiveness to gram-negative bacterial infections (101). Some herpesvirus genes protect cells from undergoing apoptosis to prolong the lives of infected cells (256, 265). One effect of the inhibition of apoptosis is the promotion of tumor cell survival, potentially interfering with anticancer chemotherapy (150). The large series of immune evasion molecules helps herpesviruses establish life-long latency interrupted by recurrent reactivations, despite an intact immune system of the host.

Herpesvirus infections affect cytokine–chemokine networks (156). Cytokines and chemokines play important roles in the first line of defense against human herpesvirus infections and also contribute significantly to the regulation of acquired immune responses. HCMV infection induces a proinflammatory cytokine profile, with production of interleukin (IL)-1β, IL-6, IL-12, tumor necrosis factor (TNF)-α, interferon (IFN)-α/β, and IFN-γ (156) and prostaglandin E_2 (PGE_2) (154). EBV infection stimulates the production of IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-8, IL-18, TNF-α, IFN-α/β, IFN-γ, monokine induced by IFN-γ (MIG), IFN-γ-inducible protein 10 (IP-10) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (156). On primary HSV infection, the host responds by producing IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, IFN-α/β, IFN-γ, GM-CSF, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β, monocyte chemoattractant protein 1 (MCP-1) and regulated upon activation normal T-cells expressed and secreted (RANTES) (156). Proinflammatory cytokine and chemokine activities normally serve a positive biological goal by aiming to overcome infection or invasion by infectious agents. IFN-γ, TNF-α and IL-6 exert particularly high antiviral activity. However, by a diverse array of strategies, herpesviruses are able to interfere with cytokine production or divert potent antiviral cytokine responses (6, 155, 256). The extensive built-in redundancy of the cytokine system and the elaborate efforts by herpesviruses to undermine or exploit its function testify to the importance of cytokines in the antiviral host defense. It is of clinical significance that cytokines may exert detrimental effects when a challenge becomes overwhelming, or with a chronic pathophysiologic stimulus.

T-helper lymphocyte type 1 (Th1) proinflammatory immune responses aim to clear the host of intracellular pathogens, such as herpesviruses. Th1 cytokines favor the development of a strong cellular immune response, whereas Th2 cytokines favor a strong humoral immune response, and some of the type 1 and type 2 cytokines are cross-regulatory. In an effort to counteract ongoing inflammation, the initial proinflammatory response triggers the release of anti-inflammatory TGF-β and IL-10, a Th2 cytokine that

antagonizes Th1 proinflammatory responses (88). HCMV (123) and EBV (206) also encode unique homologs of IL-10 capable of inhibiting the production of TNF, IL-1 and other cytokines in macrophages and monocytes (254), and of preventing the activation and polarization of naive T lymphocytes towards protective gamma interferon-producing effectors (33). Moreover, herpesviruses can block the interferon signal transduction pathway, which limits the direct and indirect antiviral effects of the interferons (256). Viruses also display great inventiveness when it comes to diverting potent antiviral cytokine and chemokine responses to their benefit (256). PGE_2, which is a major mediator of the periodontal inflammatory response (73), increases rapidly in response to exposure of cells to herpesviruses, bacterial lipopolysaccharide, and IL-1β and TNF-α cytokines (261); however, PGE_2 may under certain circumstances serve to support HCMV replication (154, 278). In sum, herpesvirus infections induce a multiplicity of interconnected immunomodulatory reactions, and various stages of the infectious process may display different levels of specific inflammatory cells and mediators, underscoring the complexity of herpesvirus–host interactions.

Herpesviruses can cause serious infectious diseases and be tumorogenic (Table 3). Herpesvirus diseases occur primarily in individuals having an immune system that is immature or suppressed by drug treatment or coinfection with other pathogens. In immunocompetent persons, complications of an acute HCMV infection are rare, except in newborns, where HCMV represents the major infectious cause of pregnancy complications and birth defects (7). About 10% of HCMV-infected newborns may show low birth weight, jaundice, hepatosplenomegaly, skin rash, microcephaly or chorioretinitis (15). Congenital HCMV infection is the leading infectious cause of mental retardation and sensorineural deafness (194). In 1992, it was estimated that approximately 40,000 newborns annually in the USA were infected prenatally with HCMV and that up to 7000 of these newborns developed permanent central nervous damage as a result of the infection (69). Approximately one-third of newborns with symptomatic congenital HCMV infection born to mothers with recurrent HCMV infection or to mothers with primary HCMV infection during pregnancy may be premature (< 37 weeks' gestation) and small for their gestational age (25). In adolescents and young adults, primary HCMV infection causes about 7% of cases of the mononucleosis syndrome and may manifest symptoms almost indistinguishable from those of EBV-induced mononucleosis.

HCMV is capable of manifesting disease in nearly every organ system in immunocompromised patients. HCMV is the most common life-threatening infection in HIV-infected patients (82). Necrotizing retinitis is a relatively common HCMV-induced complication in untreated HIV-infected persons (248). Also, rather than *Helicobacter pylori*, HCMV may be the main causative pathogen of peptic ulcers in some AIDS patients (35). Salivary HCMV DNA occurs at an elevated rate with xerostomia in HIV-infected patients with low CD4 counts, suggesting HCMV may be a potential cause of salivary gland dysfunction in these patients (81). The introduction of the highly active antiretroviral therapy (HAART) has provided a means of reconstituting the immune system in HIV-infected individuals, allowing the HCMV infection to be controlled (242).

Organ transplantation has become a widely accepted treatment modality for end-stage diseases. With the escalation in the number of patients undergoing immunosuppressive therapy following solid organ or bone marrow transplantation, HCMV activation and resulting disease has become a major clinical problem in transplant recipients. HCMV is the most common infectious reason for transplant rejection, including bone marrow or stem cell grafts (37), and a relationship has been sought between periodontal HCMV and renal transplant complications (166). HCMV infection seems also to be a significant risk factor for the development of bacterial septic infection in liver transplant patients (163, 182), and for causing colonization of the oropharynx by gram-negative bacilli in renal transplant patients (138).

HCMV and HSV have for two decades been epidemiologically associated with the development of primary atherosclerosis, postangioplasty restenosis, and post-transplantation arteriosclerosis (169). Both vascular smooth muscle and endothelial cells are targets for HCMV primary infection and may serve as potential sites of HCMV latency. HCMV DNA sequences have been detected in atheromatous plaques (87) and in the wall of atherosclerotic vessels (104, 219). A PCR-based study identified genomes of HCMV in 40%, EBV in 80% and HSV-1 in 80% of atherosclerotic aortic tissue, compared to 4%, 13% and 13%, respectively, of nonatherosclerotic aorta controls (219). HCMV-infected cardiac transplant patients are prone to develop accelerated atherosclerosis (2). Animal research has shown the Marek's disease virus, an avian herpesvirus, to be capable of inducing atherosclerotic lesions in infected chickens (61). Murine CMV is able to produce atherosclerosis in experimental mice (103). Although animal

experiments on cardiovascular disease do not replicate exactly the human disease, they may provide valuable suggestions on causality. HCMV and HSV-1 may affect atherosclerosis directly or indirectly (121). Direct effects on vascular wall cells may include cell lysis, transformation, lipid accumulation, proinflammatory changes, and augmentation of procoagulant activity. Indirect systemic effects may involve induction of acute-phase proteins, establishment of a prothrombotic state, hemodynamic stress caused by tachycardia, increased cardiac output, or a regional inflammatory activation in response to systemic cytokinemia. It is theorized that herpesvirus infections, usually in combination with other risk factors, such as hypertension, smoking, hyperlipidemia, obesity, and family history, promote atherogenesis and trigger acute coronary events. The possibility that HCMV and other herpesviruses give rise to cardiovascular disease and periodontitis in an independent manner further complicates studies on the relationship between the two diseases, and raises questions about the notion of periodontitis being a direct risk factor of ischemic heart disease (223, 229). Similar reservations are applicable to the proposed relationship between periodontitis and atherosclerosis-associated ischemic craniovascular events (52).

HCMV and EBV appear with increased frequency in synovial fluid and tissue of autoimmune chronic arthritis, pointing to a possible viral factor in the disease (147). Furthermore, HCMV has been identified in diseases that have a bacterial component, including inflammatory bowel disease, enterocolitis, esophagitis, pulmonary infections, sinusitis, acute otitis media, dermal abscesses, and pelvic inflammatory disease (28, 224). Activation of HCMV and other herpesviruses may play roles in oral ulceration of the aphthous type (183, 191, 247). HCMV has also been associated with cervical carcinoma and adenocarcinomas of the prostate and the colon (51). However, it should be cautioned that the presence of herpesvirus DNA in various disease entities does not prove causality in itself. The difficulty in providing true causal evidence for the role of herpesviruses in disease lies in inadequate knowledge about molecular aspects of herpesviruses and the pathogenic mechanisms of herpesvirus-associated pathosis.

The primary route of EBV acquisition is through salivary exchange in the oropharynx (195). The virus is the main causative agent of infectious mononucleosis, which is a relatively common clinical manifestation of a primary EBV infection in adolescents and young adults. EBV has also been implicated in multiple sclerosis and various enigmatic syndromes, and seems to play a role in the development of oral hairy leukoplakia. Oral hairy leukoplakia is associated with EBV productive and nonproductive infection of tongue epithelial tissue (266), EBV-encoded nuclear antigen (EBNA)-2 protein function (268), and an EBV-related decrease in oral epithelial Langerhans cells (267). EBV can contribute to oncogenesis, as evidenced by its frequent occurrence in certain tumors arising in lymphoid or epithelial tissue, including B-lymphocyte neoplasms, such as Burkitt's lymphoma, post-transplant B-cell lymphoma and Hodgkin's disease, certain forms of T-cell lymphoma, and some types of epithelial tumors, including undifferentiated nasopharyngeal carcinoma and a portion of gastric carcinomas. EBV may also be involved in the pathogenesis of aggressive types of non-Hodgkin lymphomas affecting gingiva (277), particularly in HIV-infected individuals (213). Recently, EBV (141) and HCMV (200) have been associated with cases of breast cancer. EBV may induce tumors by influencing survival mechanisms of B-lymphocytes, but environmental, genetic, and iatrogenic cofactors are most likely also participants in EBV-related oncogenesis. That EBV may adopt different forms of latent infection in different tumor types is a reflection of the complex interplay between the virus and the host cell environment.

HSV is the cause of some of the most frequently encountered clinical infections in humans. HSV-1 usually causes orolabial disease, and HSV-2 is associated more frequently with genital and newborn infections. Most HSV clinical infections give rise to mild and self-limiting disease of the mouth and lips or at genital sites, but can be life-threatening when affecting neonatals and the central nervous system, especially in immunocompromised hosts (105, 270). Varicella-zoster virus (VZV) causes chickenpox (varicella), after which it establishes latency and can subsequently reactivate in adults to cause shingles (herpes zoster). Serious central nervous system complications can follow both primary infection and reactivation of VZV (77). Although HHV-6 is generally asymptomatic, the virus has been associated with exanthem subitum, febrile convulsions and encephalitis in infants and immunocompromised adults, and may play a role in multiple sclerosis, the Guillain-Barre syndrome, and acute disseminated encephalomyelitis (48). HHV-7 has not been shown to cause a specific disease, but is associated with febrile convulsions and has been implicated in a few cases of exanthem subitum and as a cause of encephalitis (48). HHV-8 is implicated in Kaposi's sarcoma, the plasma-cell variant of multicentric

Castleman's disease, and pleural effusion lymphoma (93). Herpesviruses can also give rise to other types of medical and orofacial infections and tumors, especially in immunocompromised hosts (205, 207).

Treatment of herpesvirus infections can be difficult because few options exist (127). Presently available antiherpesvirus drugs can produce clinical improvement, but suffer from poor oral bioavailability, low potency, development of resistance, and dose-limiting toxicity. Nucleic acid molecules are emerging as new antiviral tools in antisense therapy, in which an antisense oligonucleotide to mRNA of genes involved in pathogenesis selectively modulates gene expression. Conventional vaccination with attenuated herpesviruses or herpesviral proteins fails to prime efficient immunologic protection, presumably because critical antigens are not presented effectively *in vivo*. Development of novel herpesviral vaccines and vaccination technologies are of high priority, and several promising herpesviral vaccine candidates are currently in clinical trials (180, 273). The prime goal of a vaccine should be to prevent primary infection, but vaccines may also be used to modify the course of established persistent herpesvirus infections by so-called postinfective immunization or therapeutic vaccination.

Herpesviruses in periodontal disease

Studies during the past 10 years have associated herpesviruses with human periodontitis. Table 4 describes the distribution of herpesviruses in biopsy specimens from clinically healthy and inflamed gingiva of adult (chronic) periodontitis patients living in Los Angeles. DNA of 2–6 herpesviruses was demonstrated in all 14 biopsies from periodontitis sites. In contrast, HCMV only occurred in two and EBV-type 1 (EBV-1) in three biopsies from 11 healthy gingival sites. HSV, HCMV, EBV-1, EBV-type 2 (EBV-2) and HHV-7 showed significant associations with periodontitis. HHV-6 and HHV-8 were only detected in biopsies from periodontitis lesions. Three of four biopsies yielding HHV-8 originated from patients with confirmed HIV infection; the HIV-status of the fourth HHV-8-positive subject was unknown.

Table 4. Herpesviruses in gingival biopsies from periodontitis and clinically healthy sites in adults[a]

Herpesviruses	Periodontitis (14 subjects)	Healthy periodontium (11 subjects)	*P*-values (chi-squared test)
HSV	8 (57)[b]	1 (9)	0.04
EBV-1	11 (79)	3 (27)	0.03
EBV-2	7 (50)	0 (0)	0.02
HCMV	12 (86)	2 (18)	0.003
HHV-6	3 (21)	0 (0)	0.31
HHV-7	6 (43)	0 (0)	0.04
HHV-8	4 (29)[c]	0 (0)	0.17

[a]Adapted from Contreras et al. (42).
[b]No. (%) of virally positive samples.
[c]Three patients were confirmed HIV-positive.

Table 5 lists the occurrence of subgingival HCMV, EBV and HSV DNA in periodontitis patients from different countries. In Turkey, HCMV was detected in 44% of chronic periodontitis lesions and in 14% of healthy periodontal sites ($P < 0.05$), EBV-1 in 17% of periodontitis lesions and in 14% of healthy sites, and HSV in 7% of periodontitis lesions but in no healthy study site (211). Another study from Turkey identified HCMV in 68% of chronic periodontitis lesions and in 33% of gingivitis lesions (252). In 62 Chinese patients, Li et al. (132) found EBV in 58% of disease-active periodontitis sites, but only in 23% of quiescent periodontitis sites and in 19% of gingivitis sites. In Japan, Idesawa et al. (108) detected EBV in 49% of chronic periodontitis lesions and in 15% of healthy periodontal sites. Studies of periodontitis in Taiwanese adult patients showed subgingival HSV monoinfection and HSV-HCMV coinfection to be associated with increased periodontal pocket depth and attachment loss, and elevated frequency of gingival bleeding but relatively little dental plaque (133). In Italy, HSV-1 (208) and HHV-7 (31) have been related to periodontal disease. Israeli subjects revealed HSV antigens in 39% of biopsies from clinically healthy gingiva (8). In France, Madinier et al. (139) detected EBV DNA in eight of 20 gingival specimens but, despite the potential of EBV to replicate in oral mucosa (9), only in one specimen from nasal, laryngeal, and oral mucosa, suggesting inflamed gingiva serves as a reservoir for EBV. Even though herpesvirus carriage varies by age, country, region within country, and population subgroups (235), studies from the various countries all report on a high prevalence of herpesvirus DNA in periodontitis lesions, attesting to the robustness of the herpesvirus–periodontitis association.

Kamma et al. (114) investigated the occurrence of DNA of HCMV, EBV-1 and selected periodontal pathogenic bacteria in 16 patients with aggressive periodontitis from Greece (Table 6). In each patient,

Table 5. Prevalence of herpesvirus DNA in periodontitis patients from various countries

Study	Country	Periodontal status	Herpes simplex virus type 1	Epstein-Barr virus[a]	Cytomegalovirus
Contreras et al. (42)	USA	Advanced chronic periodontitis	57% (periodontitis) 9% (healthy or slight gingivitis)	79% (periodontitis) 27% (healthy or slight gingivitis)	86% (periodontitis) 18% (healthy or slight gingivitis)
Ting et al. (255)	USA	Aggressive localized periodontitis	55% (periodontitis) 9% (healthy)	64% (periodontitis) 18% (healthy)	73% (periodontitis) 18% (healthy)
Michalowicz et al. (151)	Jamaica	Localized periodontitis	No data	33% (aggressive) 45% (incipient) 17% (healthy/gingivitis)	73% (aggressive) 40% (incipient) 22% (healthy/gingivitis)
Kamma et al. (114)	Greece	Generalized periodontitis	35% disease-(active) 9% (disease-stable)	44% (disease-active) 13% (disease-stable)	59% (disease-active) 13% (disease-stable)
Saygun et al. (210)	Turkey	Generalized periodontitis	78% (aggressive) 0% (healthy)	72% (aggressive) 6% (healthy)	72% (aggressive) 0% (healthy)
Kubar et al. (124)	Turkey	Generalized periodontitis	No data	89% (aggressive) 46% (chronic)	78% (aggressive) 46% (chronic)
Ling et al. (133)	Taiwan	Chronic periodontitis	31%	4%	52%
Li et al. (132)	China	Chronic periodontitis	No data	58% (disease-active) 23% (quiescent) 19% (gingivitis)	No data
Idesawa et al. (108)	Japan	Chronic periodontitis	No data	49% (saliva of periodontitis patients) 15% (saliva of healthy subjects)	No data

[a]Most studies report on EBV type 1.

Table 6. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr virus type 1 (EBV-1) in progressing and stable periodontitis sites of 16 patients with aggressive periodontitis patients[a]

Items	32 disease-active periodontitis sites	32 disease-stable periodontitis sites	*P*-values (chi-squared test)
Mean pocket probing depth in mm	5.9 ± 0.8	5.2 ± 1.0	Not significant
Bleeding upon probing, *n* (%) positive sites	31 (96.9%)	19 (59.4%)	< 0.001
% teeth exhibiting alveolar bone loss	41.3 ± 6.3	43.9 ± 6.2	Not significant
HCMV, *n* (%) positive sites	19 (59.4%)	4 (12.5%)	< 0.001
EBV-1, *n* (%) positive sites	14 (43.8%)	4 (12.5%)	0.01
HCMV and EBV-1 coinfection, *n* (%) positive sites	9 (28.7%)	0 (0%)	0.004
D. pneumosintes, *n* (%) positive sites	20 (62.5%)	6 (18.8%)	< 0.001
P. gingivalis, *n* (%) positive sites	23 (71.9%)	12 (37.5%)	0.01
D. pneumosintes and *P. gingivalis* coinfection, *n* (%) positive sites	15 (46.9%)	0 (0%)	< 0.001

[a]Adapted from Kamma et al. (114).

subgingival samples were collected from two progressing and two stable periodontitis sites with similar depth and gingival inflammation. The study revealed that herpesviruses can be detected in some but not in other periodontitis lesions of the same individual. HCMV, EBV-1 and HCMV-EBV-1 coinfection were statistically associated with disease-active periodontitis. All periodontitis sites that demonstrated HCMV-EBV-1 coinfection and all but one site that showed *P. gingivalis-D. pneumosintes* coinfection revealed bleeding upon probing (114), a clinical sign of elevated risk for disease progression (128). Some of the *Dialister* strains may have belonged to the new species *D. invisus* (53). Patients with an HCMV-EBV-1 periodontal coinfection exhibited, on average, a more rapid progression of periodontitis than patients with a herpesvirus monoinfection. Other studies have also demonstrated a strong association between subgingival *P. gingivalis*, *D. pneumosintes* and *P. gingivalis-D. pneumosintes* co-occurrence, and disease-active periodontitis (114, 230, 234). In experimental mice, a murine CMV-*P. gingivalis* combined infection produced distinct liver and spleen damage and a higher mortality rate than monoinfections by either MCMV or *P. gingivalis*, pointing to an important pathogenic interaction between MCMV and *P. gingivalis* (245). In parallel control *Escherichia coli*-MCMV coinfection experiments, the mortality and pathological findings were similar to those observed in mice infected with MCMV only (245). The ability of herpesviruses to induce immunosuppression may set the stage for enhanced proliferation of subgingival *P. gingivalis*, *D. pneumosintes* and other periodontopathic bacteria, and increase the risk of periodontal disease progression.

Herpesviruses do not appear to be only passive bystanders to gingival inflammation in periodontitis lesions. Kamma et al. (114) showed that, even if no difference was observed in the level of gingival inflammation, herpesviruses occurred more frequently in actively progressing than in stable periodontitis sites. Kubar et al. (125) found increased periodontal pocket depth and attachment loss in aggressive periodontitis sites with HCMV presence, compared to periodontitis sites with similar degree of clinical inflammation but with no detectable HCMV.

Yapar et al. (275) described a close relationship between herpesviruses and aggressive periodontitis, detecting HCMV in 65%, EBV-1 in 71% and HCMV-EBV coinfection in 47% of the deep lesions studied. In aggressive periodontitis lesions, subgingival specimens averaged 4000–10,000 HCMV copies/ml (124, 125) and gingival tissue specimens yielded up to 750,000 HCMV copies (124). The same research group from Ankara, Turkey, detected a lower qualitative and quantitative occurrence of herpesviruses in chronic periodontitis lesions (124, 211). The predilection of herpesviruses for aggressive periodontitis emphasizes the need for a careful assessment of the periodontal disease status in clinical studies of periodontal herpesviruses.

Michalowicz et al. (151) studied the presence of subgingival HCMV, EBV-1, *P. gingivalis* and *A. actinomycetemcomitans* in 15 adolescents with localized aggressive periodontitis, 20 adolescents with incidental periodontal attachment loss, and 65 randomly selected healthy controls. The study subjects were Afro-Caribbeans living in Jamaica. The most efficient multivariate model for localized aggressive periodontitis included HCMV (Odds Ratio = 6.6; 95% confidence limits: [1.7, 26.1]) and *P. gingivalis* (Odds Ratio = 8.7; 95% confidence limits: [1.7, 44.2]). The odds of having localized aggressive periodontitis increased multiplicatively when both HCMV and *P. gingivalis* were present compared to harboring neither of the two infectious agents (Odds Ratio = 51.4; 95% confidence limits: [5.7, 486.5]). Apparently, HCMV and *P. gingivalis* are independently and strongly associated with localized aggressive periodontitis in Jamaican adolescents, and the two infectious agents seem to act synergistically to influence the risk for both the occurrence and the severity of the disease.

Ting et al. (255) studied the relationship between HCMV activation and disease-active vs. disease-stable periodontitis in 11 patients with aggressive juvenile periodontitis between the ages of 10 and 23 years living in Los Angeles (Table 7). The presence of mRNA of the HCMV major capsid protein, which is an indication of an active HCMV infection, was detected in deep pockets of all five HCMV-positive patients with early disease (aged 10–14 years), but only in one of three HCMV-positive patients older than 14 years, and not in any shallow test sites. The study found HCMV reactivation in some and HCMV latency in other periodontal sites of the same patient, pointing to site-specificity in oral HCMV transcription state. HCMV activation was exclusively identified in periodontal sites showing no visible radiographic alveolar crestal lamina dura, a sign of possible periodontal disease progression (188). Gingiva of aggressive periodontitis lesions tends to show high levels of T-suppressor cells (148) and Langerhans cells (149), which are potential carriers of the HCMV

Table 7. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr type 1 (EBV-1) in deep and shallow periodontal sites of 11 localized aggressive periodontitis patients[a]

Items	5 disease-active periodontitis sites *n* (%) viral-positive sites	4 disease-stable periodontitis sites *n* (%) viral-positive sites	11 shallow periodontal sites *n* (%) viral-positive sites
HCMV	5 (100%)	2 (50%)	2 (18%)
HCMV active infection	5 (100%)	0 (0%)	0 (0%)
EBV-1	3 (60%)	3 (75%)	2 (18%)
HCMV and EBV-1 coinfection	3 (60%)	1 (25%)	2 (18%)
Presence of *A. actinomycetemcomitans*	5 (100%)	0 (0%)	Not done

[a]Adapted from Ting et al. (255).

Table 8. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr type 1 (EBV-1) in ANUG sites and normal periodontal sites of Nigerian children with and without malnutrition[a]

Herpesviruses	ANUG + malnutrition (22 subjects) *n* (%) viral-positive sites	Normal oral health + malnutrition (20 subjects) *n* (%) viral-positive sites	*P*-values (chi-squared test)
HCMV	13 (59.0%)	0 (0%)	< 0.001
EBV-1	6 (27.3%)	1 (5.0%)	0.13
HCMV and EBV-1 coinfection	8 (36.4%)	0 (0%)	0.009

[a]Adapted from Contreras et al. (40).

genome. Infiltrating cells of aggressive periodontitis lesions in juveniles have revealed a viral morphogenesis phenomenon by electron microscopic examination (29). Periodontal sites demonstrating HCMV reactivation also tend to exhibit elevated levels of *A. actinomycetemcomitans*, a major pathogen of the disease (233). Apparently, HCMV activation together with *A. actinomycetemcomitans* constitutes an important pathogenetic feature of localized aggressive periodontitis lesions in U.S. patients.

To explain the discrete nature of tissue breakdown in localized aggressive periodontitis, it is hypothesized that an active HCMV infection in tissue surrounding the tooth germs damages the root surface structure during the time of root formation of permanent incisors and first molars at 3–5 years of age. HCMV infections of infants are known to have the potential to cause changes in tooth morphology (63, 243), and teeth affected by localized aggressive periodontitis frequently show cemental hypoplasia (23). Also, DNA virus particles within odontogenic cells of developing teeth in hamsters have been related to fibrolytic and osteolytic lesions in the periodontal ligament and adjacent alveolar bone (71). It is further hypothesized that localized aggressive periodontitis patients experience reactivation of periodontal herpesviruses due to puberty-related hormonal changes, the effect of which may be overgrowth of resident periodontopathic bacteria and subsequent tissue breakdown around teeth with weakened periodontium.

Acute necrotizing ulcerative gingivitis (ANUG) affects immunocompromised, malnourished and psychosocially stressed young individuals, and the disease may occasionally spread considerably beyond the periodontium and give rise to the life-threatening infection termed noma/cancrum oris (161). It is estimated that 770,000 people are currently afflicted by noma sequelae (16). Table 8 shows the distribution of herpesviruses in ANUG-affected and non-ANUG-affected children 3–14 years of age from Nigeria (40). A significantly higher occurrence of DNA of HCMV and other herpesviruses was detected in ANUG lesions of malnourished children than in non-ANUG, normal, and malnourished children. In Europe and the U.S.A., ANUG affects mainly adolescents, young adults, and HIV-infected individuals, and virtually never young children. The occurrence of ANUG in children in Africa may be due to an acquisition of herpesviruses in early childhood (178), malnutrition that may promote herpesvirus

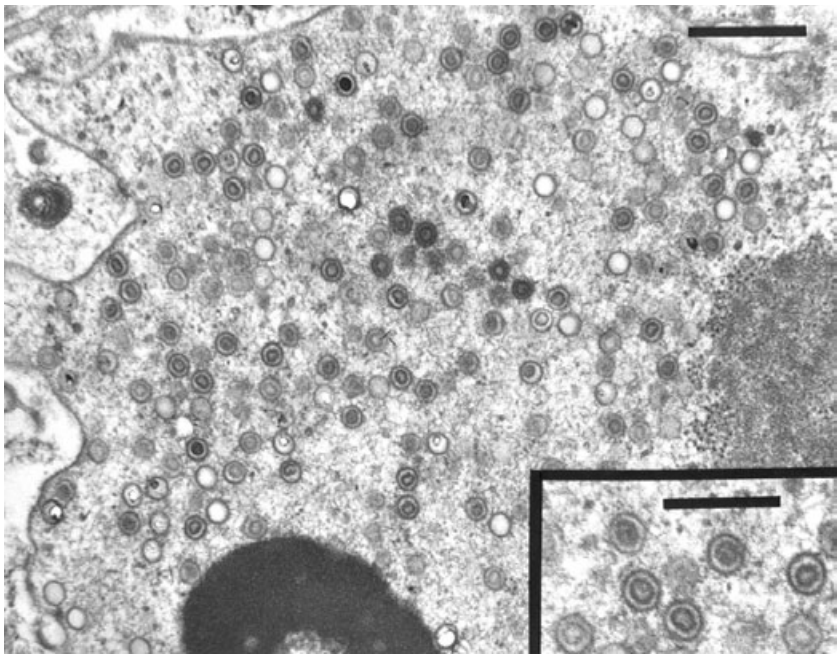

Fig. 3. Transmission electron microscopic view of herpesvirus-like virions in gingival epithelial cells of HIV-associated necrotizing ulcerative periodontitis. Bar = 0.5 μm; inset bar = 0.25 μm. Obtained from Cobb et al. (39) with the permission of the author.

activation (59), and the presence of particularly virulent periodontal bacteria (62). Maxillary osteonecrosis and severe periodontal destruction have also been described in middle-age American individuals who were systemically healthy but positive for the varicella-zoster virus (153, 185).

Periodontitis in HIV-infected patients may resemble that of periodontitis of non-HIV-infected individuals, or may be associated with profuse gingival bleeding or necrotic gingival tissue (100). HIV-induced immunosuppression is known to facilitate herpesvirus reactivation (64). Electron microscopic examination has revealed herpesvirus-like particles in 57% of biopsies from necrotic gingival papillae of HIV-associated periodontitis (39) (Fig. 3). Also, significantly more herpesvirus species have been detected in gingival specimens from HIV-periodontitis lesions than from periodontitis lesions of non-HIV patients (41). HCMV occurred in 81% of the HIV-associated periodontitis lesions and was the most common herpesvirus species identified (41). In HIV-positive individuals, HCMV has also been implicated in acute periodontitis (50), periodontal abscess formation and osteomyelitis (20), and refractory chronic sinusitis (258). EBV DNA has been detected in gingival papillae (137, 139), and EBV reactivation has been related to rapid gingival recession in HIV-infected patients (174). Contreras et al. (41) identified EBV-2 DNA in 57% of biopsies from HIV-periodontitis lesions, which agrees with previous findings of an unusually high incidence of EBV-2 in HIV-infected patients (213, 274). Moreover, Contreras et al. (41) found HHV-8 DNA, the Kaposi sarcoma virus, in periodontitis lesions of 24% of HIV-infected individuals having no clinical signs of Kaposi sarcoma, but not in periodontitis sites of non-HIV-infected individuals. Kaposi sarcoma lesions in gingiva have been linked to severe alveolar bone loss (39). Triantos et al. (257) have identified HHV-8 in the oral mucosa of HIV-infected and immunosuppressed oncologic patients from Greece. HHV-8 has tropism for and is able to infect and replicate *in vitro* in cultured oral epithelial cells (55). In HIV-infected patients, HCMV, EBV, HSV and HHV-8 DNA can be found in saliva (22, 68), and have been related to widespread gingival and mucosal inflammation (66) and oral ulcerative lesions (66, 110, 192, 249).

HCMV and EBV-1 are present in a variety of other types of severe periodontal disease, including Papillon–Lefèvre syndrome periodontitis (262), Fanconi's anemia periodontitis (167), and periodontal abscess formation (212). Down's syndrome patients demonstrate high prevalence of HCMV infection (49) and periodontitis lesions of these patients usually harbor several herpesviruses (85). In renal transplant patients, active HCMV replication has been detected in sites with gingival overgrowth and increased pocket depth (166). EBV has been identified in hyperplastic gingiva of cardiac transplantation patients with a history of cyclosporine use (170), and in odontogenic and nonodontogenic tumors (111). An acute HSV-1 infection can give rise to gingival recession, as observed in a 26-year-old male patient who suddenly developed severe gingival inflammation and vesicle formation and, within a few hours, experienced a marked destruction of the gingival

tissue (186). In patients with acute myeloid leukemia, HSV may be an important pathogen of oral mucosal ulcerations (214). Viruses other than herpesviruses can also reside in the human periodontium, but their relationship to destructive periodontal diseases remains unclear (18, 30, 65, 116, 140, 143, 144, 176, 199, 250). Hormia et al. (102) suggested that the periodontium serves as a reservoir for human papillomavirus. Viruses have also been related to periodontal disease in primates (236), cats (99, 136, 193), mice (220), and hamsters (71).

Herpesviruses may interfere with periodontal healing. In guided tissue regeneration, Smith MacDonald et al. (237) recorded an average gain in clinical attachment of 2.3 mm in four periodontal sites that revealed either HCMV or EBV DNA, compared with a mean clinical attachment gain of 5.0 mm in 16 virus-negative sites ($P = 0.004$). By infecting and altering the function of fibroblasts and other periodontal cells, herpesviruses may compromise the regenerative potential of the periodontal ligament. Undiagnosed herpesvirus infections in the human periodontium may help explain why barrier membrane-associated treatment is unsuccessful in some patients. Moreover, 11 of 15 (73%) HSV-1 seropositive patients, but only 7 of 15 (47%) matched controls experienced dry socket complications after tooth extraction (92). Tooth extraction in experimental rats can reactivate a latent HSV-1 infection, resulting in delayed healing of the extraction socket (90, 91). In order to mimic the human situation, studies on periodontal regeneration and healing may have to be performed in herpesvirus-infected animals.

Data are available on means of controlling periodontal herpesviruses. Saygun et al. (210–212) and Pacheco et al. (172) reported that antimicrobial periodontal therapy can greatly reduce the herpesviral load in the periodontium, probably because the persistence of periodontal herpesviruses depends on the presence of gingival inflammatory cells. HCMV infects periodontal monocytes/macrophages and T-cells, and EBV infects B-cells (45), and since inflammatory cells have a lifespan of up to a few months (177), an extended periodontal presence of herpesviruses may require repeated influx of infected cells or, possibly, a herpesvirus-mediated inhibition of apoptosis (279). The ability of thorough antimicrobial therapy to markedly reduce or eliminate periodontal herpesviruses may in part be responsible for a positive therapeutic outcome. However, the extent to which eradicating periodontal herpesviruses may translate into healing beyond that obtained by controlling the periodontopathic bacteria needs to be established. Moreover, Saygun et al. (209) and Idesawa et al. (108) showed that periodontal treatment and oral hygiene follow-up reduced periodontal as well as salivary HCMV and EBV counts, sometimes to undetectable levels, which may help control herpesviral transmission from individual to individual and associated oral and nonoral diseases.

Herpesviruses are also involved in the pathogenesis of periapical symptomatic lesions (201–204, 231, 232). Symptomatic periapical lesions exhibit a significantly higher frequency of HCMV and EBV active infections than asymptomatic lesions of similar radiographic size (202, 232). Although HCMV appears to be the more important endodontopathogenic herpesvirus, HCMV and EBV may often serve as copathogens in severe cases of periapical disease (231). It has been suggested adding HCMV and probably EBV to the list of putative pathogenic agents in symptomatic periapical pathosis (231).

Pathogenesis of herpesvirus-associated periodontal disease

It seems clear that periodontal tissue breakdown occurs more frequently and progresses more rapidly in herpesvirus-infected than in herpesvirus-free periodontal sites. Herpesviruses may cause periodontal pathosis as a direct result of virus infection and replication, or as a consequence of virally induced impairment of the periodontal immune defense, resulting in heightened virulence of resident bacterial pathogens (43). It is assumed that the ability of herpesviruses to express cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation from latency, and tissue tropism is of relevance for the development of periodontitis.

Herpesviruses may cause a direct cytopathic effect on fibroblasts, keratinocytes, endothelial cells, inflammatory cells, and possibly bone cells. Ongradi et al. (171) found that phagocytic and bactericidal capacities of periodontal neutrophils, cells of key importance in the periodontal defense (260), were significantly impaired in subjects who carried herpesviruses in oral lymphocytes and epithelial cells, as compared to virus-free persons. In addition, herpesvirus infection of fibroblasts and other key periodontal cells may hamper tissue turnover and repair following regenerative periodontal therapy (237). Also, herpesvirus infection and damage of periodontal pocket epithelium may contribute to gingival bleeding, as suggested by a high prevalence of HCMV

and EBV DNA in periodontal sites exhibiting bleeding upon probing (108, 114). However, herpesviruses can also occur with minimal gingival bleeding, as seen in localized aggressive periodontitis (255) and in some chronic periodontitis lesions (133).

Periodontal herpesvirus infections may lead to overgrowth of periodontopathic bacteria. In adult periodontitis, the presence of subgingival HCMV or EBV-1 DNA is related to an elevated occurrence of the periodontal pathogens *P. gingivalis*, *T. forsythia, D. pneumosintes*, *P. intermedia*, *P. nigrescens*, *C. rectus* and *T. denticola* (44, 210, 230, 234). Localized aggressive periodontitis lesions with active HCMV infection tend to yield elevated *A. actinomycetemcomitans* counts (255). Studies in Finland and Russia found positive associations between serum antibodies against HSV and serum antibodies against *P. gingivalis* and *A. actinomycetemcomitans* (263). Herpesviruses may perturb inflammatory cells involved in the periodontal defense, thereby predisposing to bacterial superinfection, or may affect the adhesion potential of periodontopathic bacteria, possibly in a species-specific manner. Teughels et al. (253) found that *A. actinomycetemcomitans* strains showed 70% (52–107%) higher and *P. gingivalis* strains 39% (36–42%) lower ability to adhere to and invade HCMV-infected HeLa epithelial cells compared to HeLa cells not infected by HCMV.

Proinflammatory cytokines play both beneficial and harmful roles in viral diseases. Herpesviruses can induce altered and, maybe, overzealous inflammatory mediator and cytokine responses in host cells attempting to counter the viral attack (156). HCMV infection can up-regulate IL-1β, TNF-α and other cytokine expression of monocytes and macrophages (156, 256, 269). Lipopolysaccharide from resident gram-negative bacteria can also induce cytokine responses in inflammatory cells and may act synergistically with HCMV in stimulating IL-1β gene transcription, resulting in markedly increased IL-1β levels at periodontal sites (269). Increased gingival concentration of proinflammatory cytokines has been associated with enhanced susceptibility to destructive periodontal disease (173). EBV may act as a potent polyclonal B-lymphocyte activator, capable of inducing proliferation and differentiation of immunoglobulin secreting cells, features associated with the progression of some types of periodontal disease (74).

Herpesviruses may produce tissue injury as a result of immunopathologic responses. HCMV can modulate antigen-specific T-lymphocyte functions, resulting in relative increases in $CD8^+$ suppressor cells, which in turn may lead to an impairment of cell-mediated immunity (215, 271). Consistent with immune responses of a herpesvirus infection, aggressive periodontitis has been related to low $CD4^+/CD8^+$ ratios (120, 142) and, within the $CD8^+$ lymphocytes, a shift towards cytolytic T (Tc) lymphocytes (184). The Tc effector cells, which execute their function by direct cytotoxicity or by releasing antiviral cytokines, comprise the first order response of the adaptive immune system in the recovery from primary viral infections. Depending on individual circumstances, the action of cytolytic effector functions can be beneficial, detrimental or neutral to host tissue.

Figure 4 proposes an infectious disease model for the development of periodontitis based on herpesvirus-bacteria–host interactive responses. Herpesvirus infection of periodontal sites may be important in a multistage pathogenesis by altering local host responses. Initially, bacterial infection of the gingiva causes inflammatory cells to enter gingival tissue, with periodontal macrophages and T-lymphocytes harboring latent HCMV and periodontal B-lymphocytes harboring latent EBV (45). IgA antibodies against HCMV, EBV, and HSV in gingival crevice fluid seem to originate mainly from local plasma cell synthesis rather than from passive transudation from serum, which is a further indication of a gingival herpesvirus presence (96–98). Reactivation of herpesviruses from

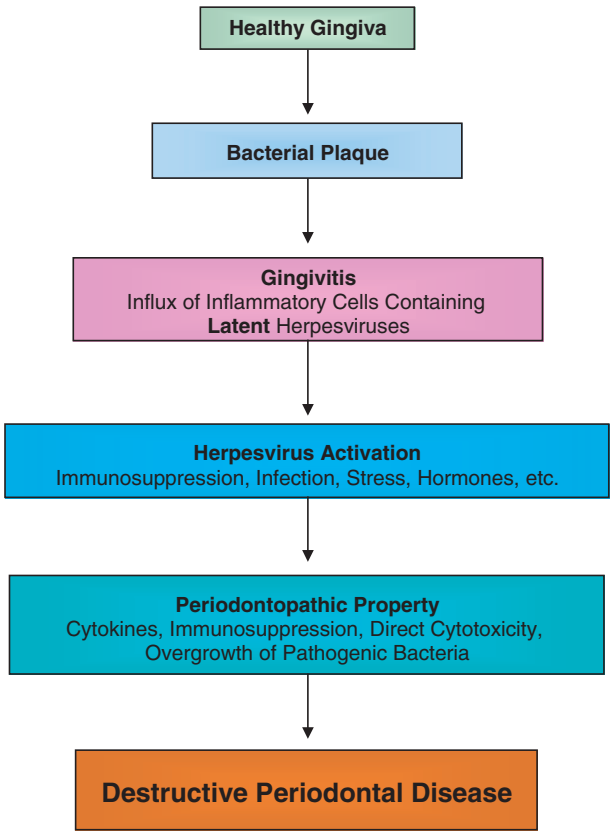

Fig. 4. Herpesviruses in destructive periodontal disease.

latency may occur spontaneously or during periods of impaired host defense, resulting from immunosuppression, infection, physical trauma, hormonal changes, etc. Herpesvirus-activating factors are also known risk factors/indicators for periodontal disease (168, 190). Herpesviral activation leads to increased inflammatory mediator responses in macrophages, and probably also in connective tissue cells within the periodontal lesion. After reaching a critical virus load, activated macrophages and lymphocytes may trigger a cytokine/chemokine 'storm' of IL-1β, TNF-α, IL-6, prostaglandins, interferons, and other multifunctional mediators, some of which have the potential to propagate bone resorption (118, 126). Several of the herpesvirus-associated cytokines and chemokines are prominent in periodontal lesions (80). Herpesvirus-induced immune impairment may also cause an upgrowth of resident gram-negative anaerobic bacteria (203, 224), whose lipopolysaccharide together with HCMV, as discussed above, can induce cytokine and chemokine release from various mammalian cells, and may act synergistically in stimulating IL-1β gene transcription (269). In a vicious circle, the triggering of cytokine responses may activate latent herpesviruses, and in so doing may further aggravate periodontal disease. Similarly, medical infections by HCMV can lead to increased susceptibility to bacterial and fungal infections and enhance the severity of existing microbial infections (24). It is conceivable that herpesviruses rely on coinfection with periodontal bacteria to produce periodontitis and, conversely, periodontopathic bacteria may depend on viral presence for the initiation and progression of some types of periodontitis.

A periodontal herpesvirus infection may partly explain the more rapidly advancing type of periodontitis that is detected in young than in adult individuals. Typically, the periodontal disease course in adolescents and young adults is aggressive with a relatively short period of tissue destruction (12). In adults, the disease course is more often slow and frequently associated with significant gingival inflammation and accumulations of plaque and calculus (12). These observations may suggest that aggressive periodontitis in young patients requires less infectious agent stimulus to trigger a progressive disease response than the more chronic type of adult periodontitis. However, progressive periodontitis may appear in HIV-infected adults (162, 244) and in aging individuals (134), probably because of suppressed cellular immunity by virtue of illness, therapies, or simply old age (58, 145).

It is common for primary and recurrent episodes of herpesvirus clinical infections to exhibit considerably different signs and symptoms. Pathosis occurring at primary infection tends to be severe in immunologically immature young people and in immunocompromised individuals, and mild-to-moderate in adults with preexisting herpesvirus immunity from past infection. For example, the varicella-zoster virus causes chickenpox during primary infection and shingles during an endogenous relapse of the primary varicella infection. Herpes simplex virus may cause acute gingivostomatitis during primary infection and epidermal or mucosal ulcers during viral recrudescence, and EBV and HCMV can give rise to mononucleosis during primary infection and a variety of relatively mild diseases during viral recrudescence (21). Similar to acute herpesvirus diseases, aggressive periodontitis may preferentially occur in immunologically immature young people, or in immunocompromised or aging individuals who are unable to mount an adequate host response against established herpesviruses and therefore will experience frequent or long-lasting herpesvirus reactivation (58). The subsequent latency period then represents the time required for the herpesviruses to overcome antiviral responses of the periodontium. In agreement with this hypothesis, virtually all established risk factors/indicators of periodontitis are immunosuppressive with potential to activate latent herpesviruses (168). If so, some types of aggressive and chronic periodontitis are basically not different diseases but merely a continuous spectrum of diseases, whose clinical expression depends on the presence of a periodontal herpesvirus infection and the specific immunity of the host, ranging from aggressive periodontitis in patients with inadequate immune response at one end, to chronic, nonprogressing periodontitis in patients who are immunocompetent at the other end, with intermediary clinical disease types between these two extremes of immune function.

Herpesvirus infections can cause both cytopathogenic and immunopathogenetic effects (227), and although the relative contribution of the two pathogenic mechanisms to destructive periodontal disease is unknown, it is likely that the early stages of periodontitis in immunologically naive hosts mainly comprise cytopathogenic events, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. Clustering of aggressive periodontitis in families (11) may arise from a transmission of herpesviruses among individuals in the same household rather than from a genetic predisposition, although the disease development may involve both pathogenetic components.

Periodontitis =

High herpesvirus load (inflammatory level) at periodontal sites +
Activation of herpesviruses in the periodontium +
Inadequate anti-viral T-cytotoxic cell response +
Presence of specific periodontal pathogenic bacteria +
Inadequate protective anti-bacterial antibody response +
A time period sufficiently long to produce tissue breakdown

Fig. 5. Pathogenic determinants of severe periodontitis.

One of the greatest challenges in confirming or refuting a role for herpesviruses in human periodontitis is their ubiquitous nature and the relatively rare occurrence of progressive periodontitis. This dilemma is apparent not only for periodontitis but also for the expanding spectrum of human diseases with which herpesviruses have been associated (224). It is likely that periodontal breakdown has a polymicrobial causation and depends upon the simultaneous occurrence of a number of infectious disease events, including at least (i) herpesvirus presence at periodontal sites, (ii) reactivation of latent periodontal herpesviruses, (iii) inadequate antiviral cytotoxic T-lymphocyte response, (iv) presence of specific pathogenic bacteria, and (v) insufficient level of protective antibacterial antibodies (Fig. 5). Reactivation of herpesviruses in periodontal sites may comprise a particularly important pathogenetic event (227). Presumably, the pathogenic determinants of periodontitis cooperate with each other in destructive constellations relatively infrequently and primarily during periods of impaired host defense. Also, the periodontal pathogenic determinants have to interact for a period of time that is sufficiently long to produce clinical breakdown.

Conclusion and perspectives

Even though bacteria are recognized to be indispensable for the development of periodontitis, and although current hypotheses on the etiopathogenesis of periodontitis correctly emphasize the importance of assessing bacterial and host factors collectively, bacterial–host interaction alone seems insufficient in explaining important clinical characteristics of the disease. It is not understood why periodontitis tends to progress in a localized pattern in many patients, the propensity to bilateral symmetry of tissue breakdown, and the intermittent exacerbation of the disease in individual teeth. It is particularly troubling that no detailed explanation exists as to the pathogenic events that trigger the conversion of a gingivitis lesion to periodontitis or a stable periodontitis site to a disease-active lesion. No unequivocal association has been established with cytokine polymorphisms or HLA haplotypes and periodontitis, although HLA-DR4 carriers may be at elevated risk for the disease (165). Variation in clinical manifestations of periodontal disease is almost certainly the result of differences in type and load of infectious agents and associated host responses. In that regard, the simultaneous occurrence of periodontal herpesvirus infection and progressive periodontitis is probably not a fortuitous event. Herpesvirus periodontal infections may cause direct damage to periodontal tissues, or impair the resistance of the periodontium, thereby permitting subgingival overgrowth of pathogenic bacteria (227).

Henle-Koch postulates of disease etiology address monocausal infectious diseases and are not readily applicable to multicausal infectious diseases such as periodontitis, which may result from a synergistic interaction among different pathogenic agents that individually may not lead to disease. The question of coincidence or a causal nexus between herpesviruses and periodontitis can be appraised on the basis of Hill's criteria of causality (94). The measures for strength of association, consistency, temporal sequence, biologic plausibility, and analogy seem to be met (94). Amongst the many arguments for a herpesvirus involvement in human periodontal disease are the following observations:

- PCR amplification of nucleic acid sequences of HCMV, EBV and other herpesviruses in severe periodontitis lesions of adolescents and adults has been robustly reported by independent laboratories in various countries.
- Herpesvirus-positive periodontitis lesions harbor increased levels of periodontopathic bacteria.
- There exists an apparent association between HCMV active infection and progressing periodontitis.
- An association between herpesviruses and acute necrotizing gingivitis has been demonstrated in malnourished children in Nigeria.
- Periodontal inflammatory cells contain nucleic acid sequences of herpesviruses.
- Herpesvirus infection of periodontal inflammatory cells has the potential to profoundly alter the host defense.
- Herpesviruses have the potential to increase the expression of tissue-damaging cytokines and chemokines in periodontal inflammatory and connective tissue cells.

Table 9 summarizes pathomorphologic characteristics of periodontitis that may be explained by a combined herpesvirus–bacteria etiological model, but

Table 9. The likelihood of herpesviruses and bacteria explaining the disease characteristics of periodontitis

Periodontitis features	Herpesviruses + Bacteria	Bacteria alone
Dental plaque amount and level of dental care not commensurate with disease severity. (36, 46)	Yes (herpesvirus active infection in the periodontium is not related to dental plaque amount). (255)	Yes (increased occurrence of specific species of bacterial pathogens in certain plaques). (226)
Localized and bilateral symmetry of tissue breakdown. (157)	Yes (herpesvirus infection exhibits tissue tropism, and tissue around similar teeth may show similar propensity to attract herpesviruses).	No.
Intermittent exacerbation of disease. (78, 239)	Yes (alterations between periods of herpesvirus latency and reactivation [240], which may correspond to disease stability and progression, respectively).	Maybe (temporary increase of periodontopathic bacteria due to nonherpesviral effects).
Cemental hypoplasias in teeth with aggressive juvenile periodontitis. (23)	Yes (active HCMV infection at the time of root development, which may cause alterations in the tooth surface). (224)	No.
Familial predisposition to disease. (17)	Yes (transmission of herpesviruses within a family). (4)	Yes (transmission of pathogenic bacteria within a family). (13)
Increased disease prevalence in lower socioeconomic groups. (56, 60)	Yes (higher rates of herpesvirus infection in individuals in lower socioeconomic groups). (32)	Maybe (individuals in lower socioeconomic groups may harbor increased levels of periodontopathic bacteria). (221)
Increased alveolar bone loss in institutionalized compared to noninstitutionalized mentally retarded individuals. (70)	Yes (high rate of herpesvirus transmission in institutionalized individuals). (216)	Unlikely (poor oral hygiene in institutionalized individuals). (129)
Occlusal trauma as a risk indicator of disease. (84)	Yes (trauma may induce herpesvirus reactivation).	Unlikely (slightly increased occurrence of periodontopathic bacteria with increased mobility). (79)
Immunodeficiency predisposes to increased incidence/prevalence of disease. (181)	Yes (immunosuppression is an important event in herpesvirus reactivation). (240)	Unlikely (some pathogenic bacteria possess immunosuppressive properties). (217, 218)
Old age as a risk indicator of disease. (134, 244)	Yes (reduced immune capacity [8] and increased herpesvirus occurrence with increasing age). (4)	Maybe (increased acquisition of pathogenic bacteria over time). (228)
HIV-infection as a risk indicator of disease. (244)	Yes (most HIV-infected patients harbor several periodontal herpesviruses that have the potential to reactivate frequently due to the immunosuppression). (41)	Unlikely (HIV and non-HIV patients harbor similar periodontopathic microbiota). (187)
Psychosocial stress as a risk indicator of disease. (131, 244)	Yes (stress can induce herpesvirus reactivation). (119, 246)	Maybe (host-derived nutrients in gingival crevice fluid of stressed individuals may stimulate[or inhibit] the growth of selected bacterial species). (135, 197)
Hormonal influences on periodontal disease. (146)	Yes (hormones and progesterone may increase the susceptibility to herpesvirus infections). (117)	Maybe (sex hormones may serve as growth factors for some periodontopathic bacteria). (122)

Table 9. Continued

Periodontitis features	Herpesviruses + Bacteria	Bacteria alone
Cigarette smoking as a risk indicator of disease. (106, 196)	Yes (tobacco products can interact with and possibly reactivate periodontal herpesviruses [175] or act synergistically with HCMV to enhance the sensitivity of peripheral blood lymphocytes to genetic damage). (5)	Unlikely (some anaerobic periodontal bacteria may occur at increased levels in smokers). (83)
Disease progression in the presence of elevated antibacterial antibodies. (57)	Yes (herpesvirus active infection is not controlled by antibacterial antibodies).	Possibly (if antibodies are directed against noncritical antigens, or against nonaccessible bacteria in biofilms, or are part of immunopathologic mechanisms of tissue destruction).
Predominance of T-lymphocytes in relatively stable and B-lymphocytes in progressive periodontitis lesions. (75)	Yes (HCMV and HSV reside in T-lymphocytes and EBV resides in B-lymphocytes). (45)	Unlikely, if not an immunopathologic mechanism of tissue breakdown is postulated. (276)
Defective neutrophil functions associated with aggressive disease. (47)	Yes (herpesviruses may infect and perturb neutrophils). (1, 76, 171)	Unlikely (some bacterial species may perturb neutrophils). (259)
Occurrence of $CD8^+$ and Th1-type lymphocytes in periodontitis. (251)	Yes (herpesvirus active infection leads to increased level of cytotoxic $CD8^+$ cells). (3, 74)	Unlikely (a few bacterial species may stimulate T-suppressor cells). (218)
Possible relationship between periodontal disease and major medical disorders (coronary heart disease, cerebrovascular disease, low birth weight infants). (272)	Yes (herpesviruses may induce both periodontitis and medical disorders; if so, periodontitis and medical disorders may not exhibit a direct causal relationship). (223)	Still to be resolved. (229)

probably not by a model based solely on a bacterial causation of the disease. Prolonged periods of latency interspersed with periods of activation of herpesvirus infections may in part be responsible for the burst-like episodes of periodontitis disease progression. Tissue tropism of herpesvirus infections may help explain the localized pattern of tissue destruction in most types of periodontitis. Frequent reactivation of periodontal herpesviruses may account for the rapid periodontal breakdown in some patients showing little dental plaque. The absence of a herpesvirus infection or of viral reactivation may explain why some individuals carry periodontopathic bacteria while still maintaining periodontal health.

The apparent importance of herpesviruses in periodontal disease may have practical consequences in addition to theoretical interest. As discussed above, effective treatment of gingival inflammation can reduce gingival (172, 212) and salivary (108, 209) herpesvirus loads, and may help diminish the risk of transmitting herpesviruses to other individuals. On the other hand, antiviral chemotherapeutics have a limited, short-term effect on oropharyngeal herpesvirus shedding and are probably ineffective in treating periodontitis. Vaccination is on the horizon as a means of preventing colonization or reactivation of human herpesviruses (10, 180). The impact of antiherpesvirus vaccines on destructive periodontal disease constitutes a future research topic of great interest. As new antiherpesvirus interventions become available, dental professionals may be able to significantly enhance the outcome of periodontal prevention and therapy. Also, although of limited usefulness in the routine diagnosis of uncomplicated periodontal disease, tests to monitor the state and level of viral replication may serve a valuable diagnostic purpose in severe periodontal infections in immunocompromised patients.

In summary, destructive periodontal disease is a heterogeneous group of pathoses characterized by a predominance of specific infectious agents in the face of inadequate local host defenses. Predisposing factors of periodontal tissue destruction are becoming better understood, but the magnitude of the effects of the most commonly reported risk factors

has not been adequately quantified in population-based studies. Resolving the many questions about the etiopathogenesis of periodontal diseases may require a readiness to give up bacteria as a single-cause of periodontitis development. The frequent occurrence of herpesviruses in various types of severe periodontal disease makes the participation of herpesvirus species in the etiology of periodontitis a distinct possibility. It is theorized that herpesvirus-associated periodontitis has its most severe course during the time of inadequate antiherpesvirus immunity at the initial disease phase, and then tapers off after the establishment of effective herpesvirus-specific cellular immune responses. The sooner the host develops adequate immunity against periodontal herpesviruses, the more localized the periodontal destruction may become. Periodontal disease relapses may preferentially occur in individuals with diminishing antiherpesvirus immunity. Synergistic interactions between periodontal herpesviruses and bacteria may enhance the risk of tissue breakdown. Mammalian viruses other than herpesviruses may also be involved in destructive periodontal disease. Recognizing a pathophysiologic relationship between mammalian viruses and periodontal disease has the potential to extend our insight into mechanisms of periodontal tissue breakdown and bridge the knowledge gap, on the molecular level, between gingivitis and periodontitis and between stable and progressive periodontitis. As we move into an era of thinking of a network of causation in periodontitis, the need is growing for well-designed studies to delineate the relative importance of the various types of infectious agents, the multiple and complex pathogenic pathways, and the genetic and environmental factors contributing to the disease. Based on current information, it seems reasonable to add human periodontitis to the list of infectious diseases that have HCMV, EBV, and maybe other viruses as probable contributory causes.

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Systemic Diseases Caused by Oral Infection

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INTRODUCTION

The theory of focal infection, which was promulgated during the 19th and early 20th centuries, stated that "foci" of sepsis were responsible for the initiation and progression of a variety of inflammatory diseases such as arthritis, peptic ulcers, and appendicitis (120). In the oral cavity, therapeutic edentulation was common as a result of the popularity of the focal infection theory. Since many teeth were extracted without evidence of infection, thereby providing no relief of symptoms, the theory was discredited and largely ignored for many years. Recent progress in classification and identification of oral microorganisms and the realization that certain microorganisms are normally found only in the oral cavity have opened the way for a more realistic assessment of the importance of oral focal infection. It has become increasingly clear that the oral cavity can act as the site of origin for dissemination of pathogenic organisms to distant body sites, especially in immunocompromised hosts such as patients suffering from malignancies, diabetes, or rheumatoid arthritis or having corticosteroid or other immunosuppressive treatment. A number of epidemiological studies have suggested that oral infection, especially marginal and apical periodontitis, may be a risk factor for systemic diseases.

The teeth are the only nonshedding surfaces in the body, and bacterial levels can reach more than 10^{11} microorganisms per mg of dental plaque. Human endodontal and periodontal infections are associated with complex microfloras in which approximately 200 species (in apical periodontitis) (140) and more than 500 species (in marginal periodontitis) (97) have been encountered. These infections are predominantly anaerobic, with gram-negative rods being the most common isolates. The anatomic closeness of these microfloras to the bloodstream can facilitate bacteremia and systemic spread of bacterial products, components, and immunocomplexes.

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BACTEREMIA

The incidence of bacteremia following dental procedures such as tooth extraction, endodontic treatment, periodontal surgery, and root scaling has been well documented (4, 12, 25, 29, 33, 53, 75, 83, 100, 108). Bacteremia after dental extraction, third-molar surgery, dental scaling, endodontic treatment, and bilateral tonsillectomy has been studied by means of lysis-filtration of blood samples with subsequent aerobic and anaerobic incubation (53). Bacteremia was observed in 100% of the patients after dental extraction, in 70% after dental scaling, in 55% after third-molar surgery, in 20% after endodontic treatment, and in 55% after bilateral tonsillectomy. Anaerobes were isolated more frequently than facultative anaerobic bacteria. Another study (117) involving 735 children undergoing treatment for extensive dental decay found that 9% of the children had detectable bacteremias before the start of dental treatment. In addition, a variety of hygiene and conservative procedures, including brushing of the teeth, increased the prevalence of bacteremias from 17 to 40%. Anesthetic and surgical procedures increased the occurrence of bacteremias from 15 to 97%. One recent study by Debelian et al. (26) used phenotypic and genetic methods to trace microorganisms released into the bloodstream during and after endodontic treatment back to their presumed source, the root canal. Microbiological samples were taken from the root canals of 26 patients with asymptomatic apical periodontitis of single-rooted teeth. Blood was drawn from the patients during and 10 min after

endodontic therapy. All root canals contained anaerobic bacteria. In group I, where the first three root canal reamers were used to a level 2 mm beyond the apical foramen of the tooth, *Propionibacterium acnes*, *Peptostreptococcus prevotii*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Saccharomyces cerevisiae* were recovered from the blood. In group 2, where instrumentation ended inside the root canal, *P. intermedia*, *Actinomyces israelii*, *Streptococcus intermedius*, and *Streptococcus sanguis* were isolated from the blood.

As stated above, dissemination of oral microorganisms into the bloodstream is common, and less than 1 min after an oral procedure, organisms from the infected site may have reached the heart, lungs, and peripheral blood capillary system (65).

There are more than 10^{13} microbes on all surfaces of the body, yet the underlying tissues and the bloodstream are usually sterile. In the oral cavity there are several barriers to bacterial penetration from dental plaque into the tissue: a physical barrier composed of the surface epithelium; defensins, which are host-derived peptide antibiotics, in the oral mucosal epithelium; an electrical barrier that reflects the E_h difference between the host cell and the microbial layer; an immunological barrier of antibody-forming cells; and the reticuloendothelial system (phagocyte barrier) (78, 81, 147). Under normal circumstances, these barrier systems work together to inhibit and eliminate penetrating bacteria. When this state of equilibrium is disturbed by an overt breach in the physical system (e.g., trauma), the electrical system (i.e., hypoxia), or immunological barriers (e.g., through neutropenia, AIDS, or immunosuppressant therapy), organisms can propagate and cause both acute and chronic infections with increased frequency and severity (79). With normal oral health and dental care, only small numbers of mostly facultative bacterial species gain access to the bloodstream. However, with poor oral hygiene, the numbers of bacteria colonizing the teeth, especially supragingivally, could increase 2- to 10-fold (80) and thus possibly introduce more bacteria into tissue and the bloodstream, leading to an increase in the prevalence and magnitude of bacteremia.

The purpose of this review is to evaluate the current status of oral infections, especially periodontitis, as a causal factor for systemic diseases.

PATHWAYS LINKING ORAL INFECTION TO SECONDARY NONORAL DISEASE

Three mechanisms or pathways linking oral infections to secondary systemic effects have been proposed (136). These are metastatic spread of infection from the oral cavity as a result of transient bacteremia, metastatic injury from the effects of circulating oral microbial toxins, and metastatic inflammation caused by immunological injury induced by oral microorganisms.

Metastatic infection. As previously discussed, oral infections and dental procedures can cause transient bacteremia. The microorganisms that gain entrance to the blood and circulate throughout the body are usually eliminated by the reticuloendothelial system within minutes (transient bacteremia) and as a rule lead to no other clinical symptoms than possibly a slight increase in body temperature (65, 136). However, if the disseminated microorganisms find favorable conditions, they may settle at a given site and, after a certain time lag, start to multiply.

Metastatic injury. Some gram-positive and gram-negative bacteria have the ability to produce diffusible proteins, or exotoxins, which include cytolytic enzymes and dimeric toxins with A and B subunits. The exotoxins have specific pharmacological actions and are considered the most powerful and lethal poisons known (51). Conversely, endotoxins are part of the outer membranes released after cell death (51, 93). Endotoxin is compositionally a lipopolysaccharide (LPS) that, when introduced into the host, gives rise to a large number of pathological manifestations. LPS is continuously shed from periodontal gram-negative rods during their growth in vivo (93).

Metastatic inflammation. Soluble antigen may enter the bloodstream, react with circulating specific antibody, and form a macromolecular complex. These immunocomplexes may give rise to a variety of acute and chronic inflammatory reactions at the sites of deposition (136, 145).

Possible pathways of oral infections and nonoral diseases are listed in Table 1.

TABLE 1. Possible pathways of oral infections and nonoral diseases[a]

Pathway for oral infection	Possible nonoral diseases
Metastatic infection from oral cavity via transient bacteremia	Subacute infective endocarditis, acute bacterial myocarditis, brain abscess, cavernous sinus thrombosis, sinusitis, lung abscess/infection, Ludwig's angina, orbital cellulitis, skin ulcer, osteomyelitis, prosthetic joint infection
Metastatic injury from circulation of oral microbial toxins	Cerebral infarction, acute myocardial infarction, abnormal pregnancy outcome, persistent pyrexia, idiopathic trigeminal neuralgia, toxic shock syndrome, systemic granulocytic cell defects, chronic meningitis
Metastatic inflammation caused by immunological injury from oral organisms	Behçet's syndrome, chronic urticaria, uveitis, inflammatory bowel disease, Crohn's disease

[a] Compiled from references 109 and 115.

PERIODONTAL DISEASE AFFECTS SUSCEPTIBILITY TO SYSTEMIC DISEASE

Most studies concerning the relationship between oral infection and systemic diseases are related to periodontal disease, by far the most common oral infection. The term periodontal disease is used to describe a group of conditions that cause inflammation and destruction of the attachment apparatus of the teeth (i.e., gingiva, periodontal ligament, root cementum, and alveolar bone). Periodontal disease is caused by bacteria found in dental plaque, and about 10 species have been identified as putative pathogens in periodontal disease, mainly gram-negative rods. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Bacteroides forsythus* are the gram-negative bacteria most commonly associated with periodontitis (3, 50, 131). Periodontitis lesions exhibit gingival inflammation as well as destruction of the periodontal ligament and alveolar bone. This leads to bone loss and apical migration of the junctional epithelium, resulting in the formation of periodontal pockets.

In a recent review article (111), Page proposed that periodontitis may affect the host's susceptibility to systemic disease in three ways: by shared risk factors, by subgingival biofilms acting as reservoirs of gram-negative bacteria, and through the periodontium acting as a reservoir of inflammatory mediators.

Shared risk factors. Factors that place individuals at high risk for periodontitis may also place them at high risk for systemic diseases such as cardiovascular disease. Among the environmental risk factors and indicators shared by periodon-

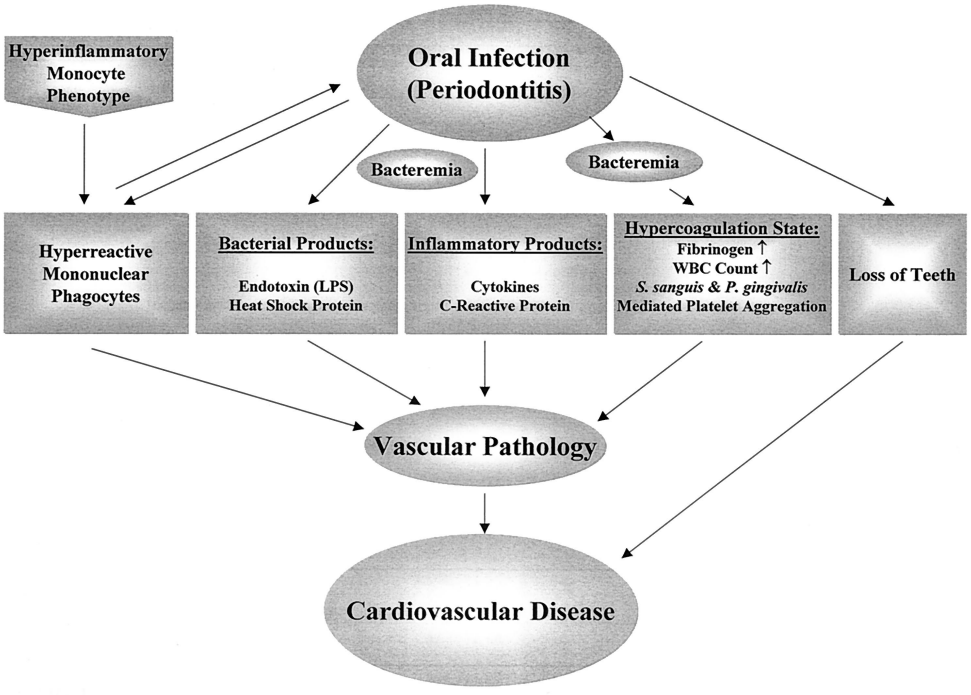

FIG. 1. Proposed mechanisms linking oral infection and periodontal disease to cardiovascular disease.

titis and systemic diseases, such as cardiovascular disease, are tobacco smoking, stress, aging, race or ethnicity, and male gender (111). Studies demonstrating genetic factors shared by periodontitis, cardiovascular disease, preterm labor, and osteoporosis have not yet been performed but may be fruitful (111).

Subgingival biofilms. Subgingival biofilms constitute an enormous and continuing bacterial load. They present continually renewing reservoirs of LPS and other gram-negative bacteria with ready access to the periodontal tissues and the circulation. Systemic challenge with gram-negative bacteria or LPS induces major vascular responses, including an inflammatory cell infiltrate in the vessel walls, vascular smooth muscle proliferation, vascular fatty degeneration, and intravascular coagulation (86, 87). LPS upregulates expression of endothelial cell adhesion molecules and secretion of interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and thromboxane, which results in platelet aggregation and adhesion, formation of lipid-laden foam cells, and deposits of cholesterol and cholesterol esters.

Periodontium as cytokine reservoir. The proinflammatory cytokines TNF-α, IL-1β, and gamma interferon as well as prostaglandin E_2 (PGE_2) reach high tissue concentrations in periodontitis (111). The periodontium can therefore serve as a renewing reservoir for spillover of these mediators, which can enter the circulation and induce and perpetuate systemic effects. IL-1β favors coagulation and thrombosis and retards fibrinolysis (19). IL-1, TNF-α, and thromboxane can cause platelet aggregation and adhesion, formation of lipid-laden foam cells, and deposition of cholesterol. These same mediators emanating from the diseased periodontium may also account for preterm labor and low-birth-weight infants (111).

SYSTEMIC DISEASES ASSOCIATED WITH ORAL INFECTION

Cardiovascular Disease

Cardiovascular diseases such as atherosclerosis and myocardial infarction occur as a result of a complex set of genetic and environmental factors (56). The genetic factors include age, lipid metabolism, obesity, hypertension, diabetes, increased fibrinogen levels, and platelet-specific antigen Zwb ($P1^{A2}$) polymorphism. Environmental risk factors include socioeconomic status, exercise stress, diet, nonsteroidal anti-inflammatory drugs, smoking, and chronic infection. The classical risk factors of cardiovascular disease such as hypertension, hypercholesterolemia, and cigarette smoking can only account for one-half to two-thirds of the variation in the incidence of cardiovascular disease (120).

Among other possible risk factors, evidence linking chronic infection and inflammation to cardiovascular disease has been accumulating (116, 134, 141). It is clear that periodontal disease is capable of predisposing individuals to cardiovascular disease, given the abundance of gram-negative species involved, the readily detectable levels of proinflammatory cytokines, the heavy immune and inflammatory infiltrates involved, the association of high peripheral fibrinogen, and the white blood cell (WBC) counts (66).

There are several proposed mechanisms (Fig. 1) by which periodontal disease may trigger pathways leading to cardiovascular disease through direct and indirect effects of oral bacteria. First, evidence indicates that oral bacteria such as *Streptococcus sanguis* and *Porphyromonas gingivalis* induce platelet aggregation, which leads to thrombus formation (55). These organisms have a collagen-like molecule, the platelet aggregation-associated protein, on their surface (57). When *S. sanguis* is injected intravenously into rabbits, a heart attack-like series

of events occur. Possibly, antibodies reactive to periodontal organisms localize in the heart and trigger complement activation, a series of events leading to sensitized T cells and heart disease (55). Furthermore, one or more periodontal pathogens have been found in 42% of the atheromas studied in patients with severe periodontal disease (V. I. Haraszthy, J. J. Zambon, M. Trevisan, R. Shah, M. Zeid, and R. J. Genco, J. Dent. Res. Spec. Iss. 77, p. 666, abstr. 273, 1998). In one recent study, Deshpande et al. (27) showed that *P. gingivalis* can actively adhere to and invade fetal bovine heart endothelial cells, bovine aortic endothelial cells, and human umbilical vein endothelial cells. Invasion efficiencies of 0.1, 0.2, and 0.3% were obtained with bovine aortic endothelial cells, human umbilical vein endothelial cells, and fetal bovine heart endothelial cells, respectively. Potempa et al. (J. Potempa, T. Imamura, and J. Travis, J. Dent. Res. Spec. Iss. 78, p. 180, abstr. 593, 1999) studied proteolytic enzymes referred to as gingipains R, which are released in large quantities from *P. gingivalis*. After entering the circulation, gingipains R can activate factor X, prothrombin, and protein C, promoting a thrombotic tendency through the ultimate release of thrombin, subsequent platelet aggregation, conversion of fibrinogen to fibrin, and intravascular clot formation.

The second factor in this process could be an exaggerated host response to a given microbial or LPS challenge, as reflected in the release of high levels of proinflammatory mediators such as PGE_2, TNF-α, and IL-1β (54, 106). These mediators have been related to interindividual differences in the T-cell repertoire and the secretory capacity of monocytic cells. Typically, peripheral blood monocytes from these individuals with the hyperinflammatory monocyte phenotype secrete 3- to 10-fold-greater amounts of these mediators in response to LPS than those from normal monocyte phenotype individuals (54, 106). Several investigators have suggested that genes that regulate the T-cell monocyte response and the host-microbe environment can directly trigger and modulate the inflammatory response. Patients with certain forms of periodontal disease, such as early-onset periodontitis and refractory periodontitis, possess a hyperinflammatory monocyte phenotype (54, 106, 125).

A third mechanism possibly involves the relationship between bacterial and inflammatory products of periodontitis and cardiovascular disease. LPS from periodontal organisms being transferred to the serum as a result of bacteremias or bacterial invasion may have a direct effect on endothelia so that atherosclerosis is promoted (113). LPS may also elicit recruitment of inflammatory cells into major blood vessels and stimulate proliferation of vascular smooth muscle, vascular fatty degeneration, intravascular coagulation, and blood platelet function. These changes are the result of the action of various biologic mediators, such as PGs, ILs, and TNF-α on vascular endothelium and smooth muscle (5, 137). Fibrinogen and WBC count increases noted in periodontitis patients may be a secondary effect of the above mechanisms or a constitutive feature of those at risk for both cardiovascular disease and periodontitis (71).

Periodontitis as an infection may stimulate the liver to produce C-reactive protein (CRP) (a marker of inflammation), which in turn will form deposits on injured blood vessels. CRP binds to cells that are damaged and fixes complement, which activates phagocytes, including neutrophils. These cells release nitric oxide, thereby contributing to atheroma formation (40; S. Al-Mubarak, S. G. Ciancio, A. Al-Suwyed, W. Hamouda, and P. Dandona, J. Dent. Res. Spec. Iss. 77, p. 1030, abstr. 3192, 1998). In a study of 1,043 apparently healthy men, baseline plasma concentrations of CRP predicted the risk of future myocardial infarction and stroke (116). Ebersole et al. (35) found that patients with adult periodontitis have higher levels of CRP and haptoglobin than subjects without periodontitis. Both CRP and haptoglobin levels decline significantly after periodontal therapy. Loos et al. (B. G. Loos, J. Hutter, A. Varoufaki, H. Bulthus, J. Craandijk, R. A. M. Huffels, F. J. Hoek, and U. Van Der Velden, J. Dent. Res. Spec. Iss. 77, p. 666, abstr. 274, 1998) described 153 systemically healthy subjects consisting of 108 untreated periodontitis patients and 45 control subjects. Mean plasma CRP levels were higher in the periodontitis patients. Patients with severe periodontitis had significantly higher CRP levels than mild-periodontitis patients, and both had significantly higher levels than the controls. Another recent study (41) evaluated the relationship of cardiovascular disease and CRP. Groups of adults who had neither periodontal nor cardiovascular disease, one of these diseases, or both of them were assembled. In those with both heart disease and periodontal disease, the mean level of CRP (8.7 g/ml) was significantly different from that (1.14 g/ml) in controls with neither disease. The authors also showed that treatment of the periodontal disease caused a 65% reduction in the level of CRP at 3 months. The level remained reduced for 6 months.

Recently, a specific heat shock protein, Hsp65, has been reported to link cardiovascular risks and host responses (67, 150–153). Heat shock proteins are important for the maintenance of normal cellular function and may have additional roles as virulence factors for many bacterial species (154). In animal studies, Xu et al. (153) demonstrated that immunization of rabbits with bacterial Hsp65 induces atherosclerotic lesions. A subsequent large-scale clinical study found a significant association between serum antibody levels to Hsp65 and the presence of cardiovascular disease (152). Their theory, consistent with their clinical findings, is that bacterial infection stimulates the host response to Hsp65, which is a major immunodominant antigen of many bacterial species. The interaction between expressed Hsp65 and the immune response induced by bacterial infection is hypothesized to be responsible for the initiation of the early atherosclerotic lesion (153). It has been suggested that chronic oral infection stimulates high levels of Hsp65 in subjects with high cardiovascular risk (81). Thus, if antibodies directed towards bacterial heat shock proteins cross-react with heat shock proteins expressed in the host tissue, especially if they are found in the lining of blood vessels, then some oral species might well be the link between oral infection and cardiovascular disease (81).

Finally, oral infection can also cause tooth loss. Evidence has shown that edentulous persons with and without dentures and dentate individuals with missing teeth change their eating habits (13, 14, 101, 143, 146). They may thereby avoid certain nutritious foods because of difficulty in chewing and select high-calorie, high-fat food. When the foods cannot be well pulverized, this has an adverse effect on the internal absorption of nutrients. Such dietary preferences would predispose such individuals to the type of high-fat foods that are recognized as risk factors for cardiovascular disease (148). In dentate individuals with many missing teeth, the diet-induced elevation of serum low-density lipoprotein has been shown to upregulate monocytic responses to LPS (96). In these subjects, one would have both the diet-induced sensitization of monocytes and the plaque-laden teeth that could provide the LPS challenge to these cells. Instead of having hyperresponsive monocytes reacting to any LPS introduced from the plaque, there would be elevated secretion of inflammatory cytokines by monocytes stimulated by elevated low-density lipoprotein levels. This interaction between LPS and monocytes may explain the severity

TABLE 2. Adjusted odds ratios for cardiovascular disease for patients with periodontal disease

Study	Yr	Odds ratio	
		Total cardiovascular disease	Fatal cardiovascular disease/stroke
DeStefano et al. (28)	1993	1.29	1.46
Beck et al. (5)	1996	1.5	1.9/2.8
Joshipura et al. (63)	1996	1.67	
Grau et al. (45)	1997		2.6

of gram-negative infections in certain diabetic patients (96), but it could also be operating in individuals who change to a high-fat diet because of missing teeth. Thus, all the mechanisms by which poor oral hygiene and periodontal disease may contribute to cardiovascular disease described above could also come into play as a result of certain dietary changes secondary to missing teeth (81).

Adjusted odds ratios for cardiovascular disease, fatal cardiovascular disease, and stroke for patients with periodontal disease are given in Table 2.

Coronary heart disease: atherosclerosis and myocardial infarction. Atherosclerosis has been defined as a progressive disease process that involves the large- to medium-sized muscular and large elastic arteries. The advanced lesion is the atheroma, which consists of elevated focal intimal plaques with a necrotic central core containing lysed cells, cholesterol ester crystals, lipid-laden foam cells, and surface plasma proteins, including fibrin and fibrinogen (9). The presence of atheroma tends to make the patient thrombosis prone because the associated surface enhances platelet aggregation and thrombus formation that can occlude the artery or be released to cause thrombosis, coronary heart disease, and stroke. Overall, about 50% of deaths in the United States are attributed to the complications of atherosclerosis and resulting cardiovascular diseases (5). A recent preliminary report indicates that atherosclerotic plaques are commonly infected with gram-negative periodontal pathogens, including *A. actinomycetemcomitans* and *P. gingivalis* (J. J. Zambon, V. I. Haraszthy, S. G. Grossi, and R. J. Genco, J. Dent. Res. Spec. Iss. 76, p. 408, abstr. 3159, 1997).

A myocardial infarction is the damaging or death of an area of the heart muscle resulting from a reduced blood supply to that area. Myocardial infarction is almost always due to the formation of an occlusive thrombus at the site of rupture of an atheromatous plaque in a coronary artery (9).

The associations between oral conditions and atherosclerosis and coronary heart disease are listed in Table 3.

Stroke. Stroke is a cerebrovascular disease that affects blood vessels supplying blood to the brain. It occurs when a blood vessel bringing oxygen and nutrients to the brain bursts or is clogged by local thrombus formation or by aggregates of bacteria and fibrin from other sources such as the heart. In an average population, the annual incidence of new strokes is 2 per 1,000 (21). Studies on the pathology of stroke indicate that 80 to 85% of these lesions are due to cerebral infarction; 15 to 20% are caused by hemorrhage (21).

The inflamed periodontium releases inflammatory cytokines, LPS, and bacteria into the systemic circulation, and they may promote atherosclerosis and affect blood coagulation, the function of platelets, and PG synthesis, thereby contributing to the onset of stroke. In a case-control study (135), 40 patients under the age of 50 with cerebral infarction and 40 randomly selected community controls matched for sex and age were compared for dental status. Poor oral health, as assessed by total dental index and orthopantomography, was more common in the patients with cerebral infarction than in individuals of the control group.

Another cross-sectional study of 401 veterans showed that several dental and oral conditions were significantly associated with the diagnosis of a cerebral vascular accident when included in a multivariate logistic regression model with and without many of the known risk factors for cerebral vascular accident (81).

Infective Endocarditis

Infective endocarditis is a bacterial infection of the heart valves or the endothelium of the heart. It occurs when bacteria in the bloodstream lodge on abnormal heart valves or damaged heart tissue. Endocarditis occurs rarely in people with normal hearts. However, people who have certain preexisting heart defects are at risk for developing endocarditis when a bacteremia occurs (9).

Infective endocarditis is a serious and often fatal systemic disease that has been associated with dental diseases and treatment. There are over 1,000 case reports associating dental procedures or disease with the onset of endocarditis (32). Three controlled studies have recently been conducted, all showing an association of dental procedures with bacterial endocarditis (31, 72, 144). In addition, multiple animal models (rats, rabbits, and pigs) have shown that oral bacteria and even dental extraction can create histologic evidence of endocarditis under experimental conditions (34, 110). It appears that dental procedures, especially extractions and possibly scaling, meet currently accepted epidemiological criteria for causation of endocarditis (58, 119). No other systemic diseases or condi-

TABLE 3. Associations between oral conditions and atherosclerosis/coronary heart disease[a]

Study	Yr	Design	Association	Adjusted measure
Mattila et al. (88)	1989	Case-control	Total dental index and heart attack	OR = 1.3
Mattila et al. (89)	1993	Case-control	Total dental index and atheromatosis	OR = 1.4
DeStefano et al. (28)	1993	Cohort	PI, OHI, and admissions	RR = 1.2
			Death due to CHD	RR = 1.7
Mattila et al. (90)	1995	Follow-up	Total dental index and new events	HR = 1.2
Beck et al. (5)	1996	Cohort	PD, bone levels, and new CHD	OR = 1.5
			Fatal CHD	OR = 1.9
			Stroke	OR = 2.8
Joshipura et al. (63)	1996	Cohort	Tooth loss and CHD	RR = 1.7
Genco et al.[b]	1997	Cohort	Bone loss and new CHD	OR = 2.7

[a] Abbreviations: CHD, coronary heart disease; PI, plaque index; PD, probing depth; OR, odds ratio; HR, hazard ratio; RR, relative risk.
[b] R. Genco, S. Chadda, S. Grossi, R. Dunford, G. Taylor, W. Knowler, and D. Pettitt, J. Dent. Res. Spec. Iss. 76, p. 408, abstr. 3158, 1997.

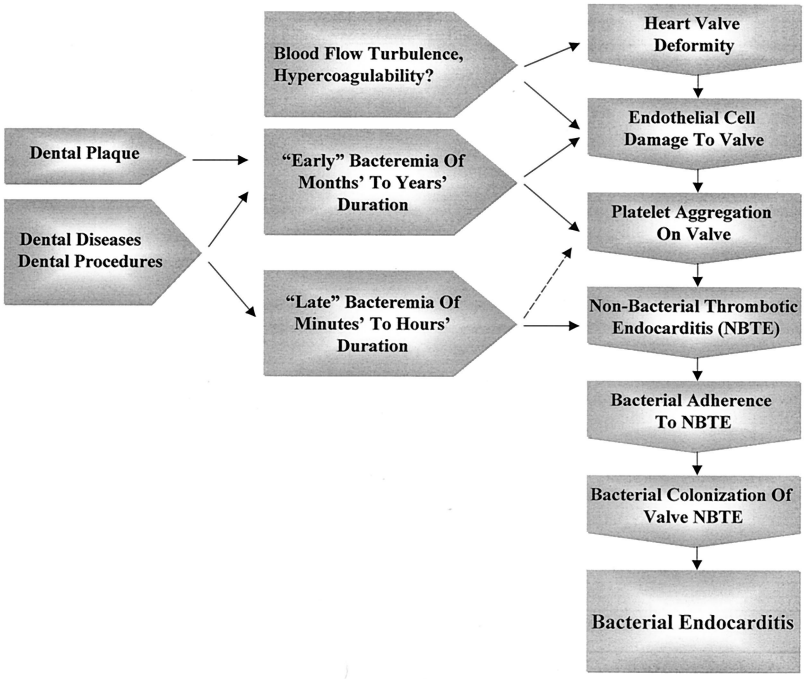

FIG. 2. Proposed causal model of dentally associated endocarditis (adapted from ref. 32 with permission of the publisher).

tions have been studied so extensively, although several other disorders may be linked to dental diseases.

Drangsholt (32) searched the world biomedical literature from 1930 to 1996 and concluded that the incidence of infective endocarditis varies between 0.70 and 6.8 per 100,000 person-years; over 50% of all infective endocarditis cases are not associated with either an obvious procedural or infectious event 3 months prior to developing symptoms; 8% of all infective endocarditis cases are associated with periodontal or dental disease without a dental procedure; the risk of infective endocarditis after a dental procedure is probably in the range of 1 per 3,000 to 5,000 procedures; and over 80% of all infective endocarditis cases are acquired in the community, and the associated bacteria are part of the host's endogenous flora. A new causal model (Fig. 2) of dental disease- and procedure-associated endocarditis has been proposed (32) that involves early and late bacteremia. The early bacteremia may "prime" the endothelial surface of the heart valves over many years and promote early valve thickening. This renders the valves susceptible to late adherence and colonization with bacteria. The late bacteremia may work over days to weeks and allows bacterial adherence and colonization of the valve, resulting in the characteristic fulminant infection.

Bacterial Pneumonia

Pneumonia is an infection of pulmonary parenchyma caused by a wide variety of infectious agents, including bacteria, fungi, parasites, and viruses. Pneumonia can be a life-threatening infection, especially in the old and immunocompromised patient, and is a significant cause of morbidity and mortality in patients of all ages. Total pneumonia mortality in low-risk individuals over 65 years of age is 9 per 100,000 (0.009%), whereas in high-risk individuals who are likely to aspirate, the mortality can be almost 1,000 per 100,000 (1%) or higher (102). Pneumonias can be broadly divided into two types, community acquired and hospital acquired (nosocomial). These types of pneumonia differ in their causative agents.

The lung is composed of numerous units formed by the progressive branching of the airways. The lower respiratory tracts are normally sterile, despite the fact that secretions from upper respiratory tracts are heavily contaminated with microorganisms from the oral and nasal surfaces. Sterility in the lower respiratory tract is maintained by intact cough reflexes, by the action of tracheobronchial secretions, by mucociliary transport of inhaled microorganisms and particulate material from the lower respiratory tract to the oropharynx, and by immune and nonimmune defense factors (30, 73, 122). The defense factors are present in a secretion which also contains surfactant and other proteins such as fibronectin, complement, and immunoglobulins, which coat the pulmonary epithelium. The lung also contains a rich system of resident phagocytic cells which remove microorganisms and particulate debris (122).

Microorganisms can infect the lower respiratory tracts by four possible routes: aspiration of oropharyngeal contents (94), inhalation of infectious aerosols (139), spread of infection from contiguous sites (73), and hematogenous spread from extrapulmonary sites of infection (37).

Most commonly, bacterial pneumonia results from aspiration of oropharyngeal flora into the lower respiratory tract, failure of host defense mechanisms to eliminate them, multiplication of the microorganisms, and subsequent tissue destruction (8). It is likely that most pathogens first colonize the surfaces of the oral cavity or pharyngeal mucosa before aspiration (8). These pathogens can colonize from an exogenous source or emerge following overgrowth of the normal oral flora after antibiotic treatment. Common potential respiratory pathogens (PRPs) such as *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and *Haemophilus influenzae* can colonize the oropharynx and be aspirated into the lower airways. Other species thought to comprise the normal oral flora, including *A. actinomycetemcomitans* and anaerobes such as *P.*

gingivalis and *Fusobacterium* species, can also be aspirated into the lower airways and cause pneumonia (122).

Generally accepted risk factors that predispose an individual to nosocomial pneumonia include the presence of underlying diseases such as chronic lung disease, congestive heart failure, or diabetes mellitus; age >70 years; mechanical ventilation or intubation; a history of smoking; previous antibiotic treatment; immunosuppression; a long preoperative stay; and prolonged surgical procedures (20, 22, 129, 139).

Pneumonia can result from infection by anaerobic bacteria. Dental plaque would seem to be a logical source of these bacteria, especially in patients with periodontal disease. Such patients harbor a large number of subgingival bacteria, particularly anaerobic species. Among the oral bacterial species implicated in pneumonia are *A. actinomycetemcomitans* (155), *Actinomyces israelii* (98, 158), *Capnocytophaga* spp. (85), *Eikenella corrodens* (62), *Prevotella intermedia*, and *Streptococcus constellatus* (127).

There are several proposed mechanisms to explain the propensity for PRPs to colonize the oropharynx of susceptible patients. First, compromised individuals such as diabetics and alcoholics may be prone to oropharyngeal colonization by PRPs (122). These individuals are thought to be more likely to aspirate and are also known to be at greater risk of periodontal disease (48). Thus, the extensive dental plaque of these subjects may provide surfaces to which PRPs might adhere to provide a reservoir for infection to distal portions of the respiratory tract (69).

Second, the oral surface of subjects at high risk for pneumonia, such as hospitalized patients, may somehow become modified to provide receptors for the adhesion of PRPs (122). Poor oral hygiene increases the plaque load and therefore the level of hydrolytic enzymes in saliva. The source of these enzymes has been attributed to plaque bacteria (82, 99, 156) or polymorphonuclear leukocytes, which enter the saliva through the inflamed gingival sulcus (6, 7, 18, 44). These proteolytic enzymes may alter the characteristics of the mucosal surfaces, resulting in increased colonization by pathogenic bacteria (15, 43).

Limeback (74) noted a relationship between poor oral hygiene and aspiration pneumonia among elderly residents of chronic care facilities. He subsequently found that the nursing homes with the least number of dental visits had the most deaths due to pneumonia.

A study by Scannapieco et al. (123) has shown that individuals with respiratory disease ($n = 41$) have significantly higher oral hygiene index scores than subjects without respiratory disease ($n = 193$; $P = 0.044$). Logistic regression analysis of data from these subjects, which considers age, race, gender, smoking status, and simplified oral hygiene index (OHI), suggests that subjects having the median OHI value are 1.3 times more likely to have a respiratory disease than those with an OHI of 0. (OHI is a composite index which scores debris and calculus deposition on tooth surfaces.) Similarly, subjects with the maximum OHI value are 4.5 times more likely to have a chronic respiratory disease than those with an OHI of 0.

In another study, Scannapieco and Ho (F. A. Scannapieco and A. W. Ho, J. Dent. Res. Spec. Iss. 78, p. 542, abstr. 3491, 1999) examined data from the National Health and Nutrition Examination Survey III. The results showed that subjects ($n = 13,792$) with a mean periodontal attachment loss (which evaluates the loss of supporting tissues of the teeth) of 2.0 mm have a higher risk of chronic lung disease than those who have a mean attachment loss of <2.0 mm (odds ratio = 1.43, 95% confidence interval [CI] = 1.08 to 1.90), adjusting for age, gender, race or ethnicity, education, income, frequency of dental visits, smoking, and alcohol consumption.

Loesche and Lopatin (81) have studied oral and dental conditions in over 350 elderly individuals that may predispose individuals for aspiration pneumonia. They used the periodontal disease score as the outcome and compared the upper tertile of the periodontal disease score with the lower tertiles. The individuals with "definite" aspiration pneumonia were 3.3 times more likely to have a higher periodontal disease score (95% CI = 1.06 to 10.3; $P = 0.05$) than the individuals without pneumonia. The odds ratio pattern and wide CIs suggest that an important association exists between poor periodontal status and aspiration pneumonia.

Low Birth Weight

Pregnancy can influence gingival health. Changes in hormone levels during pregnancy promote an inflammation termed pregnancy gingivitis (77). This type of gingivitis may occur without changes in plaque levels (70). Oral contraceptives may also produce changes in gingival health. Some birth control pill users have a high gingival inflammation level but a low plaque level. Birth control pills may cause changes such as alteration of the microvasculature, gingival permeability, and increased synthesis of estrogen PGs (64).

Oral infections also seem to increase the risk for or contribute to low birth weight in newborns. Low birth weight, defined as a birth weight of <2,500 g, is a major public health problem in both developed and developing countries. The incidence of preterm delivery and low birth weight has not decreased significantly over the last decade and remains at about 10% of all live births in the United States (105).

Low birth weight in preterm infants remains a significant cause of perinatal morbidity and mortality. Compared to normal-birth-weight infants, low-birth-weight infants are more likely to die during the neonatal period (92, 126), and low-birth-weight survivors face neurodevelopment disturbances (11, 38), respiratory problems (49, 91), and congenital anomalies (17, 142). They also demonstrate more behavioral abnormalities as preschoolers (133) and may have attention deficit hyperactivity disorder (10). For low birth weight, all these factors need further elucidation.

Risk factors for preterm low-birth-weight infants include older (>34 years) and younger (<17 years) maternal age, African-American ancestry, low socioeconomic status, inadequate prenatal care, drug, alcohol, and/or tobacco abuse, hypertension, genitourinary tract infection, diabetes, and multiple pregnancies. Although increasing efforts have been made to diminish the effects of these risk factors through preventive interventions during prenatal care, they have not reduced the frequency of preterm low-birth-weight infants (107).

Evidence of increased rates of amniotic fluid infection, chorioamnion infection, and chorioamnionitis supports an association between preterm birth or low birth weight and infection during pregnancy (105). Histologically, the chorioamnion is often inflamed, even in the absence of any bacterial infection in the vagina (vaginosis) or cervical area. This suggests that distant sites of infection or sepsis may be targeting the placental membranes. Vaginosis, caused by gram-negative, anaerobic bacteria, is a significant risk factor for prematurity and is usually associated with the smallest, most premature neonatal deliveries (59, 60). The biological mechanisms involve bacterially induced activation of cell-mediated immunity leading to cytokine production and the ensuing synthesis and release of PG, which appears to trigger preterm labor (59). Elevated levels of cytokines (IL-1, IL-6, and TNF-α) have been found in

the amniotic fluid of patients in preterm labor with amniotic fluid infection (118). These cytokines are all potent inducers of both PG synthesis and labor. Intra-amnionic levels of PGE_2 and TNF-α rise steadily throughout pregnancy until a critical threshold is reached to induce labor, cervical dilation, and delivery (107).

As a remote gram-negative infection, periodontal disease may have the potential to affect pregnancy outcome. During pregnancy, the ratio of anaerobic gram-negative bacterial species to aerobic species increases in dental plaque in the second trimester (70). The gram-negative bacteria associated with progressive disease can produce a variety of bioactive molecules that can directly affect the host. One microbial component, LPS, can activate macrophages and other cells to synthesize and secrete a wide array of molecules, including the cytokines IL-1β, TNF-α, IL-6, and PGE_2 and matrix metalloproteinases (23, 105). If they escape into the general circulation and cross the placental barrier, they could augment the physiologic levels of PGE_2 and TNF-α in the amniotic fluid and induce premature labor.

Human case-control studies have demonstrated that women who have low-birth-weight infants as a consequence of either preterm labor or premature rupture of membranes tend to have more severe periodontal disease than mothers with normal-birth-weight infants (105).

A case-control study of 124 pregnant or postpartum mothers was performed, using mothers with normal-birth-weight babies as controls (107). Assessments included a broad range of known obstetric risk factors, such as tobacco and drug use, alcohol consumption, level of prenatal care, parity, genitourinary infections, and nutrition. Each subject received a periodontal examination to determine the clinical attachment level. Mothers of preterm low-birth-weight infants and primiparous mothers of preterm low-birth-weight infants ($n = 93$) had significantly worse periodontal disease than the respective mothers of normal-birth-weight infants (controls). Multivariate logistic regression models, controlling for other risk factors and covariates, demonstrated that periodontal disease is a statistically significant risk factor for preterm low birth weight, with adjusted odds ratios of 7.9 and 7.5 for all preterm low-birth-weight cases and primiparous preterm low-birth-weight cases, respectively. These data indicate that periodontal disease represents a previously unrecognized and clinically significant risk factor for preterm low birth weight as a consequence of either preterm labor or premature rupture of membranes.

In another 1:1 matched case-control study (55 pairs), the hypothesis that poor oral health of the pregnant woman is a risk factor for low birth weight was evaluated (24). The effect of the periodontal and dental caries status of the woman on the birth weight of the infant was evaluated at the time of delivery by conditional logistic regression analysis, while controlling for known risk factors for low birth weight. Mothers of low-birth-weight infants are shorter, less educated, and married to men of lower occupational class, have fewer areas of healthy gingiva and more areas with bleeding and calculus, and gain less weight during the pregnancy. Conditional logistic regression analyses indicate that mothers with more healthy areas of gingiva (odds ratio [OR] = 0.3, 95% CI = 0.12 to 0.72) and those who are taller (OR = 0.86, 95% CI = 0.75 to 0.98) have a lower risk of giving birth to a low-birth-weight infant. The authors conclude that poor periodontal health of the mother is a potential independent risk factor for low birth weight.

In a recent case-control study, 48 case-control subjects had their gingival crevicular fluid (GCF) levels of PGE_2 and IL-1β measured to determine whether mediator levels are related to current pregnancy outcome (104). In addition, the levels of four periodontal pathogens were measured by using microbe-specific DNA probes. The results indicate that GCF PGE_2 levels are significantly higher in mothers of preterm low-birth-weight infants than in mothers of normal-birth-weight infants (controls) (131.4 ± 21.8 versus 62.6 ± 10.3 ng/ml [mean ± standard error], respectively, at $P = 0.02$). Furthermore, among the primiparous mothers of preterm low-birth weight infants, there is a significant inverse association between birth weight (as well as gestational age) and GCF PGE_2 levels at $P = 0.023$. These data suggest a dose-response relationship for increased GCF PGE_2 as a marker of current periodontal disease activity and decreasing birth weight. Four organisms associated with mature plaque and progressing periodontitis, *Bacteroides forsythus*, *P. gingivalis*, *A. actinomycetemcomitans*, and *Treponema denticola*, are detected at higher levels in mothers of preterm low-birth-weight infants than in controls. These data suggest that biochemical measures of maternal periodontal status and oral microbial burden are associated with preterm birth and low birth weight.

Offenbacher et al. (107) concluded that 18.2% of preterm low-birth-weight babies may result from periodontal disease—a previously unrecognized and clinically important risk factor for preterm birth and low birth weight.

However, it should be noted that periodontal disease pathogens are necessary but not sufficient for periodontal disease expression. The role of the host's inflammatory response appears to be the critical determinant of susceptibility and severity (103). The association between periodontal disease and low birth weight may reflect the patient's altered immune-inflammatory trait that places the patient at risk for both conditions. Thus, periodontitis may be a marker for preterm delivery susceptibility as well as a potential risk factor. Indeed, the data from animal models suggest that even if periodontal disease is not the primary cause of prematurity, in a subset of patients it may serve as a contributor to the morbidity of the condition.

Diabetes Mellitus

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to an absolute or relative deficiency of insulin. It affects more than 12 million people in the United States. Diabetes mellitus is characterized by metabolic abnormalities and long-term complications involving the eyes, kidneys, nervous system, vasculature, and periodontium (39, 76). Diabetes is commonly categorized as type 1, or insulin dependent, and type 2, non-insulin dependent. The fundamental derangement in insulin-dependent diabetes is the hypoproduction of insulin due to destruction of the beta cells of the pancreas. In non-insulin-dependent diabetes, the derangement involves resistance of target tissue to insulin action (120).

Although the precise etiology is still uncertain in both main types of primary diabetes, environmental factors interact with a genetic susceptibility to determine which of those with the genetic predisposition actually develop the clinical syndrome and the timing of its onset. Environmental factors in insulin-dependent diabetes include virus, diet, immunological factors, and pancreas disease. In non-insulin-dependent diabetes, environmental factors such as lifestyle, age, pregnancy, pancreas pathology, and insulin secretion and resistance are included (36).

Severe periodontal disease often coexists with severe diabetes mellitus. Diabetes is a risk factor for severe periodontal disease. The converse possibility that periodontal disease either predisposes or exacerbates the diabetic condition has received more and more attention. Recently, a new model was presented by Grossi and Genco (46), in which severe periodon-

tal disease increases the severity of diabetes mellitus and complicates metabolic control. They propose that an infection-mediated upregulation cycle of cytokine synthesis and secretion by chronic stimulus from LPS and products of periodontopathic organisms may amplify the magnitude of the advanced glycation end product (AGE)-mediated cytokine response that is operative in diabetes mellitus. The combination of these two pathways, infection and AGE-mediated cytokine upregulation, helps explain the increase in tissue destruction seen in diabetic periodontitis and how periodontal infection may complicate the severity of diabetes and the degree of metabolic control, resulting in a two-way relationship between diabetes mellitus and periodontal disease or infection.

It is well established that diabetics are more likely to develop periodontal disease than nondiabetics (112) and that the disease severity is related to the duration of diabetes (61, 138). One plausible biologic mechanism for why diabetics have more severe periodontal disease is that glucose-mediated AGE accumulation affects the migration and phagocytic activity of mononuclear and polymorphonuclear phagocytic cells, resulting in the establishment of a more pathogenic subgingival flora. The maturation and gradual transformation of the subgingival microflora into an essentially gram-negative flora will in turn constitute, via the ulcerated pocket epithelium, a chronic source of systemic challenge. This in turn triggers both an "infection-mediated" pathway of cytokine upregulation, especially with secretion of TNF-α and IL-1, and a state of insulin resistance, affecting glucose-utilizing pathways. The interaction of mononuclear phagocytes with AGE-modified proteins induces upregulation of cytokine expression and induction of oxidative stress. Thus, monocytes in diabetic individuals may be "primed" by AGE-protein binding. Periodontal infection challenge to these primed phagocytic cells may, in turn, amplify the magnitude of the macrophage response to AGE-protein, enhancing cytokine production and oxidative stress. Simultaneously, periodontal infection may induce a chronic state of insulin resistance, contributing to the cycle of hyperglycemia, nonenzymatic irreversible glycation, and AGE-protein binding and accumulation, amplifying the classical pathway of diabetic connective tissue degradation, destruction, and proliferation. Hence, the relationship between diabetes mellitus and periodontal disease or infection becomes two way. A self-feeding two-way system of catabolic response and tissue destruction ensues, resulting in more severe periodontal disease and increased difficulty in controlling blood sugar (46).

Certain metabolic end products such as glycated hemoglobin are thought to contribute to the degenerative retinal and arterial changes commonly found in diabetic subjects. The concentration of glycated hemoglobin in serum is a direct function of the time that hemoglobin is exposed to elevated glucose levels (120). A longitudinal study (128) of diabetes and periodontal disease has been carried out in the Pima tribe, an Indian population in the United States having a prevalence of non-insulin-dependent diabetes of about 50%. This is the highest reported prevalence of non-insulin-dependent diabetes in the world (68). Poor glycemia control was defined as the occurrence of glycated hemoglobin of 9% or more at follow-up. The results indicated that severe periodontitis at baseline is associated with increased risk of poor glycemic control at follow-up 2 or more years later. These findings suggest that severe periodontitis may be an important risk factor in the progression of diabetes, and control of periodontal infection is essential to achieve long-term control of diabetes mellitus. Grossi and Genco (46) reexamined the studies that addressed the effect of periodontal treatment on metabolic control of diabetes mellitus (1, 16, 47, 95, 124, 132, 149). Six of these studies included type 1 patients, and two studies (16, 47) included type 2 patients. Periodontal treatment was divided into two groups, mechanical treatment only and with systemic antibiotics as an adjunct to mechanical treatment. The results show that the effect of periodontal treatment on diabetic metabolic control is dependent on the mode of therapy. When mechanical periodontal treatment alone is provided, regardless of the severity of periodontal disease or degree of diabetes control, the treatment outcome is strict improvement in periodontal status or a local effect. On the contrary, when systemic antibiotics are included with mechanical therapy, an improvement in diabetes control, measured as a reduction in glycated hemoglobin or reduction in insulin requirements, is achieved. Therefore, one may propose that control of the chronic gram-negative periodontal infection should be part of the standard treatment of the diabetic patient.

RELATIONSHIP BETWEEN ORAL INFECTION AND SYSTEMIC DISEASES AND FUTURE STUDIES

As mentioned above, a large number of publications have suggested that oral infection, especially periodontitis, are a potential contributing factor to a variety of clinically important systemic diseases. Endocarditis has been studied most extensively. It appears that dental procedures and oral infection meet currently accepted epidemiological criteria for causation of endocarditis (58, 119). However, there is still not sufficient evidence to claim a causal association between oral infection and other systemic diseases.

Epidemiological research (cross-sectional and longitudinal studies) can identify relationships but not causation. If some types of periodontal disease merely constitute an oral component of a systemic disorder or have etiological features in common with systemic diseases, periodontal and systemic diseases might frequently occur together without having a cause-effect relationship (130).

Therefore, further research must be done before the potential for oral infections to cause damage in other sites of the body can definitely be established. Slots (130) defined the criteria for causal links between periodontal disease and systemic diseases. These criteria also indicate the directions that future research in this area should take. The prevalence and incidence of the systemic disease in question should be significantly higher in periodontitis patients than in periodontally healthy ones (retrospective research); the onset of the systemic disease should follow the onset of periodontitis (prospective research); the removal or reduction of periodontitis should decrease the incidence of the medical disease (intervention research); the microorganism(s) of the systemic disease should be the same species, biotype, serotype, and genotype as the oral microorganism(s) (research on specific etiologic agents); appropriate experimental animals with periodontitis or with inoculated microorganism should develop more systemic disease than periodontally healthy animals (animal research); and the postulated association between periodontal disease and systemic disease should be biologically feasible (research on pathogenic mechanisms).

If the criteria listed above can be satisfied, then a causal relationship between periodontal disease and systemic disease is probable. Nevertheless, so much information is accessible at the moment that it seems justified to state that good oral health is important not only to prevent oral disease but also to maintain good general health.

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Identification of Pathogen and Host-Response Markers Correlated With Periodontal Disease

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Background: Periodontitis is the major cause of tooth loss in adults and is linked to systemic illnesses, such as cardiovascular disease and stroke. The development of rapid point-of-care (POC) chairside diagnostics has the potential for the early detection of periodontal infection and progression to identify incipient disease and reduce health care costs. However, validation of effective diagnostics requires the identification and verification of biomarkers correlated with disease progression. This clinical study sought to determine the ability of putative host- and microbially derived biomarkers to identify periodontal disease status from whole saliva and plaque biofilm.

Methods: One hundred human subjects were equally recruited into a healthy/gingivitis group or a periodontitis population. Whole saliva was collected from all subjects and analyzed using antibody arrays to measure the levels of multiple proinflammatory cytokines and bone resorptive/turnover markers.

Results: Salivary biomarker data were correlated to comprehensive clinical, radiographic, and microbial plaque biofilm levels measured by quantitative polymerase chain reaction (qPCR) for the generation of models for periodontal disease identification. Significantly elevated levels of matrix metalloproteinase (MMP)-8 and -9 were found in subjects with advanced periodontitis with Random Forest importance scores of 7.1 and 5.1, respectively. The generation of receiver operating characteristic curves demonstrated that permutations of salivary biomarkers and pathogen biofilm values augmented the prediction of disease category. Multiple combinations of salivary biomarkers (especially MMP-8 and -9 and osteoprotegerin) combined with red-complex anaerobic periodontal pathogens (such as *Porphyromonas gingivalis* or *Treponema denticola*) provided highly accurate predictions of periodontal disease category. Elevated salivary MMP-8 and *T. denticola* biofilm levels displayed robust combinatorial characteristics in predicting periodontal disease severity (area under the curve = 0.88; odds ratio = 24.6; 95% confidence interval: 5.2 to 116.5).

Conclusions: Using qPCR and sensitive immunoassays, we identified host- and bacterially derived biomarkers correlated with periodontal disease. This approach offers significant potential for the discovery of biomarker signatures useful in the development of rapid POC chairside diagnostics for oral and systemic diseases. Studies are ongoing to apply this approach to the longitudinal predictions of disease activity. *J Periodontol 2009;80:436-446.*

KEY WORDS

Diagnosis; periodontal disease; saliva.

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Periodontal disease is the leading cause of tooth loss in adults.[1] Periodontitis is initiated by tooth-associated microbial biofilms triggering an altered host response leading to soft tissue inflammation and alveolar bone loss. Periodontal infections are implicated in a variety of other diseases, such as cardiovascular disease, stroke, and aspiration pneumonia, whereby the microbial biofilm serves as a "slow-delivery system" of oral pathogens adhering to teeth, leading to a chronic microbial challenge and downstream effects of an altered host response.[2] Diagnostic methods used in clinical practice today lack the ability to detect the onset of inflammation and to identify those patients who are susceptible to future disease progression. Oral fluid–based point-of-care (POC) diagnostics are commonly used in medicine and, more recently, are being adapted for the potential "chairside" determination of oral diseases.[3] The latest clinical applications use new "lab-on-a-chip" (LOC) technologies as rapid POC diagnostic tests for systemic infectious diseases[4,5] and periodontal disease.[6] The human salivary proteome project, supported by the United States National Institute of Dental and Craniofacial Research, Bethesda, Maryland, has generated further emphasis on the use of proteomic markers for disease diagnosis.[7] The identification of the proteomic content of human saliva in diagnostic tests, assessing the fingerprint of different human illnesses, generally suggests the probability that multianalyte detection approaches will surpass conventional clinical diagnostic procedures using single biomarkers.

The use of oral fluids in oral-based diagnostics have proven to be easy to use for POC application[8] in the detection of oral cancer[9,10] or human immunodeficiency virus infection.[11] Furthermore, the use of microfluidic devices as examples of LOC technology offers significant potential for rapid saliva diagnosis for widespread public health purposes.[6,12] However, for periodontal disease determination, most research has focused primarily on gingival crevicular fluid (GCF) biomarkers that provide local disease status, but it represents a cumbersome, difficult-to-use approach for clinical application.[13] Easy-to-access saliva contains locally and systemically derived mediators of periodontal disease and, thus, offers significant potential for the assessment of periodontal disease status and risk.[14]

Although a single specific target biomarker for periodontal disease has not been identified, combinations of putative biomarkers of disease have been evaluated in GCF and demonstrated significant potential as panels of targets for the development of an oral fluid fingerprint of periodontal disease status. Given the multifaceted pattern of periodontal disease as a continuum of infection to inflammatory dysregulation and subsequent bone loss, specific biomarkers, such as matrix metalloproteinase (MMP)-8, interleukin (IL)-1β and -6, and type I collagen pyridinoline cross-linked telopeptide (ICTP), have been assessed in GCF singularly for disease identification.[15] This approach of developing "biologic phenotypes" that consider the microbial and inflammatory response may be useful in the development of patient disease classifications with implications in targeted therapeutics.[16,17]

Here we demonstrate the validation of multiple proinflammatory and bone-specific biomarkers from whole saliva coupled with microbial biofilm pathogens for the identification of periodontal disease. This unique combinatorial approach resulted in robust predictions of periodontitis in human subjects.

MATERIALS AND METHODS

Subjects

This clinical study was approved by the University of Michigan Health Sciences Institutional Review Board and registered with the clinical trials database of the National Institutes of Health, Bethesda, Maryland. Research subjects were recruited from September 2005 through June 2006. Upon receiving written consent, 100 human subjects aged 18 years and older were evaluated at the Michigan Center for Oral Health Research. All subjects possessed ≥20 teeth and had received no periodontal treatment or antibiotic therapy for medical or dental reasons 3 months prior to the investigation. In addition, the subjects did not previously undergo any long-term use of medications affecting periodontal status, such as anti-inflammatory drugs.

Subjects were enrolled into a healthy/gingivitis population (n = 50) or a periodontitis population (n = 49; one patient dropped out at experimental baseline). Subjects from the healthy and gingivitis population exhibited <3 mm of attachment loss, no periodontal probing depth (PD) >4 mm, and no radiographic alveolar bone loss. Periodontitis subjects exhibited at least four sites with evidence of radiographic bone loss, at least four sites with attachment loss >3 mm, and at least four sites with PD >4 mm (Fig. 1).

Subjects were excluded if they possessed a history of metabolic bone diseases, autoimmune diseases, unstable diabetes, or postmenopausal osteoporosis. Women who were pregnant were also excluded from the study.

Clinical Measures

All teeth except third molars were assessed for periodontal clinical measures by two calibrated examiners (CR and JK). Clinical parameters, including PD, clinical attachment level (CAL), and bleeding on probing (BOP), were measured at six sites per tooth. Other clinical assessments included dichotomous measures of plaque accumulation (PI) and gingival redness index (GRI), as previously described by Haffajee et al.[18]

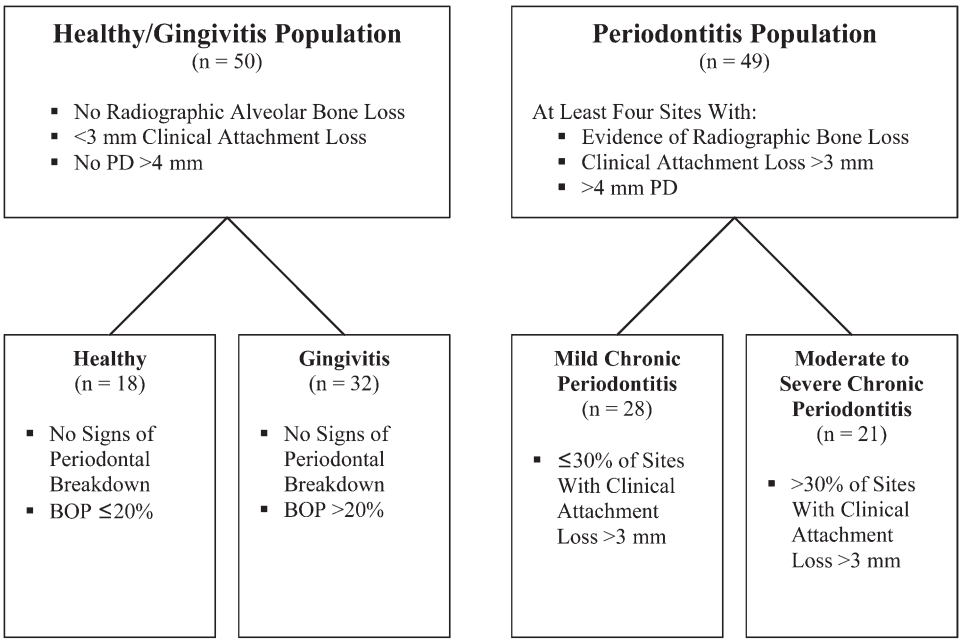

Figure 1.
Stratification of the low-risk population and the disease-susceptible population into four groups based on clinical attachment loss, PD, RBL, and BOP.

Standardized periapical digital radiographs[#] were taken in the posterior dentition of all subjects using a parallel technique for the determination of alveolar bone height. Using a computer software measurement tool,[**] the interproximal alveolar bone levels of both premolars and first and second molars were measured on a digital computer screen by one calibrated examiner (LR). The distance from the alveolar bone crest to the cemento-enamel junction or the restorative margin reference was recorded as the radiographic alveolar bone level (RBL).

Quantitative Polymerase Chain Reaction (qPCR) Microbial Plaque Biofilm Analysis

Plaque biofilm collection. Subgingival plaque biofilm was collected from the mesio-buccal surfaces of all teeth and immediately placed into labeled vials containing 500 µl stabilizing buffer to prevent mRNA degradation,[††] as previously described.[19] After vortexing for 30 seconds, the samples were stored at 4°C until they were sent to the laboratory for analysis.

Detection of oral bacteria colonization in plaque biofilm samples. The detection of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Campylobacter rectus*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Tannerella forsythia* (previously *T. forsythensis*), and *Treponema denticola* in pooled plaque samples was evaluated by real-time qPCR, as described,[20,21] using primers specific for the hypervariable segments of the 16S rRNA genes of each bacterium (Table 1). The percentage of the total flora for each species was calculated by dividing the number of target organisms by the total number of bacteria as determined by qPCR using 16S rRNA primers that reacted with all bacterial species. Data were represented using a patient-based assessment.

Whole Saliva Collection

Unstimulated whole saliva was collected with passive drooling into sterile plastic tubes from all subjects at the beginning of the screening appointment.[22] The collection was completed as soon as 2 ml whole saliva was collected or 15 minutes of sampling time had elapsed. Subsequently, the samples were placed on ice, aliquotted, and supplemented with a proteinase inhibitor combination of 1% aprotinin and 0.5% phenylmethylsulphonyl fluoride prior to storage at –80°C.

Protein Biomarker Assays

Protein biomarker levels were determined using colorimetric-based enzyme-linked immunosorbent assays (ELISAs), fluorescence-based protein microarrays, and radioimmunoassay (RIA), run according to manufacturer protocols. ELISAs[‡‡] were used for measurement of MMP-8 and -9, calprotectin, and osteoprotegerin (OPG). Detection of the cytokines IL-1β, -2, -4, -5, -6, -10, and -13, tumor necrosis factor-alpha (TNF-α), and interferon (IFN)-γ was accomplished using a protein microarray.[§§] The concentration of ICTP was determined using an RIA.[‖‖]

Prior to each assay, whole saliva samples were thawed at room temperature and microcentrifuged for 5 minutes to obtain cell-free supernatant for analysis. For ELISAs, absorbance measurements were collected using a primary signal at 450 nm with background subtraction of the 540-nm signal. A fluorescence scanner[¶¶] was operated to collect Cy5

Schick Technologies, Long Island City, NY.
** Emago Advanced, Oral Diagnostic Systems, Amsterdam, The Netherlands.
†† RNA Protect, Ambion, Austin, TX.
‡‡ R&D Systems, Minneapolis, MN.
§§ Whatman, Florham Park, NJ.
‖‖ Immunodiagnostic Systems, Fountain Hills, AZ.
¶¶ Molecular Devices, Sunnyvale, CA.

Table 1.

Primers for qPCR Analysis of Plaque Biofilm Bacteria

Bacterial Species	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
A. actinomycetemcomitans	GGCACGTAGGCGGACCTT	ACCAGGGCTAAAGCCCAATC
C. rectus	TTTCGGAGCGTAAACTCCTTTTC	TTTCTGCAAGCAGACACTCTT
F. nucleatum	ACCAGCGTTTGACATCTTAGGAATG	AGCCATGCACCTGTCTTTAG
P. intermedia	AGATTGACGGCCCTATGGGT	CCGGTCCTTATTCGAAGGGTA
P. gingivalis	CATAGATATCACGAGGAACTCCGATT	AAACTGTTAGCAACTACCGATGTGG
T. forsythia	GGGTGAGTAACGCGTATGTAACCT	ACCCATCCGCAACCAATAAA
T. denticola	CGTTCCTGGGCCTTGTACA	TAGCGACTTCAGGTACCCTCG
Universal	CCATGAAGTCGGAATCGCTAG	GCTTGACGGGCGGTGT

fluorescence signal from the cytokine protein microarrays. Data collection of the protein microarray signals was performed using software.[##]

Statistical Analysis

Basic demographics were summarized with means and proportions for each subject group; between-group comparisons were made with a one-way analysis of variance. Biomarker levels were summarized with medians for each group; between-group comparisons were made with a Kruskal-Wallis test. Areas under the curve (AUCs) for receiver operating characteristic (ROC) curves were estimated non-parametrically.[23] Thresholds for biomarkers were preselected as those values for which sensitivity and specificity were as equal as possible. ROC curves and corresponding AUCs for multiple biomarker combinations were based upon predicted probabilities of diseased subjects from a logistic regression model in which a subject's biomarker levels were dichotomized as being above or below their corresponding thresholds. Furthermore, the biomarkers and microbial gene biofilm levels were ranked in importance via Random Forest methods.[24] Statistical significance was defined as $P \leq 0.05$.

RESULTS

Fifty-seven female (74% white) and 42 male (81% white) subjects, ranging in age from 20 to 77 years, were enrolled in the study. Following the recording of periodontal clinical and radiographic parameters, the 99 subjects were stratified and subdivided into four groups, according to previously described disease categories, prior to the analysis of the data (Fig. 1).[25,26]

From the healthy and gingivitis population, 18 subjects were stratified as healthy (group A), with no signs of periodontal breakdown and with BOP ≤20%. Thirty-two subjects were categorized as having gingivitis (BOP >20%) and no alveolar bone loss (group B). From the periodontitis population, 28 subjects exhibiting ≤30% of sites with CAL >3 mm were classified as having mild chronic periodontitis (group C), and 21 subjects were labeled as having moderate to severe chronic periodontitis (group D); CAL >3 mm was found in >30% of sites.

Dental and periodontal data (Table 2) were significantly different among the four groups for the mean number of teeth (25 to 28; $P<0.001$), BOP (12% to 64%; $P<0.001$), GRI (13% to 56%; $P<0.001$), accumulation of plaque (13% to 56%; $P<0.001$), mean PD (1.49 to 3.03 mm; $P<0.001$), sites with PD >4 mm (0% to 20%; $P<0.001$), mean CAL (0.59 to 2.93 mm; $P<0.001$), and mean RBL (1.89 to 4.33 mm; $P<0.001$). Additionally, the prevalence of smoking was significantly higher in groups C and D (36% and 81%, respectively; $P<0.001$). The demographics for gender and ethnicity were balanced among the four groups. However, mean age was statistically significantly different among the four groups (range, 42 to 53 years; $P=0.02$).

Data from our analysis of putative biomarkers of periodontal disease are shown in Table 3. Because the majority (>70%) of the subjects did not have detecable protein levels of IL-5 and IFN-γ in their whole saliva, these proteins were not included (data not shown).

Compared to the healthier individuals, the median levels of protein concentrations of MMP-8 ($P<0.001$), MMP-9 ($P=0.001$), and calprotectin ($P=0.023$) were increased in subjects with advancing stages of periodontal disease. Increased levels of OPG demonstrated a significant ability to predict health ($P=0.007$; Table

GenePix Pro, MDS Analytical Technologies, Toronto, ON.

Table 2.

Patient Demographics and Clinical Parameters Stratified by Level of Disease

	Group A (healthy)	Group B (gingivitis)	Group C (mild chronic periodontitis)	Group D (moderate to severe chronic periodontitis)	*P* Values Comparing A Through D		*P* Values Comparing A and B Versus C and D
					Overall	Trend	
Subjects (n)	18	32	28	21	NA	NA	NA
Males (%)	56	41	39	38	0.67	0.32	0.47
Whites (%)	78	78	68	86	0.54	0.80	0.78
Smokers (%)	0	19	36	81	<0.001	<0.001	<0.001
Mean number of teeth	28	27	26	25	<0.001	<0.001	<0.001
Mean age (years)	45	42	53	50	0.02	0.03	0.002
Sites with BOP (%)	12	31	52	64	<0.001	<0.001	<0.001
Sites with gingival redness (%)	13	22	49	56	<0.001	<0.001	<0.001
Sites with plaque (%)	24	26	57	61	<0.001	<0.001	<0.001
Mean PD (mm)	1.49	1.65	2.29	3.03	<0.001	<0.001	<0.001
Sites with PD >4 mm (%)	0	0	7	20	<0.001	<0.001	<0.001
Mean CAL (mm)	0.59	0.72	1.69	2.93	<0.001	<0.001	<0.001
Mean RBL (mm)	1.89	2.00	3.13	4.33	<0.001	<0.001	<0.001

NA = not applicable.

3). Various trends were noted for other biomarkers, including ICTP and IL-1 and -6, but these were used to rank significant. Random Forest methods were ranking the importance of MMP-8 with a score of 7.1 and OPG with a score of 6.3, reflecting the highest importance level among the biomarkers in this dataset.

Further analysis was done using a subset of biomarkers demonstrating high Random Forest importance scores, relatively low *P* values, and high AUCs. The diagnostic properties of specific thresholds that gave nearly equal levels of sensitivity and specificity for our selection of biomarkers were selected as cutoff values. MMP-8 and -9 and calprotectin demonstrated significant abilities to predict disease category (odds ratios [ORs] were 5.3 for MMP-8 and -9 and 2.7 for calprotectin) (Table 4).

Table 3 shows the median levels as a percentage of selected red and orange complex organisms for their ability to identify periodontal disease category. A greater diagnostic ability of these organisms was demonstrated compared to the salivary biomarkers. When comparing the healthy/gingivitis group to the periodontitis group, *T. denticola*, *P. gingivalis*, *T. forsythia*, *P. intermedia*, and *C. rectus* exhibited significant differences ($P < 0.001$); *F. nucleatum* and *Eikenella corrodens* did not. When the diagnostic properties were evaluated for the pathogens demonstrating significant differences between the groups, good sensitivity and specificity for disease category were shown (Table 4). ORs (2.7 to 21.6) were also found to be significant for *T. denticola*, *P. gingivalis*, *T. forsythia*, *P. intermedia*, and *C. rectus* (Table 4).

Multianalyte assessments were performed using various combinations of salivary biomarkers and plaque biofilm levels (Fig. 2). For example, when MMP-8 and calprotectin were combined to predict high-risk periodontal status, an AUC of 0.74 was found with a corresponding OR = 3.9 (95% confidence interval [CI]: 1.3 to 11.6). When multiple biomarkers were combined, such as MMP-8, OPG, and ICTP, the AUC increased to 0.75 with OR = 10.1 (95% CI: 1.2 to 84.8; Fig. 2C). When the microbial biofilm was combined with the biomarkers, the predictive values increased markedly. Figure 2D depicts the combination of MMP-8 and *T. denticola* with a resultant AUC of 0.88 (OR = 24.6; 95% CI: 5.2 to 116.6). Further improvements in the OR were noted when several pathogens were combined. Given the relatively small sample of 99 subjects, the OR could not be determined for many combinations because in all cases, periodontal disease category was correctly identified when comprehensive combinations were

Table 3.

Median Levels (ranges) and Diagnostic Ability of Salivary Biomarkers and Plaque Biofilm Pathogens

Biomarker	Group A Healthy (median [range])	Group B Gingivitis (median [range])	Group C Mild Chronic Periodontitis (median [range])	Group D Moderate to Severe Chronic Periodontitis (median [range])	*P* Values Comparing A Through D	*P* Values Comparing A and B Versus C and D	AUC	Importance Score via Random Forest
MMP-8 (ng/ml)	23.6 (2.5 to 322.5)	54.1 (1 to 473.9)	129.9 (8.5 to 978.9)	203.8 (10.1 to 2,681.1)	<0.001	<0.001	0.75	7.1
OPG (pg/ml)	2.3 (1.4 to 6.6)	2.7 (1.2 to 6.2)	1.9 (0.2 to 10.1)	1.6 (0.5 to 11.8)	0.056	0.007	0.62	6.3
MMP-9 (ng/ml)	106.4 (10 to 1,185.7)	225.8 (4.9 to 1,732.2)	301.6 (4.6 to 3,348.1)	780.8 (10.4 to 9,778.2)	0.002	0.001	0.72	5.1
Calprotectin (ng/ml)	3.0 (1.3 to 10)	3.5 (0 to 24.6)	4.3 (0 to 17.8)	5.4 (1.7 to 97.6)	0.082	0.023	0.68	4.7
IL-1β (pg/ml)	158.6 (0 to 6,000)	206.7 (0 to 3,856.8)	247.5 (24.1 to 3,120)	462.2 (15.7 to 6,000)	0.157	0.059	0.72	3.7
ICTP (ng/ml)	0.9 (0 to 4)	0.8 (0 to 4)	0.6 (0 to 5.4)	0.9 (0 to 13.9)	0.195	0.185	0.58	3.2
IL-6 (pg/ml)	0.0 (0 to 1,915)	22.1 (0 to 8,784.9)	14.6 (0 to 5,259.7)	88.7 (0 to 10,816.9)	0.127	0.092	0.71	2.2
IL-10 (pg/ml)	881.4 (0 to 11,088.8)	120.6 (0 to 45,488.9)	1,153.1 (0 to 24,581.4)	1,445.1 (0 to 30,633.1)	0.618	0.329	0.68	1.9
TNF-α (pg/ml)	9.8 (0 to 1,788.3)	0.0 (0 to 3,720.5)	8.1 (0 to 4,370.2)	0.0 (0 to 8,212.7)	0.483	0.954	0.64	1.8
IL-13 (pg/ml)	14.3 (0 to 83,151.1)	0.0 (0 to 92,423.8)	0.0 (0 to 76,046)	169.9 (0 to 75,445.2)	0.780	0.783	0.64	1.5
IL-4 (pg/ml)	0.0 (0 to 5,315.1)	0.0 (0 to 6,579.3)	54.4 (0 to 14,588)	69.5 (0 to 11,714.3)	0.377	0.086	0.71	1.3
IL-2 (pg/ml)	0.0 (0 to 3,718.1)	0.0 (0 to 6,000)	8.0 (0 to 6,205.5)	0.0 (0 to 14,400.1)	0.421	0.178	0.69	1.2
T. denticola (%)	0.11 (0 to 0.54)	0.10 (0 to 2.95)	1.53 (0 to 5.25)	2.34 (0.79 to 6.63)	<0.001	<0.001	0.86	13.7
P. gingivalis (%)	0.05 (0 to 0.9)	0.04 (0 to 0.66)	0.53(0 to 2.36)	1.00 (0.43 to 3.24)	<0.001	<0.001	0.84	9.6
T. forsythia (%)	0.09 (0 to 0.88)	0.07 (0 to 0.8)	0.71 (0 to 3.16)	1.26 (0.11 to 3.55)	<0.001	<0.001	0.85	8.4
P. intermedia (%)	0.11 (0 to 1.17)	0.20 (0 to 1.99)	0.82 (0 to 3.77)	1.85 (0 to 3.5)	<0.001	<0.001	0.79	6.7
C. rectus (%)	0.00 (0 to 1.22)	0.00 (0 to 1.18)	0.66 (0 to 2.82)	1.32 (0 to 3.34)	0.001	<0.001	0.74	4.7
F. nucleatum (%)	2.96 (0 to 8.27)	2.33 (0 to 7.32)	3.29 (0 to 10.74)	3.30 (0 to 9.56)	0.251	0.196	0.59	3.9
E. corrodens (%)	0.00 (0 to 0.96)	0.00 (0 to 1.04)	0.00 (0 to 1.32)	0.00 (0 to 0.1)	0.697	0.259	0.56	0.3

chosen and were considered infinite for these permutations (See supplementary table in online *Journal of Periodontology*). These results suggest that although the study of 99 subjects was able to determine differences in biomarker/biofilm levels to identify disease category, a much larger sample is needed to generate ORs that can be usable given the high level of accuracy demonstrated in this patient cohort.

DISCUSSION

To the best of our knowledge, this study demonstrates for the first time the ability to use host-response salivary biomarkers coupled with microbial biofilm DNA to identify individuals with different stages of periodontal disease. The results underscore the robustness of combinatorial measures of disease mediators, such as MMPs with putative periodontal pathogen genes, to more accurately identify a patient's status. These findings may allow for rapid POC diagnostics to quickly identify and screen at-risk patients in a more time-effective manner compared to extensive clinical examinations.

Our data identified key biomarkers from saliva and biofilm that represent three distinct phases of periodontitis: periodontal tissue inflammation (IL-1 and -6), matrix degradation (MMP-8 and -9), and alveolar bone turnover/resorption (osteoprotegerin and ICTP). Complementing the dataset with anaerobic pathogens (particularly *P. gingivalis*, *T. denticola*, and *T. forsythia*) augments the microbe–host influences on periodontal disease identification to clinical measures of disease status. These results represent an early approach to the identification of disease signatures for periodontitis using rapid diagnostic techniques. Given

Table 4.

Diagnostic Properties of Specific Thresholds of Selected Salivary Biomarkers and Plaque Biofilm Pathogens

Biomarker	Threshold	Above Threshold	Periodontitis		Sensitivity	Specificity	OR	95% CI
			No (n)	Yes (n)				
MMP-8 (ng/ml)	87.0	–	28	12	0.69	0.70	5.3	2.0 to 13.7
		+	12	27				
MMP-9 (ng/ml)	240.0	–	28	12	0.69	0.70	5.3	2.0 to 13.7
		+	12	27				
Calprotectin (ng/ml)	3.6	–	25	15	0.62	0.63	2.7	1.1 to 6.6
		+	15	24				
IL-6 (pg/ml)	22.4	–	24	16	0.59	0.60	2.2	0.9 to 5.3
		+	16	23				
IL-1β (pg/ml)	235.8	–	22	18	0.54	0.55	1.4	0.6 to 3.5
		+	18	21				
IL-10 (pg/ml)	520.9	–	22	18	0.54	0.55	1.4	0.6 to 3.5
		+	18	21				
OPG (pg/ml)	2.0	–	17	22	0.44	0.43	0.6	0.2 to 1.4
		+	23	17				
ICTP (ng/ml)	0.7	–	16	23	0.41	0.40	0.5	0.2 to 1.1
		+	24	16				
T. denticola (%)	0.2	–	33	7	0.82	0.83	21.6	6.8 to 68.4
		+	7	32				
T. forsythia (%)	0.1	–	32	8	0.80	0.80	15.5	5.2 to 46.4
		+	8	31				
P. gingivalis (%)	0.1	–	31	8	0.80	0.78	13.3	4.6 to 39.1
		+	9	31				
P. intermedia (%)	0.4	–	29	11	0.72	0.73	6.7	2.5 to 18
		+	11	28				
C. rectus (%)	0.1	–	25	15	0.62	0.63	2.7	1.1 to 6.6
		+	15	24				
F. nucleatum (%)	2.8	–	24	16	0.59	0.60	2.2	0.9 to 5.3
		+	16	23				
E. corrodens (%)	0.0	–	35	31	0.21	0.88	1.8	0.5 to 6.1
		+	5	8				

– = no; + = yes.

the multifactorial complexity of periodontitis as a polygenic disease, similar to cardiovascular disease and osteoporosis, the consideration of multiple checkpoints of disease (infection, inflammation, immune dysregulation, and bone resorption) can now be addressed with the use of multiple biomarkers that reflect the distinct stages of periodontitis. The periodontology field has failed to come up with a "silver bullet" or specific biomarker for periodontal disease identification. The results from this study suggest that patient disease status might be able to be determined rapidly using a combined proteomic/microbial genetic approach. The development of such methodologies may have implications for rapid POC diagnostics for oral and other systemic diseases; however, much more information will be gleaned from longitudinal investigation.[27]

During the initiation of an inflammatory response in the periodontal connective tissue, numerous cytokines, such as IL-1β and -6 and TNF-α, are released

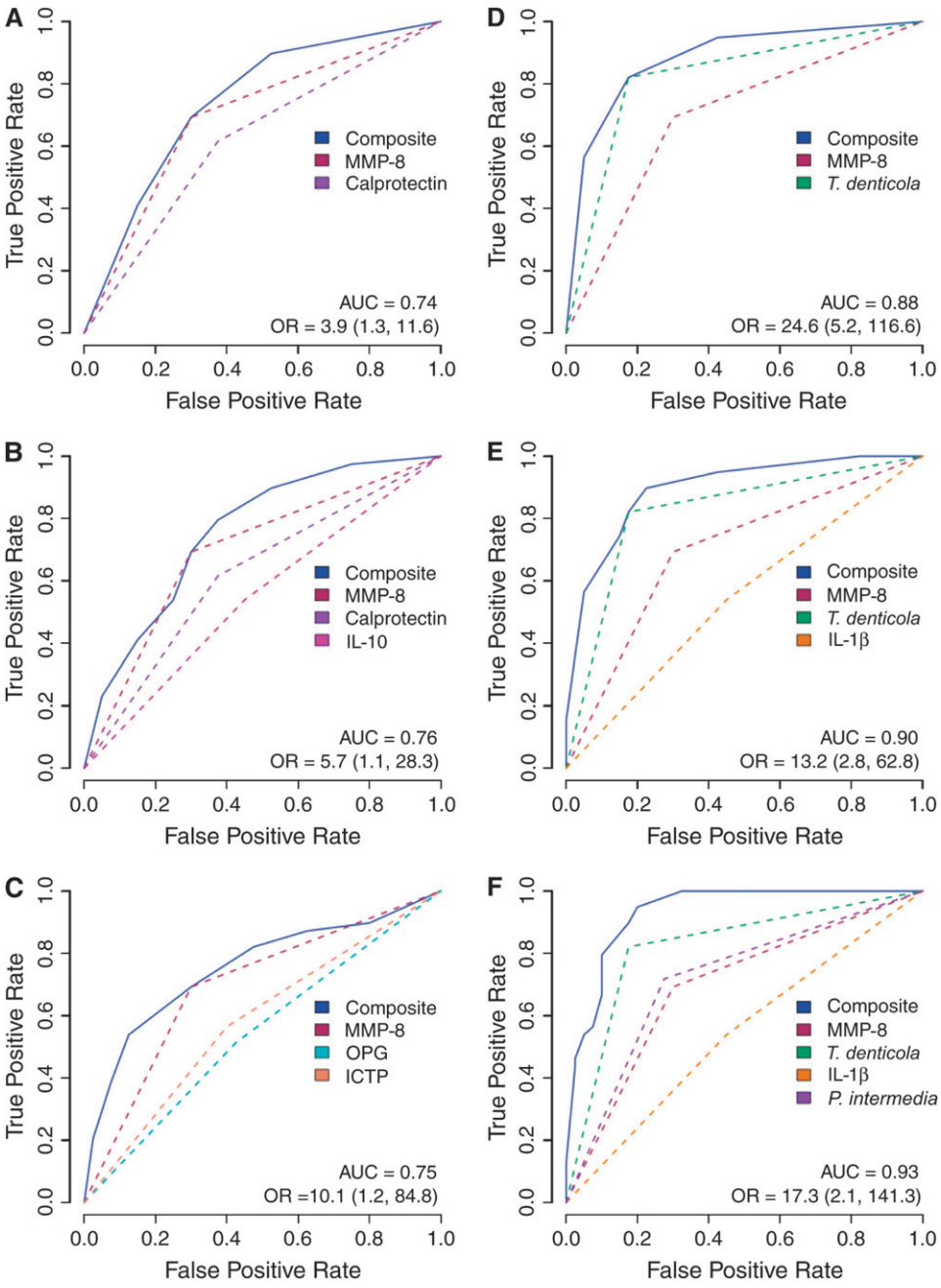

Figure 2.
A through F) *ROC of combinatorial permutations of salivary biomarkers coupled with biofilm subgingival pathogens measured by qPCR. Numbers in parentheses are 95% CIs.*

from cells of the junctional epithelia, connective tissue fibroblasts, and macrophages. Additionally, a number of enzymes, such as MMP-8 and -9 and calprotectin, are produced by PMNs and osteoclasts, leading to the degradation of connective tissue collagen and alveolar bone. During connective tissue inflammation and following bone resorption, cytokines and bone resorptive/turnover proteins migrate toward the gingival sulcus or periodontal pocket and further into GCF, where they are released into and contribute to whole saliva. Host cell–derived MMP-8 and -9 are believed to mediate, to a substantial extent, the matrix-destroying events during the stages of periodontal disease. The results from our investigation are in agreement with and extend the overall findings that MMP-8 and -9 seem to be key biomarkers that are elevated in the oral fluids of periodontal patients.[28,29]

These data support the concept of the development of periodontal signatures or biologic phenotypes for disease classification that consider the host phenotype (response to the microbial insult) and the nature of the invading pathogens that initiate periodontal disease.[16] Specific biofilm organisms or exposures may have the capacity to affect the "inflammatory set point" of the local tissues in certain patients via epigenetic mechanisms.[30,31] Thus, the use of rapid chairside POC diagnostics that identify disease in the context of the host–microbe interaction will likely lead to more rationally tailored therapeutic strategies. Offenbacher et al.[17] recently described periodontal disease at the biofilm–gingival interface (BGI) and noted from a molecular epidemiologic investigation that patients' clinical phenotypes are linked to biologic phenotypes based on antibody response, microbial biofilm levels, and GCF levels of specific proinflammatory cytokines. The identification of these BGI classifications has led to distinct categories that contain elevated antibody titers to *P. gingivalis*, *C. rectus*, and *T. denticola* immunoglobulin G as well as increased GCF concentrations of IL-1 and -6. Our data support and expand these findings using salivary-derived biomarkers for more rapid, easy-to-collect, and more global whole-mouth assessment of inflammatory and

matrix-associated markers of periodontal disease. The greatest diagnostic accuracy in disease identification was noted when MMP-8 or -9 was coupled with red-complex periodontal organisms *T. denticola*, *P. gingivalis*, or *T. forsythia*.[32] The concept of MMP-8 as a diagnostic has been well described,[6,33-36] and the linkage between red-complex bacteria and collagen destruction was reported.[37,38] The red-complex bacteria are known for their potent ability to display trypsin-like enzyme activity that is responsible for destroying collagen matrices.[39] Thus, these data substantiate the combinatorial use of MMP-destroying enzymes and corresponding initiating pathogens, such as *T. denticola*, for periodontal disease identification. The greatest usefulness of these diagnostic approaches is the development of predictive models for disease that need to be validated in large, longitudinal studies. The patients involved in this investigation were evaluated (Table 5) to determine the ability of these diagnostic approaches to predict progressive periodontal disease.[40]

Table 5.

Positive and Negative Predictive Values of Specific Thresholds of Selected Salivary Biomarkers and Plaque Biofilm Pathogens

Biomarker	Threshold	Above Threshold	Periodontitis		PPV	NPV
			No (n)	Yes (n)		
MMP-8 (ng/ml)	87.0	–	28	12	0.69	0.70
		+	12	27		
MMP-9 (ng/ml)	240.0	–	28	12	0.69	0.70
		+	12	27		
Calprotectin (ng/ml)	3.6	–	25	15	0.62	0.63
		+	15	24		
IL-6 (pg/ml)	22.4	–	24	16	0.59	0.60
		+	16	23		
IL-1β (pg/ml)	235.8	–	22	18	0.54	0.55
		+	18	21		
IL-10 (pg/ml)	520.9	–	22	18	0.54	0.55
		+	18	21		
OPG (pg/ml)	2.0	–	17	22	0.43	0.44
		+	23	17		
ICTP (ng/ml)	0.7	–	16	23	0.40	0.41
		+	24	16		
T. denticola (%)	0.2	–	33	7	0.82	0.83
		+	7	32		
T. forsythia (%)	0.1	–	32	8	0.80	0.80
		+	8	31		
P. gingivalis (%)	0.1	–	31	8	0.78	0.80
		+	9	31		
P. intermedia (%)	0.4	–	29	11	0.72	0.73
		+	11	28		
C. rectus (%)	0.1	–	25	15	0.62	0.63
		+	15	24		
F. nucleatum (%)	2.8	–	24	16	0.59	0.60
		+	16	23		
E. corrodens (%)	0.0	–	35	31	0.62	0.53
		+	5	8		

PPV = positive predictive value; NPV = negative predictive value; – = no; + = yes.

CONCLUSIONS

These data support the pairing of microbial and host-response biomarker information for more accurate periodontal diagnoses. Future clinical in-office applications of rapid POC diagnostics that can measure proteins, genes, and biofilm pathogens in saliva should lead to the development of improved disease identification and improved oral health. These studies require the longitudinal validation of these cross-sectional approaches to determine the prediction of disease activity. The patients in this trial are being monitored for the determination of disease progression to better forecast clinical disease outcomes.

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Pearls

Oral Bacteria and Cancer

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Epidemiological Associations

Over a number of years, epidemiological studies established several well-defined risk factors for cancer, including age, heredity, diet, tobacco use, chronic viral infections, and inflammation. Paradoxically, the success of these studies left little room for incorporation of any new factors or causative agents, and, consequently, the idea that a bacterial infection could contribute to cancer was generally disregarded. However, landmark studies in the early 1990s established *Helicobacter pylori* as a causative agent of gastric cancers, resulting in a paradigm shift regarding the relationship between microbial agents and cancers [1]. Indeed, in 1994, *H. pylori* became the first bacterial species to be officially recognized by the World Health Organization as a definite cause of cancer in humans. Since then, there has been a growing body of evidence supporting an association between specific microorganisms, including those in the oral cavity, and various types of cancers.

The oral cavity is inhabited by complex multispecies communities that usually exist in a balanced immunoinflammatory state with the host [2]. Certain species, such as *Porphyromonas gingivalis*, can disrupt this equilibrium, resulting in a dysbiotic host–microbiota interaction. Subsequently, other community constituents, such as *Fusobacterium nucleatum*, can become opportunistically pathogenic, and the combined effect of a dysbiotic microbial community along with a dysregulated immune response ultimately causes periodontal disease [2]. These well-studied periodontal organisms have now emerged as the focal point for the developing association between oral bacteria and cancer.

Perhaps the most likely carcinogenic link with oral bacteria is with oral squamous cell carcinoma (OSCC), one of the most common cancers worldwide. OSCC surfaces have been reported to harbor significantly higher levels of *Porphyromonas* and *Fusobacterium* compared with contiguous healthy mucosa [3]. Moreover, immunohistochemistry with *P. gingivalis* antibodies revealed higher levels of detection and intensity of staining in gingival carcinomas compared with healthy gingival tissue, although only a small number of cases were examined [4]. A striking association has also been demonstrated between *P. gingivalis* infection and pancreatic cancer. In a prospective cohort study of over 400 cases and controls, a >2-fold increase in risk of pancreatic cancer was observed among those with high levels of antibodies to *P. gingivalis*, after adjusting for known risk factors [5]. Similarly, in the extensive National Health and Nutrition Examination Survey III, orodigestive cancer mortality was found to be related to the levels of *P. gingivalis* antibodies, independent of periodontal disease [6]. Several recent studies have shown a strong association between *F. nucleatum* and colorectal cancer (CRC) [7–10]. *F. nucleatum* was found to be one of the more abundant species within and around CRC neoplasms, and levels of *F. nucleatum* correlated with the presence of lymph node metastases.

Mechanistic Basis Supporting a Role for Oral Bacteria in Cancer

Epidemiological studies associate oral bacteria temporally and spatially with certain cancers and render involvement in the initiation or progression of the disease plausible. However, it is equally plausible that early undetected cancer, or precancerous lesions, facilitate the colonization and growth of oral bacteria. If these organisms are active participants in the disease process, then a mechanistic basis that would support an etiological role should exist.

Chronic or dysregulated inflammation has long been appreciated as contributing to tumor development, in part through modulation of the tumor microenvironment [11]. Both *P. gingivalis* and *F. nucleatum* establish chronic infections that involve intracellular persistence within epithelial cells, can spread systemically and cause extra-oral infections, and have well-characterized immune disruptive properties [12]. *F. nucleatum* is strongly proinflammatory, and McCoy et al. [7] demonstrated a positive correlation between mRNA levels for several local cytokines and *Fusobacterium* species in CRC cases. Furthermore, in the $Apc^{Min/+}$ mouse model of intestinal tumorigenesis, *F. nucleatum* recruits tumor-infiltrating immune cells, thus generating a proinflammatory microenvironment that is conducive for CRC progression [13]. The inflammatory properties of *P. gingivalis* are more nuanced, and the organism can exhibit both pro- and anti-inflammatory properties, depending on the context [14,15]. In either event, *P. gingivalis* has a major disruptive effect on local immune responses in the periodontal area [2]; however, the possible link with tumor development has yet to be investigated in molecular detail. In addition to broadly based immune-disruptive properties, both *P. gingivalis* and *F. nucleatum* impinge upon several aspects of epithelial cell signaling that have relevance to cancer progression.

P. gingivalis

Cancer cells, by definition, are defective in functional cell death pathways, and tumorigenesis is initiated when cells are freed from growth restraints. Epithelial cell responses to *P. gingivalis* infection include both changes to apoptosis and cell division (Figure 1). In primary cultures of gingival epithelial cells, *P. gingivalis* is strongly antiapoptotic and, indeed, can suppress chemically induced apoptosis [16]. *P. gingivalis* activates Jak1/Akt/Stat3 signaling that controls intrinsic mitochondrial apoptosis pathways [16,17]. At the mitochondrial membrane, the activity of proapoptotic Bad is inhibited, and the Bcl2 (antiapoptotic):Bax (proapoptotic) ratio is

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increased, consequently curtailing the release of the apoptosis effector cytochrome c [18]. Further downstream, activation of both caspase-9 and the executioner caspase-3 is blocked. Remarkably, *P. gingivalis* possesses multiple mechanisms for inhibition of apoptosis in epithelial cells. Expression of microRNAs (miRs) is modulated, and up-regulation of miR-203 leads to inhibition of the negative regulator SOCS3 and subsequent suppression of apoptosis [19]. *P. gingivalis* secretes a nucleoside diphosphate kinase (NDK), which can function as an ATPase and prevent ATP-dependent apoptosis mediated through the purinergic receptor $P2X_7$ [20]. Another potential role for NDK is diminishing ATP activation of $P2X_7$ receptors on dendritic cells, which will impede activation of the NLRP3/ASC/caspase-1 inflammasome. This, in turn, will reduce secretion of IL-1β, which is important for the priming of IFNγ-producing tumor–antigen-specific $CD8^+$ T cells [21]. In concert with suppression of apoptosis, *P. gingivalis* can accelerate progression through the S-phase of the cell cycle by manipulation of cyclin/CDK (cyclin-dependent kinase) activity and reducing the level of the p53 tumor suppressor [22]. A fimbrial-deficient mutant of *P. gingivalis* does not display this activity, suggestive of a role for the FimA adhesin in elevating epithelial cell proliferation. A role for LPS (lipopolysaccharide) in the dysregulation of p53 has been established [23], and the extent to which *P. gingivalis* LPS can target p53 requires further investigation.

In both primary gingival epithelial cells and OSCC cells, *P. gingivalis* can induce the expression of the B7-H1 and B7-DC receptors [24]. These receptors are up-regulated in cells originating from a variety of cancers and contribute to chronic inflammation. Furthermore, B7-H1 expression promotes the development of regulatory T cells (Treg), which suppress effector T cells, and thus could contribute to immune evasion by oral cancers. Another impact of *P. gingivalis* on OSCC cells is in promoting cellular invasion. *P. gingivalis* infection activates the ERK1/2-Ets1, p38/HSP27, and PAR2/NF-KB pathways to induce promatrix metalloproteinase (MMP)-9 expression [25]. Gingipains, cysteine proteinases produced by *P. gingivalis*, play a dual role in this process. They both engage the PAR2 receptor and cleave the MMP-9 proenzyme into the mature active form. MMP-9 degrades basement membrane and extracellular matrix, which promotes carcinoma cell migration and invasion, thus allowing carcinoma cells to enter the lymphatic system and blood vessels for dissemination and metastatic growth at remote sites. In this manner, *P. gingivalis* may contribute to OSCC metastasis.

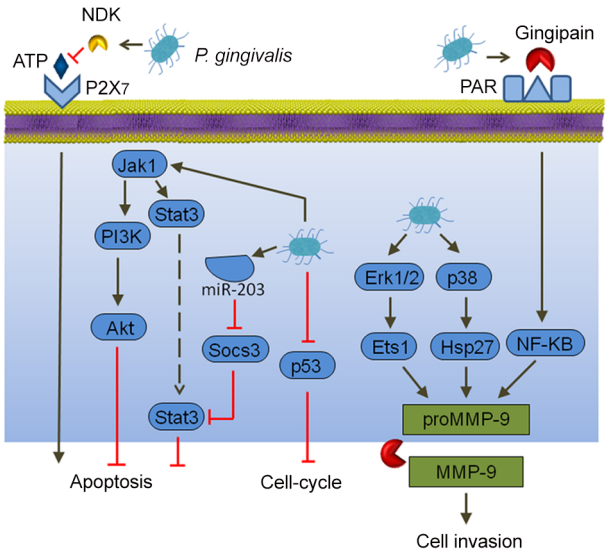

Figure 1. Interactions between *P. gingivalis* and epithelial cells that could produce an oncogenic phenotype. Extracellular *P. gingivalis* secrete gingipains, which activate Protease Activated Receptor (PAR) leading to promatrix metalloprotease (MMP)-9 production, and they also convert proMMP-9 to mature MMP-9, along with nucleoside diphosphate kinase (NDK), which cleaves ATP and prevents activation of the proapoptotic $P2X_7$ receptor. Intracellular *P. gingivalis* activate antiapoptotic Jak-Stat signaling and inhibit expression of the p53 tumor suppressor. Additionally, Erk 1/2 and p38 are activated, which also elevates proMMP-9 expression.
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F. nucleatum

Epithelial cell responses to *F. nucleatum* infection are also consistent with carcinogenesis (Figure 2). Signaling molecules targeted by *F. nucleatum* include kinases involved in cell cycle control, and, as a result, *F. nucleatum* can elevate cell proliferation and migration [26]. *F. nucleatum* also activates p38, leading to the secretion of MMP-9 and MMP-13 (collagenase 3). Similar to MMP-9, MMP-13 plays an important role in tumor invasion and metastasis. Recently, a more direct relationship between *F. nucleatum* and CRC was demonstrated whereby the fusobacterial adhesin FadA binds to E-cadherin on colon cancer cells and activates β-catenin signaling [27]. This pathway leads to increased transcriptional activity of oncogenes, Wnt, and pro-inflammatory cytokines, as well as stimulation of CRC cell proliferation. In vivo relevance was established by the finding that *fadA* gene levels in colon tissue from patients with CRC were >10-fold higher compared with normal individuals.

Conclusions

Both *P. gingivalis* and *F. nucleatum* have attributes consistent with a role in cancer development and progression. The question then

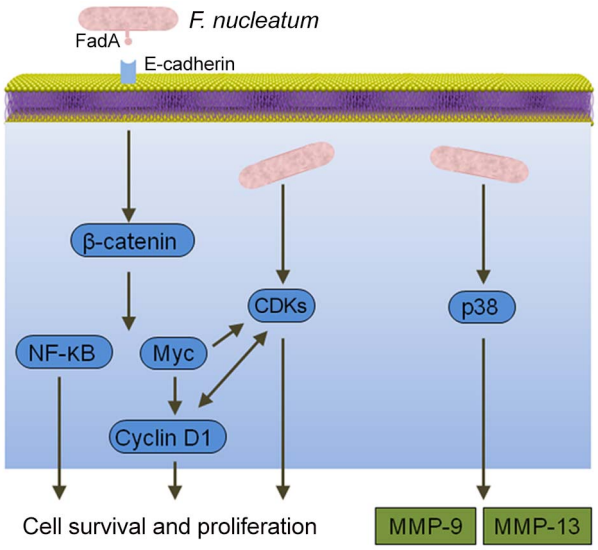

Figure 2. Interactions between *F. nucleatum* and epithelial cells that could produce an oncogenic phenotype. Binding of the FadA adhesin to E-cadherin activates β-catenin signaling, resulting in activation of genes that control cell survival and proliferation. *F. nucleatum* also activates several cyclin dependent kinases (CDKs) and p38, which controls the production of matrix metalloproteases MMP-9 and MMP-13.
doi:10.1371/journal.ppat.1003933.g002

arises as to why the widespread infection with these organisms leads to disease in only a limited number of individuals. Part of the answer may relate to the community nature of oral infections and the potential constraining influence of other bacteria. However, another consideration is the multifactorial etiology of cancer, and, within this framework, specific oral bacteria and their associated inflammatory insults may play a contributory, but not exclusive, role.

The implications of oral bacterial involvement in cancer are many. The detection of *P. gingivalis* or *F. nucleatum* in precancerous lesions could be used as a poor prognosis indicator. Improved oral hygiene and treatment of periodontitis may be useful in limiting the development or spread of cancer. Finally, since well-characterized virulence factors of *P. gingivalis* and *F. nucleatum*, such as the FimA and FadA adhesins, may function as effector molecules in the transition of normal epithelial cells to cancerous cells, they may provide novel targets for therapeutic intervention.

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ORIGINAL PAPER

Use of PCR to detect *Entamoeba gingivalis* in diseased gingival pockets and demonstrate its absence in healthy gingival sites

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Abstract Investigators using light microscopy have identified the protozoan parasite *Entamoeba gingivalis* from diseased gingival pockets for nearly 100 years. The objective of the present investigation was to develop a molecular biology approach for determining the presence of *E. gingivalis* in both diseased gingival pockets and healthy gingival sites. For this, a previously developed conventional polymerase chain reaction (PCR) was evaluated and a real-time polymerase chain reaction assay was developed. Paper points were inserted into the base of the sulcus of both diseased gingival pockets and healthy gingival sites. DNA was extracted using the QIAamp DNA mini kit, and subsequently analyzed using conventional and real-time PCR analysis. A previously described primer set specific for the small subunit ribosomal RNA gene (SSU rDNA) of *E. gingivalis* was used for the conventional PCR. For the real-time PCR, a primer set was designed to amplify a 135-bp fragment inside the SSU rDNA of *E. gingivalis*. A conventional PCR assay detected *E. gingivalis* in 27% of diseased gingival pockets. The real-time PCR using a different primer set detected protozoa in 69% of diseased pocket sites. Thus, the latter technique proved more sensitive for detection of *E. gingivalis*. No *E. gingivalis* were detected in any of the healthy gingival pocket sites using either type of PCR assay. Results support a concept that the presence of *E. gingivalis* is associated only with diseased gingival pocket sites. The newly described methodology may also serve to provide a novel eukaryotic cell marker of disease status in gingival pockets.

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Introduction

Periodontitis with its various clinical forms represents one of the most widely distributed types of oral disease. Approximately 5% to 20% of any population is affected by severe generalized periodontitis (Burt 2005). This inflammatory condition is associated with a chronic bacterial infection caused by anaerobic Gram-negative bacteria (Armitage 1999; Haake et al. 2006; Socransky 1977). For nearly 100 years, light microscopic studies have also demonstrated a high incidence of the protozoan parasite *Entamoeba gingivalis* in individuals suffering from oral disease including periodontitis (Bass and Johns 1915; Barrett 1914). This has led to a speculation that it might also be a contributing factor to periodontal disease. More recently, *E. gingivalis* was identified in all 65 subjects with destructive periodontitis, but was absent in individuals with marginal gingivitis or in excellent periodontal health (Keyes and Rams 1983). Similarly, in 1989 a clinical survey of *E. gingivalis* by multiple sampling in patients with advanced periodontal disease revealed the occurrence of *E. gingivalis* in all ten periodontal patients tested (Linke et al. 1989). However, the average protozoa prevalence (based on microscopic observation) was 62% (62 samplings were positive from a total of 100 samplings). Conversely, on occasion, some have reported the general presence of *E.*

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gingivalis in disease-free individuals (Dao et al. 1983) and in part to be age dependent and related to the amount of calculus present on teeth (Wantland and Laurer 1970). In 1983, it was also reported that there was an association between oral deterioration and *E. gingivalis*. After recovery of *E. gingivalis* from apparently healthy tissue, there would be periodontal decline unless the protozoa were eradicated in the meantime (Lyons et al. 1983). One may also encounter difficulty in identifying the protozoa (Krogstad et al. 1978) and it can be difficult to differentiate *E. gingivalis* from a macrophage (Dao 1985).

In the past decade, newer molecular biology-based identification techniques such as the polymerase chain reaction (PCR) have been successfully used to identify bacteria in the gingival pockets of patients suffering from periodontal disease (Ashimoto et al. 1996; Jervoe-Storm et al. 2005). The PCR can also document the presence of the potential bacterial pathogens before, during, and after disease treatment, and this technology is commercially available to dental practice activities (http://www.hain-lifescience.de/en/products/microbiology.html.).

The development and application of this newer technology to monitor the presence of *E. gingivalis* in periodontitis and its treatment is lacking. Based on light microscopy, there is the well documented close association of this parasite with periodontal disease and its potential to contribute to the condition. Therefore, it might be prudent to develop and use molecular biology methodology such as the PCR to detect and quantify the occurrence of *E. gingivalis* in individuals with periodontal disease. In 1996, DNA oligonucleotides were described that target the small subunit ribosomal RNA gene (SSU rDNA) of *E. gingivalis*, and they were used in the PCR to amplify the DNA of laboratory cultures of *E. gingivalis* (Kikuta et al. 1996). The primers also amplified DNA from subgingival plaque samples of patients.

In the current study, a primer set was successfully used to identify the occurrence of protozoa in gingival pockets of patients diagnosed with periodontal disease. We failed to detect *E. gingivalis* not only in the gingival tissue of healthy patients, but also within healthy gingival pocket sites of diseased patients. Additional insight into a close association between the presence of *E. gingivalis* and periodontitis was supported by development of a real-time PCR assay that allowed for a more sensitive specific detection and quantification of the parasite. *E. gingivalis* is morphologically indistinguishable from *Entamoeba histolytica* (Dao 1985) and can cause a diagnostic problem if found in the sputum of patients studied for pulmonary masses (Dao et al. 1983). Development of a real-time PCR assay for *E. histolytica* was reported in 2005 (Roy et al. 2005).

Here we report the development of a real-time PCR assay for *E. gingivalis* that was more sensitive than the conventional PCR assay for detection of this amoeba. Results of this study further demonstrated that use of molecular biology techniques for detection of *E. gingivalis* may serve to provide a novel eukaryotic cell marker of disease status in gingival pockets. The results also provide a means to further help identify a potential role for this organism in periodontal disease.

Materials and methods

Sampling methodology

The study was in accordance with compliance policy at Middle Tennessee State University. Subjects for the study were recruited from the Advanced Institute for Oral Health (Brentwood, TN). Patients were screened, examined, and selected for participation if they met criteria for sites of periodontal disease and good oral health. Only previously untreated patients presenting with periodontal disease were included in this study. Patients with a history of systemic antibiotic usage within the previous 6 weeks were excluded. Patient sample sites were organized into one of three categories: destructive periodontitis (gingival pockets ≥7 mm, marginal periodontitis (gingival pockets >4 mm), and healthy (gingival pockets <3 mm).

Prior to sampling, the supragingival plaque was removed with a sterile curette, and the sample site was dried with a sterile cotton roll. Sampling of diseased sites was performed prior to mechanical treatment of the pocket. A pair of sterile forceps was used to insert one paper point at a time down to the base of the sulcus. The area which occurs between the tooth and gingiva (gum) and the gum tissue that surrounds the tooth was considered the sulcus (an unusually deep gingival sulcus was considered a periodontal pocket). Samples were collected according to procedures used for PCR detection of bacteria periodontal pathogens from the sulcus (Micro-IDent, Hain Lifesciences, Nehren, Germany). The paper point was transferred into a microcentrifuge tube and assigned a corresponding patient letter. The collection procedure was performed once for detection of protozoa and once for bacteria detection. Samples were stored at −80°C until the DNA extraction was completed. This procedure was completed twice for most patients with the second sample (if taken) sent to Hain Lifesciences (Nehren, Germany) for the Micro-IDent test to detect and quantify bacteria. Samples from disease-free (orally healthy) patients were collected in the same manner; however, no mechanical treatment was required.

Laboratory procedures

A vial of *E. gingivalis* (ATCC 30928) from the American Type Culture Collection (Manassas, VA, USA) was shipped

in TYGM-9 medium. Based on hemocytometer counts, the culture contained 9×10^4 amoebae trophozoites/mL (the vial of xenic *E. gingivalis* from ATCC contained concentrated trophozoites, personal communication). From this, twofold serial dilutions were prepared, and DNA extracted from each dilution to form a standard curve for the real-time PCR assay (Fig. 1).

DNA was extracted from paper points using the QIAamp DNA mini kit (QIAGEN, Hilden, GR) according to the manufacturer's instructions. Extracted DNA was used immediately or stored at −80°C until use. PCR products (15 μl each) were combined with 3 μl loading dye and separated in a 1% agarose gel that included ethidium bromide.

Conventional PCR assay

Primer sets EGO-1 and EGO-2 were used to amplify the SSU rDNA gene of *E. gingivalis* (Kikuta et al. 1996), 18SF and 18SR served as universal eukaryotic (for all eukaryotic cells) SSU rDNA primers (Zhang et al. 2004), and 8F and 1540R served as universal bacterial SSU rDNA primers (Fields et al. 2005). The latter two primer sets were used as positive controls to ensure PCR inhibitors were not present in the samples. All of the samples were positive for eukaryotic and bacterial SSU rDNA, which was used as an indication of the absence of PCR inhibitors. Each 25-μl PCR reaction consisted of 2.5 μl of extracted patient DNA, 2.5 μl of the forward and reverse primers at final concentrations of 0.2 μM each, 17.5 μl of distilled water, and one PuRe Taq Ready-To-Go PCR bead (2.5 units of PuRE Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of each dNTP), stabilizers, and bovine serum albumin (GE Healthcare, Piscataway, NJ).

Real-time PCR assay

Oligonucleotide primers were designed using software from IDT Scitools (Integrated DNA Technologies, Coralville, IA). The primer pair for real-time PCR (Table 1) specifically amplified a 135-bp fragment inside the SSU rDNA of *E. gingivalis* (GenBank accession number D28490). The primers were purchased from Integrated DNA Technologies. Each 25-μl reaction consisted of 2.5 μl of patient DNA, 2.5 μl of the forward and reverse primer, 5 μl of distilled water, and 12.5 μl of Bio-Rad (Hercules, CA) iQ SYBR Green Supermix (25 units iTaq DNA polymerase, 3 mM $MgCl_2$, dNTPs, SYBR Green I, 10 nM fluorescein, buffer, and stabilizers). The *E. gingivalis* SSU rDNA was amplified in a Bio-Rad iQ5 optical system with software version 1.0 under the following conditions: 95°C for 7.5 min, 40 cycles of 95°C for 1 min, 47°C for 30 s, 72°C for 30 s, followed by a 5-min extension at 72°C. A melt curve was included to ensure that only one product was amplified. The primers did not amplify products from other eukaryotic or bacteria cells tested and melt curve analyses performed for all reactions confirmed that the melt curves matched that of the *E. gingivalis* DNA control. The melt curve began at 95°C for 5 min followed by a 0.5°C decrease in temperature every 30 s to 47°C.

Samples were evaluated as positive for the presence of *E. gingivalis* DNA if fluorescence values crossed the cycle threshold (C_T) by 35 amplicon cycles (Fig.1). The C_T value represents the PCR cycle at which fluorescence, and thus DNA, is logarithmically increasing in the reaction. The earlier the fluorescence crosses the C_T, that is the lower the C_T value, the greater the amount of DNA present in the sample. All samples were run in triplicate (as a single replicate). A single plot of C_T value vs the number of *E. gingivalis* trophozoites was made with all dilutions performed in triplicate and the C_T values were averaged. All values that did not cross the C_T were designated as negative.

Micro-IDent bacteria detection and quantification

In some patients (18 of the 26 patients), duplicate paper point samples from diseased gingival pockets were sent to Hain Lifescience (Nehren, Germany). DNA was extracted for PCR and it was used to detect and quantify a variety of anaerobic bacteria associated with periodontal disease.

Results

PCR analysis was used to determine the occurrence of *E. gingivalis* in a diseased gingival pocket for 26 individuals diagnosed with periodontal disease. A gingival pocket from disease-free individuals was analyzed also for comparative purposes. Using conventional PCR methodology, 7 of 26 (27%) diseased pocket sites tested positive for *E. gingivalis* based on the presence of a 1.4-kb PCR product (Fig. 2). Subsequently, DNA extracted from the same pockets was tested for the presence of *E. gingivalis* using real-time PCR methodology with a different primer set. Using this assay, 18 of the 26 (69%) diseased pocket sites tested positive based on the presence of a 135-bp real-time product. Comparisons of the conventional and real-time PCR results are summarized in Table 2. PCR analysis (both a conventional and real-time PCR) was also used to determine the occurrence *of E. gingivalis* in healthy gingival pockets from five disease-free individuals and in healthy gingival pocket sites of seven patients diagnosed with periodontal disease. No *E. gingivalis* were detected in any of the 12 healthy gingival pocket sites tested.

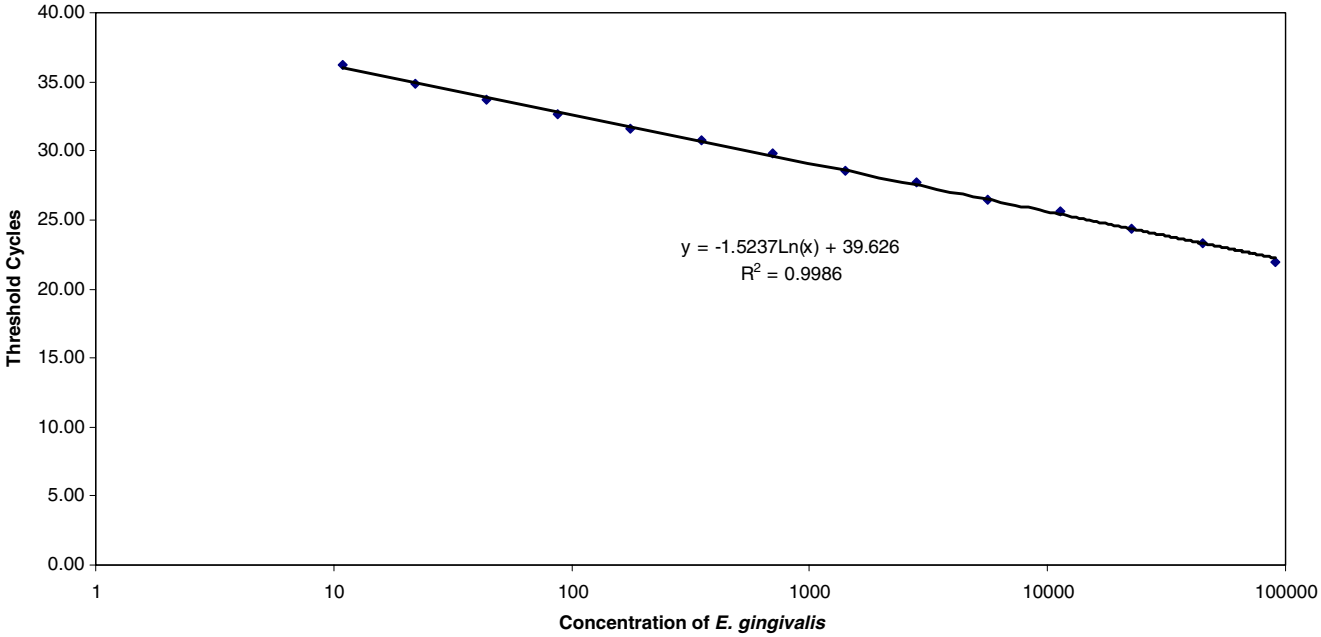

Fig. 1 The plotted C_T value for extracted DNA from twofold serial dilutions of *E. gingivalis* trophozoites. Using a C_T value of 35 as the upper limit of detection, as few as 20 trophozoites can be detected by real-time PCR

The Micro-IDent bacteria detection and quantification provided information on the potential occurrence of 11 different genera of bacteria. *Fusobacterium nucleatum/periodonticium* was reported from the diseased pocket of all of the 18 patients tested for bacteria (Table 3). The presence and relative number of the other genera of bacteria varied from one patient to another without distinct association with *E. gingivalis* (ANOVA using coded data; $P<0.0001$).

Discussion

Among the *Entamoeba* species, only *E. histolytica* is a well-documented human pathogen. The ability to document other *Entamoeba* species at diseased human sites requires accurate and efficient detection methodology. The potential to use the PCR for detection of oral amoebae that might contribute to disease development is supported by commercially available PCR technology to detect periopathogenic bacteria in the gingival sulcus (Micro-IDent, Hain Lifesciences). Riboprinting analysis has revealed phylogenetic relationships in the genus *Entamoeba* (Clark and Diamond 1997). It joined restriction site polymorphism analysis and rDNA amplification to determine sequence variation in SSU rDNA and was initially described by this term in 1991 (Clark and Diamond 1991). Two *E. gingivalis* oral isolates had the same riboprint pattern and one of these (ATCC 30928) was the same strain used in the current

Table 1 Primer sets for conventional and real-time PCR

Primer set	Sequence	Product size (bp)	Purpose
18SF	5′-GCTTGTCTCAAAGATTAAGCCATGC	1,800–2,000	Eukaryotic SSU rDNA[a] (positive control)
18SR	5′-CACCTACGGAAACCTTGTTACGAC		
8F	5′-AGAGTTTGATCCTGGCTCAG	1,500	Bacterial SSU rDNA[a] (positive control)
1540R	5′-AAGGAGGTGWTCCARCCGCA		
EGO-1	5′-GAATAGGCGCATTTCGAACAGG	1,400	*E. gingivalis* SSU rDNA[a]
EGO-2	5′-TCCCACTAGTAAGGTACTTACTC		
EGHF	5′-TACCATACAAGGAATAGCTTTGTGAATAA	135	*E. gingivalis* SSU rDNA[b]
EGHR	5′-ACAATTGTAAATTTGTTCTTTTTCT		

SSU rDNA small subunit ribosomal RNA

[a] Used in conventional PCR

[b] Used in real-time PCR

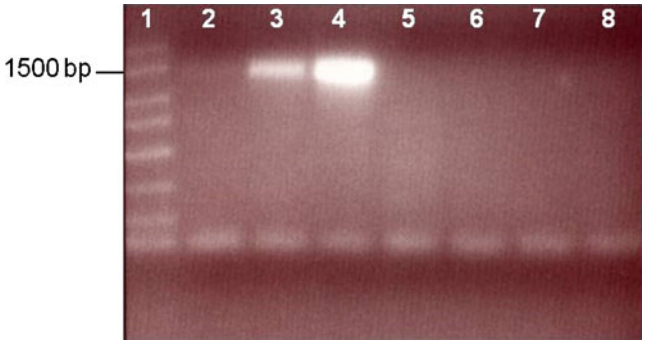

Fig. 2 Amplification of a 1.4 kb PCR product from an *E. gingivalis* laboratory culture and a positive patient sample from a diseased gingival pocket. *Lane 1* is the standard ladder (PCR marker, Sigma-Aldrich, St. Louis, MO). *Lane 2* is a negative control (water). *Lane 3* is a positive control (*E. gingivalis* from approximately 500 trophozoites of an ATCC culture). *Lane 4* is a positive patient sample, and *lanes 5–8* are negative results from patients

study. A variant riboprint was observed in an *E. gingivalis* (ATCC 30956) isolated from a uterine infection. Additionally, the oral isolates may have two forms of the SSU-rRNA gene. Thus, two ribodemes exist within *E. gingivalis* (Clark and Diamond 1997). It was concluded, however, that plural ribodemes within a species did not merit recognition as distinct species. These differences likely are not a reflection of different habitats. Rather, riboprinting suggested the presence of variant genes within an individual *E. gingivalis* isolate. Riboprinting, however, will miss a significant amount of sequence variation and the genetic distances between *Entamoeba* species based on riboprinting are large.

The role of this potential sequence variation in PCR-based detection methodology remains to be clearly defined. A previous investigation and the current study using clinical specimens support the potential of PCR technology for use to identify *E. gingivalis* in diseased clinical samples. The results for the real-time PCR increased sensitivity suggest this might be of greater preference over the conventional PCR.

A conventional PCR assay that amplified DNA from laboratory cultures of *E. gingivalis* and clinical samples has been previously reported (Kikuta et al. 1996). When tested against nine other species of protists (including *Entamoeba* species), ten species of oral bacteria, and human leukocytes, no PCR product of any length was produced. Thus, the primers used (EGO-1 and EGO-2) were considered specific for *E. gingivalis*. Subsequent PCR testing of subgingival plaque samples (one curette scrape) from eight patients with marginal periodontitis or gingivitis resulted in an amplification product in two samples. No specific DNA amplification occurred from 20 supragingival samples from healthy humans. It was concluded that the EGO-1 and EGO-2 primers detected as few as 30 *E. gingivalis* cells in reaction mixtures and could be applicable to clinical use (Kikuta et al. 1996). In this report, 6.25% (2 of 32 subgingival plaque samples) of patient samples proved positive by PCR analysis.

Table 2 Comparison of the conventional PCR and real-time PCR for detection of *E. gingivalis* in a diseased gingival pocket from 26 patients diagnosed with periodontal disease

	Positive	Negative
Conventional	7	19
Real-time PCR[a]	18[a]	8

PCR polymerase chain reaction

[a] All real-time PCR patient samples were also positive by conventional PCR

For the present study, the EGO-1 and EGO-2 primer sets were used to detect *E. gingivalis* in patients with periodontal disease. Here, 27% (7 of 26 diseased pocket sites) tested positive. No positives occurred from healthy gingival pockets (even healthy gingival pocket sites within patients suffering from periodontal disease). This further supports a concept that with conventional PCR analysis, *E. gingivalis* specifically associated with the presence of periodontal disease. The higher incidence (percent positive) of positives samples in the current study could be a reflection of differences in sample collection. In the study by Kikuta et al. (1996), subgingival plaque samples were composed of one curette scrape dispensed in 100 μl. In the present study, samples for PCR testing consisted of insertion of a paper point to the base of the sulcus for sample recovery.

For the present study, the authors successfully modified the conventional PCR assay to detect *E. gingivalis* in patients with periodontal disease. Subsequently, a real-time PCR assay was developed. Advantages of real-time PCR over conventional PCR are that it is performed in a closed system where post-PCR handling is not required, it is highly sensitive, and can be used for quantitative purposes. Since more patients were found to be positive for *E. gingivalis* with the real-time PCR assay, it should be considered to be more sensitive for the detection of *E. gingivalis*. No *E. gingivalis* were detected when healthy gingival pocket sites from periodontal disease patients were tested by conventional or real-time PCR. Additionally, no *E. gingivalis* were detected from gingival pocket sites from individuals identified as having good oral health. Collectively, the real-time PCR results also supported the concept that *E. gingivalis* were specifically associated with periodontal disease.

The possibility that *E. gingivalis* might elaborate proteolytic enzymes that could contribute to the pathogenesis of periodontitis is not a novel hypothesis (Gottlier and

Table 3 Micro-IDent detection and quantification of bacteria present in 18 different patients with *Entamoeba gingivalis*

Patient	Aa	Pg	Tf	Td	Pi	Pm	Fn	Cr	En	Ec	Cs
A	−	+	++	+	+	+	++	+	−	+	+++
B	−	++	++	+	−	+	++	++	−	(+)	(+)
C	++	−	−	−	−	+	++	−	−	−	(+)
D	−	+	++	+	+	−	++	−	−	++	(+)
E	−	(+)	++	(+)	−	(+)	++	−	++	−	(+)
K	−	−	+	−	−	(+)	++	(+)	−	+	−
L	−	−	−	+	−	(+)	+++	−	−	++	−
M	−	++	−	−	−	−	++	(+)	−	+	+
O	(+)	+	+	(+)	−	(+)	++	(+)	−	++	+
P	+++	++	++	+	−	(+)	++	+	(+)	+	(+)
Q	−	+	++	(+)	−	(+)	++	+	(+)	+	−
R	−	−	++	+	++	++	++	(+)	++	+	−
U	−	−	−	−	−	−	(+)	−	−	−	−
V	−	−	++	+	−	+	++	(+)	−	+	+
W	−	++	++	++	(+)	+	++	+	(+)	(+)	−
X	−	−	++	++	−	−	++	−	−	+	−
Y	−	++	+	−	−	(+)	++	−	−	−	−
Z	(+)	++	++	+	++	(+)	++	+	+	++	−

ANOVA showed significant differences between all 11 species ($P<0.0001$)

−=<10^4, (+)=10^4,+=<10^5, ++=<10^6, +++=>10^7

Aa Actinobacillus actinomycetemcomitans, *Fn Fusobacterium nucleatum/periodonticum*, *Pg Porphyromonas gingivalis*, *Cr Campylobacter rectus*, *Tf Tannerella forsythia*, *En Eubacterium nodatum*, *Td Treponema denticola*, *Ec Eikenella corrodens*, *Pi Prevotella intermedia*, *Cs Capnocytophaga* sp. (*gingivalis, ochracea, sputigena), Pm Peptostreptococcus micros*

Miller 1971). Previous studies with the closely related *E. histolytica* have clearly identified virulence factors that contribute to and result in tissue destruction. Cysteine proteases serve as virulence factors and are important proteolytic enzymes in parasitic protozoa (Sajid and McKerrow 2002). *E. histolytica* contains 20 cysteine protease genes and orthologous sequences were also present in *Entamoeba dispar* (Bruchhaus et al. 2003; Tillack et al. 2007). Cysteine protease inhibitors greatly impacted the ability of *E. histolytica* to produce liver abscess development in laboratory animals (Li et al. 1995; Stanley et al 1995). If *E. gingivalis* possesses similar genes to express cysteine proteinases, it would suggest a new factor to consider in treating periodontal disease.

Additional virulence factors included the galactose/N-acetyl D-galactosamine-inhibitable (Gal/GalNAc) adherence lectin (Petri et al. 2002) and phospholipases (Ravidn 1986). Lipase increases were also associated with *E. histolytica* energy metabolism adaptation to the host intestinal environment (Gilchrist et al. 2006).

During the last 20 years, a variety of laboratory studies have demonstrated that some obligate and facultative intracellular bacteria pathogens benefit from associations with facultative pathogenic amoebae in the genera *Naegleria* and *Acanthamoeba*. Amoebae can serve as host cells for bacteria in the genera *Legionella*, *Parachlamydia*, *Listreria*, and other intracellular bacteria pathogens (Greub and Raoult 2004). Amoebae can also serve as host cells to protect bacteria from detrimental environmental factors. Interaction with amoebae may promote expression of virulence traits for *Legionella pneumophilia* and *Mycobacteria avium* (Cirillo et al. 1997; Cirillo et al. 1994). The likelihood bacteria could reside within cells of *Entamoeba* species were suggested in xenically (with associated intestinal flora) cultured *E. dispar* and *E. histolytica* (Pimenta et al. 2002). Several bacteria were always identified within vacuoles of the latter. In *E. dispar*, however, only single bacterium occurred in vacuoles and on occasion bacteria were free in the cell cytoplasm. Studies addressing potential interactions between periopathogenic bacteria and *E. gingivalis* are lacking. However, since both occur in diseased gingival pockets, interactions would be expected to occur. In one in vitro study, it was reported that *Actinobacillus actinomycetemcomitans* was affected by the presence of unidentified oral amoebae. The amoebae enhanced the growth of *A. actinomycetemcomitans* in media which otherwise failed to meet nutritional requirement (Derderian 1991). The bacteria were found around the exterior of amoebae and appeared to be localized in its vacuoles. Although amoebae consume bacteria as a food source, some bacteria may survive phagocytosis and multiply within amoebae (Greub and Raoult 2004). It was suggested that this interaction could be potentially significant since bacteria harbored inside amoebae could be protected from the immune system or antibiotics which are given as a part of therapy during periodontal treatment. In the absence of periodontal disease treatments which

might eliminate *E. gingivalis*, bacteria sheltered within amoebae could exit the amoebae to reseed the tissues or the sulcus and possibly create a refractory case (Derderian 1991).

There are several challenges ahead for determining the exact role of *E. gingivalis* in periodontal disease. The organism is extremely difficult to culture and it cannot be cultured in the absence of bacteria (Gannon and Linke 1992). Some bacteria accompanying the xenic cultures are detrimental to growth of *E. gingivalis* while other bacteria are beneficial to trophozoite growth (Gannon and Linke 1992). Additional insight into the potential roles of *E. gingivalis* in periodontal health could be addressed by using molecular biology techniques to identify potential virulence factors in clinical isolates and characterize its interactions with periopathogenic bacteria in laboratory studies using in situ hybridization to detect periopathogenic bacteria that might be localized in the amoebae.

Periodontal disease may be a reflection of the interplay between several etiological agents and environmental factors. Many antibiotics given to treat periodontal disease would have no effect on protozoa. Often, periodontists observe reductions in bacteria within the gingival socket, but the patient will still have symptoms that indicate active disease. A real-time PCR assay for *E. gingivalis* could measure parasite loads and determine if treatments are efficacious in elimination of amoebae. Results of this investigation also provide a framework to help assess a potential etiological role for *E. gingivalis* in periodontal disease.

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Periodontal disease may associate with breast cancer

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Abstract

Purpose The main purpose was to evaluate the association between periodontal disease and the incidence of breast cancer in a prospective study of 3273 randomly-selected subjects aged 30–40 years at baseline.

Methods Breast cancer incidence was registered from 1985 to 2001 according to the WHO International Classification of Diseases criteria. At baseline, 1676 individuals also underwent a clinical oral examination (Group A) whereas 1597 subjects were not clinically examined but were registered (Group B). The associations between breast cancer, periodontal disease and missing molars were determined using multiple logistic regression models with several background variables and known risk factors for cancer.

Results In total 26 subjects in group A and 15 subjects in group B had breast cancer. The incidence of breast cancer was 1.75 % in subjects who had periodontal disease and/or any missing molars, and 0 in subjects who had periodontal disease but had no missing molars. For periodontally healthy subjects with no missing teeth the breast cancer incidence was 1%. For Group B the respective incidence was 0.94%. Female gender (odds ratio [OR] 13.08) and missing any molar in the mandible (OR 2.36) were explanatory variables for breast cancer. Of the subjects with periodontal disease and any missing molars in the mandible 5.5% had breast cancer in comparison to 0.5% of the subjects who had periodontal disease but no missing molars in the mandible ($p<0.02$).

Conclusions Chronic periodontal disease indicated by missing molars seemed to associate statistically with breast cancer.

Introduction

Inflammation is a key feature in many chronic diseases including cancer [1-2]. Inflammation caused by infections seems to be one of the most important preventable causes of cancer in humans [3]. Periodontal disease is characterized by chronic infection and inflammation leading to destruction of the bone surrounding the teeth. The disease may take decades to develop and it ultimately leads to tooth loss [4-5]. An estimated 15-35% of the adult population in industrialized countries suffers from this multifactor disease [6-8]. Periodontal disease involves complex interactions of host defence, bacteria, and virus. Periodontal disease is initiated by a biofilm of bacteria on the teeth which triggers an immune-inflammatory response in the adjacent host tissues [4, 9] . Numerous oral bacterial species have been associated with periodontal disease [10-11] .

Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and HCMV-EBV co-infection seems to be closely associated with disease-active periodontitis. [12-14] . Infection by HCMV or EBV seems to inhibit the macrophages to respond to bacterial challenge, and thus have pathogenic role in the development of periodontal disease. [15] The associations between infectious agents of putative bacterial pathogens, herpes viruses, Epstein-Barr virus (EBV) type 1, cytomegalovirus (CMV) seems to play an important synergistic role in the pathogenesis of chronic periodontitis. [16-18] .

Breast cancer is the most common malignancy in women worldwide and it is the leading cause of cancer-related mortality [19]. Incidence rates are high in developed countries and are also increasing in the developing world [20]. National income and health expenditures are known to affect breast cancer incidence but these factors only partly explain variations in mortality rates [20]. Viral infections such as human papilloma virus infections seem to associate with breast cancer as shown in a recent meta-analysis [21] In general, persistent and prevalent infections in populations appear to link with malignancies [22]. In Sweden breast cancer represents 29% of all cancers in the female [23]. Breast cancer also has a hereditary component [24].

The results of an epidemiological study 2001, from our group, showed that fairly young individuals with periodontal disease and missing molars are at increased risk of premature

death caused by life threatening diseases such as malignant neoplasm, cardiovascular diseases, and diseases of the digestive system [25]. Recently, periodontal disease was also found to associate with head and neck cancer in patients who never smoked or consumed alcohol [26]. Poor oral health has been statistically associated with the prevalence of many types of cancer such as pancreatic and gastrointestinal cancer [27]. Consequently, the hypothesis for the present study was that a low degree chronic inflammation such as seen in periodontal disease is involved in carcinogenesis.

For that reason the specific aim of the present investigation was to study the incidence of breast cancer from 2001 in subjects with periodontal disease and characteristic tooth loss in our 16-year prospective investigation.

Materials and Methods

Study population

In 1985 we undertook this longitudinal prospective study, which comprised a random sample cohort of 3273 individuals aged 30–40 years. The subjects were selected from a registry database of all inhabitants (n = 105,798) of Stockholm County born on the 20th of any month from 1945 to 1954 inclusive. They were informed about the purpose of the study and offered a clinical oral examination. In total, 1676 individuals (51.2%; 838 men and 838 women) underwent a detailed clinical oral examination (Group A). The remaining 1597 subjects (849 men and 748 women) did not have the clinical examination and constituted Group B. Figure 1 shows the study profile.

Clinical examination

For all subjects of the clinically examined Group A the following parameters were recorded: the number of remaining teeth excluding third molars; gingival inflammation around every tooth as assessed using the gingival index [28]; and oral hygiene status as determined by using both the dental plaque [29] and the calculus indices to assess all six surfaces of six representative teeth. Pocket depth was determined using a periodontal probe and recorded to the nearest higher millimetre for six sites of each tooth. Presence or absence of each tooth was recorded. The prevalence of periodontal disease was determined in each age group year by year from 31 years of the age to 40. All subjects in Group A also filled in a structured

questionnaire containing questions about factors such as regular dental visits and the use of tobacco. Smoking was divided into smokers and former smoker categories. The smokers reported the number of cigarettes per day, and the number of years of smoking. Former smokers reported when they had quit smoking and also the number of cigarettes per day and years of smoking. Smoking was then further analyzed as pack-years. The Ethics Committee of the Karolinska Institute and Huddinge University Hospital, Sweden, approved the study protocol. The study is in accordance with the Declaration of Helsinki.

Cancer and socio economic data

The data for cancer (malignant neoplasm) and causes of death were obtained from the Centre of Epidemiology, Swedish National Board of Health and Welfare, Sweden. The data were classified according to the WHO International Statistical Classification of Diseases and Related Health Problems ICD-7, ICD-9 and ICD-10. Socio-economic data were obtained from the National Statistics Centre, Örebro, Sweden.

Statistical analyses

Analysis of variance, chi-square test, Fisher´s exact t-test and multiple logistic regression analyses were applied when appropriate. Multiple logistic regression analysis was used to compare the incidence of cancer, according to the state of oral health at baseline, while simultaneously controlling for confounding variables. The confounding variables included age, gender, education, income, socioeconomic status, smoking habits, and dental visits. Smoking habits were dichotomized into the number of smokers (ever smokers) and the number of never smokers. The model with the confounders was correlated to the incidences of cancer. A backwards elimination method was used to control for multicollinearity (correlation between confounders). The statistical model was tested according to Cox & Snell [30] and Nagelkerke [31].

Results

In the total cohort of 3273 subjects, 41 subjects (1.3 %) were diagnosed as having breast cancer; comprising 39 women and 2 men. Breast cancer had been diagnosed at the mean age of 45.8 ± 6.8. In the clinically examined Group A comprising 1676 subjects, 26 persons (1.6%) had breast cancer; 24 women and two men; whereas in Group B with 1597 subjects, 15 women (0.9 %) had breast cancer.

The prevalence of periodontal disease in Group A significantly increased year by year, in women from 9.8 % at the age of 31 to 25.6 % at the age of 40 ($p<0.001$) and in men from 8.5% at the age of 31 to 35.0 % at the age of 40 ($p<0.001$). In total 17.1% had periodontal disease diagnosed at the 1985 baseline examination.

Of the subjects with breast cancer 12 subjects were smokers (42.8%); 5 were former smokers (17.9%), whereas 11 had never smoked (39.3%). Of the remaining 1648 subjects with no breast cancer 605 were smokers (37.7%), 432 were former smokers (26.2%), and 611 had never smoked (37.1%). For the subjects with breast cancer, a mean of 2411.3 ± 2811.4 S.D. pack-years of smoking was calculated; for those with no breast cancer the respective pack-years were 3643.0 ± 5195.7 S.D. The difference between the groups was statistically not significant (NS).

The demographic and oral health data at baseline in 1985 for subjects with and without periodontal disease are shown in Table 1. The demographic data and oral health data in 1985 for subjects with or without breast cancer in 2001 are shown in Table 2. In the multiple logistic regression model with breast cancer as the dependent variable, female gender appeared to be the principal independent predictor associated with 13.08-fold the risk for breast cancer. Those subjects with any missing molar had 2.36-fold the risk for breast cancer. The results are given in Table 3. The incidence figures for breast cancer for the clinically examined Group A showed that 1.75% of those with any missing molar had breast cancer whereas only 0 % of the subjects with no missing molar had cancer (Table 4). The difference in the prevalence of breast cancer for subjects with periodontal disease and with or without any missing molar in the mandible was significant ($p<0.02$), see Table 5.

Discussion

This study addressed the issue of chronic dental infections, and periodontal disease in particular, as a risk factor for breast cancer. Our results clearly identified periodontal disease and loss of any molar from the mandible as an independent predictor for breast cancer. Consequently, our study hypothesis based on the paradigm of chronic infection/inflammation *vs.* breast cancer was confirmed.

Some comments should be made concerning the reliability of the results. Our subjects were randomly chosen to avoid selection bias. The large subject pool was representative of the ethnically homogenous Swedish adult population, with an age range of 10 years to limit the influence of age differences. The study had a longitudinal prospective design with a cohort of subjects of whom many had periodontal disease documented at baseline 16 years earlier. The participants were born on the 20th of any month between 1945 and 1954 and therefore it was possible to get the diagnosis of breast cancer for each participant up to the year 2001 from the National Cancer Registry Centre of Epidemiology, Swedish National Board of Health and Welfare. This registry collects cancer data from all subjects in Sweden born on the 20th of any month.

Further comments should be made about the missing molars which in this study were used as a proxy for chronic dental infections. The first permanent teeth to erupt are the first and second permanent molars [32]. The newly erupted molars can be infected by oral bacteria from the primary dentition or by intra-familial transmission [33-34]. Our study subjects who had lost their molars already by 1985 had most probably been suffering from periodontal disease for many years. Namely, the reason for molar tooth extraction is either due to dental caries or periodontal disease. The reason could not be identified in our data. However, dental caries is also a chronic infectious disease leading to pulp infections and periapical abscesses with subsequent systemic spread of micro-organisms. Thus the end result of both periodontal disease and caries is a systemic infection burden and often leading to tooth extraction. Hence we felt justified to use missing molars as a proxy for long-lasting dental infections in our analyses.

The inevitable increase of periodontal disease with age poses a threat to large patient groups who then will be exposed to chronic infections with systemic consequences. Hence, periodontal disease may indeed contribute to the increasing incidence of breast cancer in middle aged and older people. The development of cancer obviously depends on differences in the characteristic carcinogenesis and thus the impact of the role infection and inflammation depends on the etiopathogenesis of the neoplasm in question.

Our findings may nevertheless have importance in the ongoing discussion about life style and the risk of cancer. The results also have public health consequences. There is a well warranted basis for prophylactic measures against the prevalence and outcome of periodontal disease in

addition to the costs it incurs to societies. Even if periodontal disease is only one contributor to the carcinogenic process a strategy to remove this burden might reduce the incidence of breast cancer worldwide.

Conclusions

This is to our knowledge the first study presenting data about the eventual risk that long-standing dental infections may present risk for breast cancer. Chronic periodontal disease indicated by missing molars seemed to associate statistically with breast cancer. If our results will be confirmed in future studies the finding should have clinical consequences in advising the patients.

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Fig. 1 Study profile

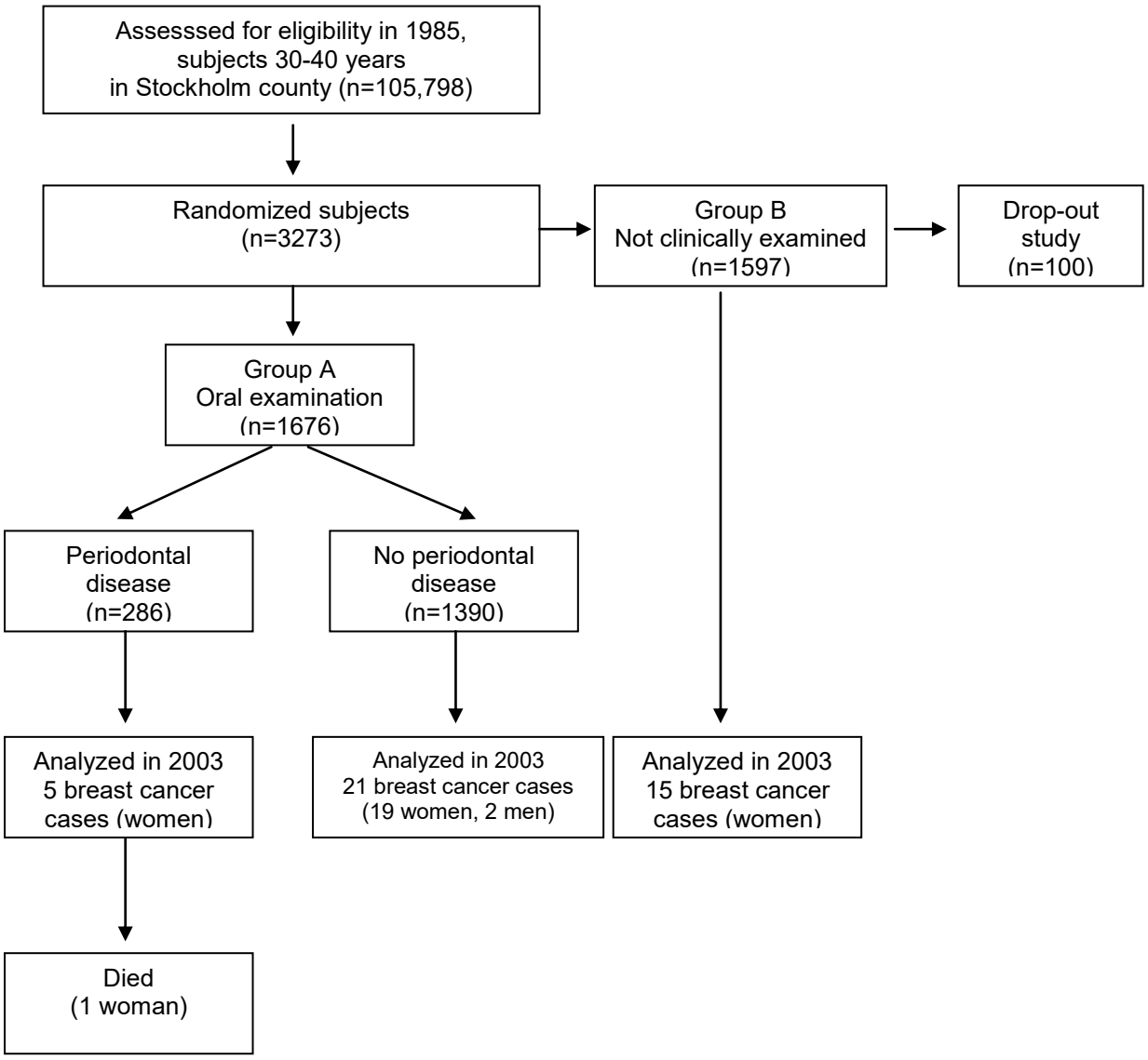

Table 1 Demographic clinical oral health data of subjects of Group A with or without diagnosed periodontal disease at the baseline examination in 1985.

	No periodontal disease (n =1390) number, mean ± SD	Periodontal disease (n = 286) number, mean ± SD	*p**
Gender (female/male)	159/825	127/851	<0.05
Age in 1985 (years)	35.6 ± 2.8	36.5 ± 2.8	<0.001
Education (compulsory/higher)	218/1172	73/286	<0.001
Smoking (pack-year)	3315.5 ± 5041.2	5060.5 ± 5455.7	<0.001
Income (Swedish Crowns x 1000)	188.5 ± 102.9	186.4 ± 89.3	NS
Plaque index[28]	0.67 ± 0.47	0.99 ± 0.53	<0.001
Gingival inflammation[29]	1.18 ± 0.47	1.76 ± 0.54	<0.001
Calculus index	0.39 ± 0.54	0.80 ± 0.73	<0.001
Missing teeth	1.19 ± 2.37	1.59 ± 2.40	= 0.01
Missing molars in the mandible	0.30 ± 0.74	0.44 ± 0.89	<0.01

* Fisher´s exact t/test or Student's t-test for unpaired samples as appropriate.
Data are expressed as mean ± SD.

Table 2 Demographic and clinical oral data for subjects with periodontitis 1985, with and without breast cancer 2001

	Periodontitis 1985 without breast cancer 2001 (n = 281) number, mean ± SD	Periodontitis 1985 with breast cancer 2001 (n = 5) number, mean ± SD	*p**
Gender (male/female)	0/161	5/125	= 0.01
Age in 1985 (years)	35.7 ± 2.9	36.6 ± 2.5	NS
Education (compulsory/higher)	70/281	3/5	NS
Smoking (pack-year)	5107.1 ± 5482.6	2445.5 ± 2814.3	NS
Income (Swedish Crowns x 1000)	178.7 ± 92.2	150.2 ± 53.0	NS
Plaque index[28]	0.90 ± 0.537	0.87 ± 0.61	NS
Gingival inflammation[29]	1.76 ± 0.54	2.12 ± 0.45	NS
Calculus index	0.80 ± 0.72	0.97 ± 0.62	NS
Number of deep pockets(>5mm)	5.1 ± 5.1	8.6 ± 3.0	NS
Number of remaining teeth	26.5 ± 2.3	23.6 ± 5.6	< 0.01
Number of missing molars	0.7 ± 1.4	2.4 ± 2.6	= 0.01
Number of missing molars in the mandible	0.4 ± 0.9	1.6 ± 1.5	= 0.003
Number of missing front teeth	0.7 ± 1.4	2.4 ± 2.6	= 0.01

Fisher´s exact t/test or Student's *t*-test for unpaired samples as appropriate.
Data are expressed as mean ± SD

Table 3 Results of the multiple logistic regression analysis. Breast cancer was the dependent variable and age, gender, education level, socio economic status, working history, yearly income, smoking in pack-years, dental appointments, dental plaque index, gingival bleeding index, and loss of any molar tooth in the mandible were used as explanatory variables.

Dependent variable	Explaining variable	ß	X^2	*p*	Odds ratio	95% confidence interval
Breast cancer	Gender (female)	2.57	12.21	<0.001	13.08	3.09 - 55.32
	Missing any molar from the mandible	0.86	4.55	0.033	2.36	1.07 - 5.21

Cox & Snell R^2= 0.017; Nagelkerke R^2 = 0.11

Table 4 Percentage of breast cancer 2001[†] for subjects in A with periodontitis and periodontitis with missing any molar as well as subjects with no periodontitis and no missing teeth and subjects in B.

A			B
(n=1676)			(n=1597)
Breast cancer			Breast Cancer
(n=26)			(n=15)
	Periodontitis		
	(n=286)		
Periodontitis	Periodontitis	No periodontitis	
and	(n=96)	(n=1390)	
missing any molar			
(n=190)			
Breast cancer	Breast cancer	Breast cancer	Breast cancer
(n=5)	(n=0)	(n=21)	(n=15)
1.75 %	0	1.51 %	0.94 %

Group A was clinically orally examined whereas Group B data refer register information only.

[†] According to International Classification of Diseases, ICD-8, ICD-9 in1985-1996 and ICD-10 in 1997-2000.

Table 5 Percentage of breast cancer in subjects with periodontal disease with or without any missing molar teeth.

	Missing molars in the mandible (n=73)	No missing molars in the mandible (n=213)	p
Percent	5.48	0.47	<0.02

17

Biocompatible Periodontal Therapy

Received……...2/28/99 Scientific Review…….....3/5/99 IAOMT Board Review…….3/17/99 Reevaluation……..…3/30/00, 10/9/05	**Biocompatible Periodontal Therapy**	**Approval…….……...3/30/00** Provisional Approval No Opinion No Approval
Explanation of IAOMT position: This SR is intended as a framework through which a comprehensive and organized Biocompatible Periodontal Therapy program may be developed to clarify and define this difficult dental discipline. As such, this SR should be a living, changing document as new information is available.		

Name of Scientific Review: Biocompatible Periodontal Therapy
Alternative name(s) of Scientific Review: n/a
This Scientific Review is related to Dentistry
This Scientific Review is a Procedure
Purpose of the Scientific Review: To clarify the proper methods of diagnosis, treatment, maintenance and prevention of periodontal disease.
Scientific Review History: Began with the work of Keyes (NIDR) and Ramfjord (U. of Michigan) in the 1960's
A brief description of the Scientific Review: The establishment of parameters for determining the etiology, diagnosis, treatment and prevention of periodontal disease and reduction of the systemic risk it poses to patients.
A specific description of this Scientific Review: See "Appendix A"
Manufacturer(s): N/A
Scientific Literature: See "Appendix C"
Legal Aspects of this Scientific Review: Defined by decades of evidence-based research and clinical studies**.** This Standard of Care will be the guideline of future protocols and preferred procedures.

Appendix A

IAOMT Committee on Periodontal Therapy

Format for series of Standards of Care

Periodontal disease is an infection --- "an invasion by pathogenic microorganisms of a bodily part in which the conditions are favorable for growth, production of toxins, and resulting injury to tissue." (Webster's II New Riverside University Dictionary). Pathogens of bacterial, protozoan, viral or fungal origin have been implicated as causal factors in periodontal disease. Its clinical symptoms and progression indicate the body's defenses are being challenged, and that the immune system is unable to adequately defend against the invaders. It is also important to note that recent research has demonstrated serious cardiovascular and other health risks associated with high levels of proteolytic enzymes and endotoxins produced by the pathogens most commonly associated with active periodontal disease.

Periodontal disease is a long-term chronic degenerative disease. It is often refractory, in that it may be periodically active or dormant depending on the effect that environmental or acquired risk factors (e.g., smoking and poor nutrition) have on host immuno-inflammatory response to the microbial challenge.

Because the understanding of periodontal disease has improved dramatically, treatment methods have been changing. Today the treatment of choice takes into account both local factors and systemic risk factors, and treats causes, not just effects. The goal is to help patients achieve optimal long-term periodontal health and maximize their resistance to periodontal infection. The treatment of choice is no longer removal of healthy or potentially healthy body parts.

Phases of Biocompatible Periodontal Therapy:

- Diagnosis
- Treatment
- Maintenance and Prevention

I. Diagnosis

A. **Clinical tests**

1. Periodontal Probing: Sulcus depth by itself does not constitute disease or health. Shallow sulci are not necessarily healthy or protective. If periodontal disease results in deeper pockets, it obviously originates in shallow pockets. Absolute probing depths are not predictive of future attachment loss. Changes in attachment >2mm over time, however, are pathologic. Probing sites exceeding 3 mm should be considered to be at greater risk, but increased pocket depth, per se, does not constitute disease and many deep pockets may be free of infection.

2. Tissue Tone: Periodontal tissue should be pink and firm, highly stippled and knife edged or it is possibly pathologic. Edema and erythema, however, are not dependable signs of periodontal disease since they may result from other causes, including systemic medications (e.g. the diuretics used by 20% of adults, anti-convulsants, and calcium channel blockers), local trauma, transient hormonal effects and other syndromes.

3. Bleeding on Probing or Manipulation: Bleeding from the gingiva is not healthy any more than bleeding from any other body tissue. It is not, however, predictive of future attachment loss as it is frequently associated with and confounded by non-periodontal causes. If blood can get out, pathogens can get in.

4. Odor or purulent discharge: A fetid odor and metallic taste are the classic symptoms of periodontal infection. Contributing factors may include poor oral hygiene, the presence of anaerobic pathogens in the depth of the sulcus, interproximal stagnation due to lack of circulation in the interdental papilla, dietary excesses or deficiencies or other systemic health problems that tend to impair host immuno-competency.

5.Recession or "notching" (abfraction): While not an indication of infection, this may be a sign of occlusal problems. Occlusal problems can overwhelm a periodontium that has lost supporting bone.

6. Mobility: Healthy teeth with a healthy periodontium are not mobile outside of physiologic limits. Malocclusion cannot initiate periodontal disease, but may exacerbate it.

7. Connective Tissue Destruction and Bone Loss: Radiographically, apical migration of the connective tissue attachment and alveolar bone loss are characterized by a lack of cortication of the interproximal alveolar crest and periodontal pocket formation. Although radiographic evidence may indicate that active periodontal infection was present at some time in the past, it does not indicate the presence of active infection nor is it predictive of future attachment loss. Dense cortication of the alveolar crest and a lack of attachment loss are generally regarded as signs of periodontal health.

B. Microbiological tests

1.Microscopic Examination:

a. Phase contrast microscopy is the quickest and most cost-effective clinical method of evaluating microbiological risk factors at individual periodontal sites.

b. Phase microscopy is the only chairside method of determining the relative immune status of patients at individual periodontal sites by analysis of local WBC counts.

c. Phase microscopy is also the only practical chairside method of determining the presence of a wide range of putative periodontal pathogens, including: protozoans (amoebae & trichomonads); treponemes (spirochetes); fungi and yeasts.

d. A number of other risk factors can be identified through microscopy, including: motile microorganisms; colonial patterns; and the relative numbers and proportions of bacterial morphotypes.

e. Approximately 5% of refractory periodontal infections cannot be diagnosed through microscopy. Such infections are often the result of incomplete or inadequate therapy, which eliminates the natural antagonists of otherwise innocuous oral microorganisms. The resultant superinfection may have no microscopically obvious morphological distinguishing characteristics.

2. Culture & Antibiotic Specificity Testing

Cultures for laboratory analysis should be taken in the following circumstances:

a. Whenever the use of systemic antibiotics is contemplated. Many periodontal pathogens are resistant to traditional antibiotics. Culture labs automatically test positive microorganisms for specific antibiotic susceptibility.

b. When phase contrast microscopy *is negative,* and there are obvious clinical signs or symptoms of periodontal disease and its progression.

C. **Diet Pattern Analysis:** If the balance of host immune response can be tipped by the chronic deficiency of a single essential micro-nutrient (e.g., scurvy and lack of vitamin C), and the microbial challenge can be intensified by a diet high in simple sugars, then some basic form of dietary adjustment is in order with the possibility of supplement addition to the diet. Trying to satisfy the body's basic vitamin and mineral needs through the diet is almost impossible without supplementation. A carefully planned Nutritional Program for the Patient is critical to the success of BPT. At a minimum, vitamin C, Coenzyme Q10, Lipoic Acid, and immune system booster and a good multi vitamin/mineral are essential.

Hair Analysis: Helpful many times, particularly with class III & IV periodontitis. This provides a measure of general nutrition status. Hair analysis is of questionable value to patients who use strong bleaching or coloring agents.

Micronutrient Analysis: When diet pattern analysis fails to reveal potential excesses or deficiencies, than a micronutrient analysis by a registered dietitian or nutritionist may be recommended

D.**Medical health Evaluation and Systemic Tests:** Systemic health problems (e.g., diabetes) can cause a deterioration of nerves and blood vessels and can dramatically affect host immuno-competency and resistance to periodontal infection. When local etiological factors and diet patterns cannot explain an impaired or exaggerated soft tissue response to common oral microorganisms, then a medical health evaluation may be recommended.

a. **Blood Tests:** A complete blood count (CBC) measures the amount of hemoglobin, the hematocrit (percentage of red blood cells), the number and kinds of white blood cells and the number of platelets. This test can indicate a wide variety of systemic conditions that may have an important impact on periodontal health. A blood glucose test can also be used to diagnose Type II diabetes (NIDDM) which may negatively affect oral immune-inflammatory response without having any other outward symptoms. Blood testing may also indicate certain nutritional deficiencies.

b. **Urine Tests:** Tests for diabetes and other systemic problems which may impact the oral immune-inflammatory response.

II. Treatment:

A. **Objectives**

1. To disinfect the mouth and eliminate periodontopathic microorganisms.
2. To remove as little healthy tissue (including cementum) as possible. Once the infection has been controlled and the body given a chance to heal itself, re-evaluate the need to excise any residual diseased or necrotic tissue
3. To remove calculus deposits which obstruct access to the base of the pocket or defect.
4. To ensure that the patient is practicing proper oral hygiene and plaque control, has a balanced dietary intake, good nutritional function and no other acquired risk factors such as tobacco use in any form.
5. To ensure proper Nutritional Supplementation to strengthen the immune response.

B. **All Appointments:** Oral health evaluation and disinfection of the periodontium and oral cavity.
Definitions: see Appendix B

C. **Nutritional Status Assessment**: Patient must be assessed and supplemented when appropriate. Periodontal disease is not simply microbial but also the result of immunosuppression. The IAOMT is in favor of elimination or limiting the use of alcohol, tobacco products and refined sugar in the diet. We further recommend the increase of healthy foods and supplements in the diet.

D. **First Appointment**

1. Pre-scaling rinse with an antimicrobial agent to reduce contaminated aerosols & general microbial loads.
2. *Gross scaling* with an ultrasonic scaler to remove bulk debris. Use an antimicrobial agent in lieu of water as coolant to further reduce microbial loads.
3. Subgingival Irrigation to deliver antimicrobial agents to the apical depth of periodontal pockets, reduce or eliminate bacteremia associated with this procedure and to reduce contaminating aerosols and general microbial loads.
4. Co-therapist: Patient is thoroughly instructed in appropriate oral hygiene techniques, including oral irrigation and brushing. Patient *must* be willing to follow a meticulous regimen of home care and proper nutrition to support professional treatment.
5. Professional Sequence of Care - Alternative Treatment Philosophies
   a) Conservative Option: Mechanical debridement and local antimicrobial agents used. Systemic antibiotics used only if local measures fail to eliminate the infection.
   b) Aggressive Option: Systemic antibiotics prescribed ASAP (after culture and antibiotic specificity testing is complete) in advanced disease for early and optimal elimination of pathogens.
   c) Home Care and Nutritional Option: Professional care instituted only after patients nutritional needs have been addressed and the patient has instituted proper oral hygiene techniques.

E. **Subsequent Appointments**

1. Re-evaluation of the effectiveness and compliance with home care measures via phase microscopy.
2. Pre-scaling irrigation with an antimicrobial agent to reduce contaminated aerosols & general microbial loads.
3. Definitive quadrant scaling. Ultrasonic scalers may substitute for traditional manual scaling. Antimicrobial agents should be used in lieu of coolant.
4. Subgingival irrigation with antimicrobial agents of all quadrants during each quadrant appointment.

5. Possible antibiotic therapy using the antibiotics indicated as effective with the results of culture and sensitivity testing.
6. When an antibiotic is given, it should be recommended to the patient that they take a Probiotic with the antibiotic according to the manufacturer's directions on the Probiotic of choice.
7. An alternative to a prescription antibiotic is Olive Leaf Extract, and/or Garlic Extract, and/or Oil of Oregano, taken according to the manufacturer's directions for a period of 30 days.

F. **Initial Therapy End Point**

1. Absence of microbiological risk factors
2. Clinical signs and symptoms consistent with health.
3. Attaining an abstract and idealized pocket depth *is not* a goal of bio-compatible periodontal therapy.

G. **Surgery**

1. Indicated as a limited therapy of last resort if areas do not respond to the above therapy
2. When surgery performed in limited areas, it will be to determine what is preventing healing

III.Maintenance:

A. Frequency:

1. Individually determined as demonstrated by clinical and microbial parameters
2. Best way to determine frequency: Phase Contrast Microscope
3. Most patients with class 3 or 4 periodontitis should be seen on a 3 month preventive basis
4. Negative microbiological risk: 1 year or four consecutive preventive appointments – 3 month interval
5. Continued microbiological risk: 2 month interval is indicated

B. **Irrigant use:** Is determined by individual needs, as above

Further Thoughts for Consideration

I. **Pre-medication**: for patients with Mitral Valve Prolapse or other valvular problems with regurgitation, or joint replacement surgery.

II. **Irrigation:** Any one needing pre-medication according to the published guidelines should be irrigated with antiseptic solution before any therapy which may lead to bleeding (Included in this is Rheumatic Heart Disease, Mitral Valve Prolapse, Prosthetic Heart Valves, Prosthetic Joint Replacement or Reconstruction, Atherosclerosis and Congenital Heart Disease).

III. **Irrigants:** should be the most effective, non-toxic substances available, which will do the job, have the least number of side effects, and which are appropriate for the patient

IV. **Root Planing:** Opposed to the outdated and unnecessary practice of root planning and complete soft tissue curettage as taught in the dental schools throughout the world. The concept of removal of healthy root structure in the name of curing disease is outdated and unnecessary. Since this is a disease caused by microbes, the circumcision of healthy tissue is to be condemned. Periodontal attachment, on one hand, inserts connective fibers into alveolar bone, and on the other, into the root surface. Removal of cementum by **overzealous** root planing, not only removes healthy tooth structure, but it also precludes periodontal re-attachment. The **Goal of Biocompatible Periodontal Therapy** is the elimination of the infections & not the elimination of healthy tooth structure.

Appendix B

DEFINITION OF IRRIGATION:

Irrigation is the process of using an oral irrigator (e.g., the Water-Pik, Viajet, or Hydrofloss) to introduce water (with or without an antiseptic solution) into the sulcus and interproximal areas to flush away microbial plaque.

- **Supragingival** irrigation may be used under high pressure when directed 90^0 to the long axis of the tooth facially & lingually. This neutralizes the proteolytic enzymes and endotoxins generated by the microorganisms in the plaque by both direct flushing or, when held for 3-4 seconds, by setting up a suction through hydrodynamic forces which disrupt the intercellular plaque matrix. It also reduces interproximal stagnation by increasing healthy gingival circulation interdentally.
- **Subgingival** irrigation is also effective when used as a delivery system to introduce antimicrobial agents directly into the gingival sulcus (0-3 mm depth) or periodontal pocket (> 3 mm depth) under the irrigator's lowest pressure. In the office, delivery of antimicrobial agents to the bottom of the sulcus or pocket is done by a trained professional with a side-port cannula. At home, the patient who has been trained by this dental professional aims a special tip directly into the sulcus or pocket.
- "**Rinsing"** is not irrigation. Rinsing or flushing the mouth cannot get into the sulcus or pocket to disrupt the plaque or neutralize pathogenic endotoxins.
- **Brushing:** Irrigation, while absolutely necessary for the biologically compatible control of periodontal infection, does not take the place of sulcular brushing, gum massage, and general cleaning of the mouth with a soft nylon brush, proxabrushes, end-tuft brushes, etc. All of these oral hygiene methods contribute to elimination of the infection and are encouraged.

Appendix C

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16) Newman and Kornman. Antibiotic /Antimicrobial Use in Dental Practice; Quintessence Publishing Co., Inc., 1990.
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18) Kennedy. How To Save Your Teeth; Health Action Press, 1993.

There are over 10 more pages of references supporting the position of the IAOMT!!

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IAOMT Member #: 557		**IAOMT Chapter:** North American

Oxygen/Ozone Applications in Dentistry

Received…….....….7/7/06 Scientific Review……...…….....7/26/06 IAOMT Board Review…….…..2/24/08 Reevaluation	**Miscellaneous** Oxygen/Ozone Applications in Dentistry	**Approval……...…….3/6/08** Provisional Approval No Opinion No Approval
Explanation of IAOMT position: The IAOMT must be careful in recommending new techniques for a dental practice. O3 therapy is one that deserves special attention due to how it lends itself to biocompatibility. This scientific review is in several categories: Root Canals & Cavitations, Restorative, Biocompatible Periodontal Therapy, and Biological Support. For this reason the category of Miscellaneous would be most appropriate.		

Name of Scientific Review: Oxygen/Ozone Applications in Dentistry
Alternative name(s) of Scientific Review: Biologic Methods for Infection Control in the Oral Cavity
This Scientific Review is related to Dentistry**.**
This Scientific Review is a procedure (and outcome based review).
Purpose of the Scientific Review: To explain the efficacy and integration of oxygen/ozone therapy in the control and elimination of infection in the oral cavity and surrounding tissues.
Scientific Review History: Our introduction to oxygen/ozone therapy occurred at Capital University of Integrative Medicine in 1998. We began to use this technology at the University and then integrated into our practices in 2001. These concepts and procedures based upon evidence based medicine/dentistry were brought into the educational field in 2002. AGD continuing education approval followed in 2005. Currently an international outcome study is under way that is fully approved by the American College of Integrative Medicine and Dentistry's - Institutional Review Board.
A brief description of the Scientific Review: Oxygen/Ozone Therapy in Dentistry offers a non-surgical intervention for the treatment of osteonecrosis, infection, and inflammation of the head, neck, and associated structures. Ozone is a naturally occurring oxidant and when exposed to biologic systems induce multiple biologic reactions. These biologic reactions result in a positive therapeutic response and outcome. Oxygen/Ozone is anti-viral, anti-bacterial, anti-fungal, and anti-parasitic. With proper application these pathogenic life forms can be controlled and eliminated with no toxicity or side-effects. Safe and effective application of oxygen/ozone for successful outcomes is the result of proper training. Training is offered at the American College of Integrative Medicine and Dentistry training facility in Saddle Brook, New Jersey. .

A specific description of this Scientific Review: Training in Ozone/oxygen therapy is necessary before one uses this therapy in practice. It is offered at the American College of Integrative Medicine and Dentistry in Saddle Brook, New Jersey. Contact the author. The science and clinical applications are presented for:

1. Ozonated water for rinsing, irrigation and topical application
2. Ozonated olive oil for topical application
3. Oxygen/Ozone gas for infusion and insufflation

Vested Financial Interest? Yes, by educational based programs

Manufacturer(s): Provide during education course

Scientific Literature:

1. Oxygen/Ozone Therapy: A Critical Evaluation, by Velio Bocci MD
2. Ozone – A New Medical Drug, by Velio Bocci MD
3. Ozone: The Revolution in Dentistry, by Edward Lynch
4. Management of Aggressive Periodontitis Using Ozonized Water, Ramzy M.I. et. al.
5. Efficacy of ozone on survival and permeability of oral microorganisms Nagayoshi M. et al
6. Integrating Oxygen/Ozone therapy into your practice. Mollica, Harris
7. Of Metalized Mouths, Mycotoxicosis, and Oxygen Ali,.Mollica, Harris Townsend Letter #263 p.73-76

Legal Aspects of this Scientific Review: Medical use of oxygen/ozone therapy has been approved in 14 states. The American College of Integrative Medicine and Dentistry has a federally approved IRB (#00004686) and a Federal Wide Assurance number 00009475 registered with the Office of Human Research Protection. The ACIMD also is a member of the Professional Responsibility in Medicine and Research Group.

Applicant Name: American College of Integrative Medicine and Dentistry, Phil Mollica		**Office Phone:** 201-587-0222
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IAOMT Member #: 882		**IAOMT Chapter:** North American

Periowave Photodisinfection System

Received……..…11.10.08 Scientific Review……... 11.24.08 IAOMT Board Review……03.26.09 Reevaluation or Revision……	**Periodontal Therapy Periowave™ Photodisinfection System**	**Approval……03.26.09** Provisional Approval No Opinion No Approval
Explanation of IAOMT position: Thorough periodontal disinfection – well done SR		

Name of Scientific Review: Periowave™ Photodisinfection System

Alternative name(s) of Scientific Review: Photodynamic Therapy for the Treatment of Periodontal Disease

This Scientific Review is related to: Dentistry and Medicine

This Scientific Review is a: Equipment and Procedure

Purpose of the Scientific Review: To facilitate the disinfection of periodontal pockets and initiate healing by eliminating many of the pathological microorganism species known to be causative in periodontal disease.

Scientific Review History: Lasers have been shown to be effective for killing all types of pathogens. With the subsequent development and use of photo-initiator chemicals or dyes to selectively target pathogens known to be associated with periodontal disease, products and procedures were developed that could effectively disinfect infected periodontal pocket sulcus sites without the use of heat. This non-heat process alleviates the potential for tissue damage and therefore the need for either anesthesia or post-operative wound healing while still maintaining effective microbial kill statistics.

This therapy, known as Photodynamic Therapy or PDT, employs a non-toxic dye, called a photosensitizer, and a low intensity visible light which, in the presence of oxygen, combine to produce cytotoxic species that are able to effectively kill all forms of microbes. Infections of the mouth are mostly localized in nature but if left untreated can lead to potentially life-threatening medical conditions. As most mouth infections are readily accessible to the products and procedures of PDT, they are therefore well suited to treatment by this modality. Applications of PDT in dentistry are growing rapidly for the treatment of oral cancer, bacterial and fungal infections, and the photodynamic diagnosis of the potential transformation of oral lesions. Periowave™ is a PDT system specifically designed and engineered for the treatment of periodontal infections and is certified for sale and use in Canada for this purpose.

A brief description of the Scientific Review: After the appropriate scaling and debridement of a patient, a proprietary photosensitizing solution is injected into an infected periodontal pocket site. The photosensitizing solution is able to selectively attach to targeted species of pathogens in the desired treatment area. A non-thermal diode laser attachment is then inserted into the pocket and illuminated for a 60 second interval to activate the solution. This photoactivation of the solution leads to the formation of reactive oxygen species which are able to break the cell walls structures of the targeted pathogens, and as in the case of the Periowave™ system, eliminate the pathogens associated with periodontal disease. This effectively disinfects the sulcus and or diseased periodontal pockets being treated, and promotes the successful healing of the affected surgical site.

A <u>specific</u> description of this Scientific Review: Biofilms play a very important and potentially destructive role in periodontal disease. They are structured communities of microorganisms that live within an encapsulated or enclosed mucilaginous, polymeric matrix secretion. This mucinous, gel like secretion permits the biofilms to attach to tooth surfaces and the epithelium of the sulcus, and acts as a protective barrier to assaults from both exogenous, (eg. antibiotics), and endogenous, (host immune responses), sources. In periodontal disease, the unique protective properties of the biofilm are even more significant, due to the secluded nature of the host site in which these microbes live, (the periodontal pocket), and makes definitive treatment of periodontal disease more difficult. While most of the 500+ known sulcular species of bacteria are thought to be commensals, (neither helping nor harming the host), there are a number of pathogenic species that are capable of triggering an immune response, even in relatively low numbers. The presence of even small numbers of disease associated microorganisms therefore, is cause for concern. Treatment modalities include the use of antibiotics, the physical manipulation of the biofilms, and the use of chemical or antimicrobial solutions to disinfect or eliminate the biofilm. A recent innovation for the treatment of biofilms/pathogens in periodontal disease is the use of lasers. Photodynamic periodontal therapy is only one out of a number of these techniques that was developed for the elimination of microbial pathogens. The Photodynamic Periodontal Therapy protocol, or PDT protocol, is a two step procedure, involving the topical application of a chemical photosensitizer, followed by the selective illumination of the target lesion with the laser light source.

Periowave™ is a PDT protocol that has been designed and marketed utilizing this technology. In the Periowave™ protocol, a non-thermal diode laser light that maintains temperatures and energy levels below destructive doses for normal hard and soft tissues is utilized. The laser light cannot by itself eliminate pathogenic species associated with periodontal infections. When the laser light is combined however, with a matching wavelength-specific photosensitizing dye, a unique chemical reaction is initiated. In the presence of oxygen, the transfer of energy from the laser light to the photosensitive dyes activates them and they are then able to transfer this energy to available oxygen molecules, generating reactive oxygen species. These reactive oxygen species have the capacity to damage proteins, lipids, nucleic acids, and other cellular components. It is these reactive oxygen species that are cytotoxic to, and can effectively kill, many if not all of the pathogens associated with periodontal disease. Photodynamic periodontal therapy, (PDT) is capable of effectively killing bacteria, including both wild and antibiotic resistant strains, viruses, yeasts, and parasites. These photosensitizing dyes by themselves are non-toxic to human hard and soft tissues and alternatively by themselves are not cytotoxic to pathogens. As chemical dyes however, they can be matched to selectively attach to only the desired pathogen species and thus avoid the potential for attachment to, and the subsequent destruction of, cells of the host organism when photoactivated. PDT has this unique advantage of dual selectivity, in that the photosensitizers can be targeted to very specific pathogenic organisms, and in addition, the illumination procedure can be localized to only the area of concern. This selectivity for microbes over host cells, the accurate delivery of the photosensitizers to the affected areas, and the dose adjustment abilities, help minimize side effects and give PDT an advantage over conventional therapies. The dyes can be controlled to selectively adhere to only pathogenic species of microbes leaving desirable bacterial species intact. It is not necessarily desirable to kill the entire microbial flora in the sulcus, as this could leave a patient open to other opportunistic infections.

When used in combination with normal routine scaling and root planning procedures, photodisinfection is shown to have significant improvements in post-operative healing success over the use of scaling and root planning alone. Photodisinfection procedures done in conjunction with scaling and root planning have been shown to statistically decrease the periodontal pocket depths of infected sites better than scaling and root planning alone. Photodisinfection has shown great promise as well, as an effective therapy for controlling bone loss in furcation areas in periodontitis.

Chemical antibacterial agents and mouthwashes are increasingly being used in prophylactic and therapeutic regimes for dental plaque-related diseases. As these agents are difficult to maintain at the therapeutic concentrations in the oral cavity and can be rendered ineffective by resistance development in the target organisms, there is a need for alternative therapies. Many pathogens are becoming resistant to antibiotics through their over-use in either systemic or topical applications. The continued reliance of antibiotics and antiseptic agents creates the potential for mutagenic processes of these microbes or the selective resistance of microbial cells. PDT procedures are an effective alternative to these traditional treatment therapies as the method of action is from oxidative

reactive processes and does not lead to bacterial-resistance. As microbial cell death is primarily a consequence of membrane photodamage, the risk for either the onset of mutagenic processes and or the selection of photoresistant cells has not been seen. This approach is therefore a useful alternative to antibiotic and antiseptics in eliminating periodontopathogenic bacteria while at the same time conserving the dwindling supply of antimicrobial agents that are effective in the treatment of serious systemic infections.

The specific Periowave™ Protocol for disinfection of infected periodontal pockets, as per the manufacturer is:

1. Irrigation: After traditional scaling and debridement, Periowave™ photosensitizing solution is irrigated into an infected periodontal pocket. To thoroughly irrigate each treatment site with Periowave™ photosensitizer solution; place the irrigation cannula at the base of the treatment site pocket and slowly deliver the solution while gently moving the cannula back-and-forth in a mesiodistal direction. Continue to irrigate the pocket until a small quantity of solution can be seen flowing over the free gingival margin. Only one treatment area or site should be irrigated at a time to prevent gingival crevicular fluids from flushing out sensitizer solution out of the pockets of concern before the illumination step can be completed.

2. Illumination: The non-thermal diode laser light tip is inserted into the periodontal pocket and activated to illuminate the solution filled treatment area for a 60 second period. To achieve optimum results, the tip should be walked around the treatment area defect during illumination in both an apico-coronal and mesio-distal direction, without allowing the tip to rise above the gingival crest. Reactive oxygen species formation follows. These reactive oxygen species; 1). damage bacterial cell membranes facilitating the killing of these pathogenic bacteria, and 2). are capable of inactivating the virulence factors associated with these pathogens. Any pockets greater than 4 mm that present with bleeding on probing should be re-treated at 3 to 6 weeks to prevent the re-establishment of biofilms during the healing process. Very deep pockets, implant sites, and refractory pockets have a better healing response after a second application of the treatment protocol at the initial treatment appointment.

Periowave™ is ideally targeted for patients with bleeding on probing and all pockets of 4 - 9 mm length that bleed on probing. Periowave™ should only be used following a thorough scaling and root planing debridement and after any moderate to severe post-instrumentation bleeding has been controlled. Successive, (recall), treatments may benefit from light manual debridement prior to treatment. Each defect should be treated separately, and contralateral sides of each tooth checked, especially at interproximals, as these sites may benefit from additional photodisinfection as well. Long-term results may be significantly enhanced by a follow-up treatment 3-6 weeks after the first treatment. Patient outcomes should include a reduction or elimination of bleeding on probing, an improvement in gingival tissue tone and texture, and a reduction in pocket depth.

Manufacturer(s):
Ondine Biopharma Corporation
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Periowave™ is strictly a minimally invasive therapy for the destruction of pathogenic flora. The unit currently sells for under $5,000.00 Canadian which is the equivalent of about $4,000.00 U.S. and with all the attachments, kits, etc, is only about $7,000.00 Canadian - total. It has the potential to be a simplified tool for the entire dental team of a biological dental office, i.e. as it is marketed, for the **hygienist** to use in office, and without dentist supervision to effect a **disinfection** of the sulcular/diseased pocket areas. It has the ability to be one more tool in a limited family of effective treatment modalities, (i.e. versus drug or chemical tharapies), for the average dentist to help his periodontally compromised patients achieve better oral health.

Distributors:
Henry Schein Canada, Patterson Dental Canada Inc., or Sinclair Dental Co. Ltd.

Scientific Literature:

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Below are the titles from three sources of information provided by the manufacturer that would indicate their successes with periodontal pocket depth reductions while utilizing their system.

☐ 1. Loebel N, Andersen R, Hammond D, Leone S, and Leone V, Ondine Biopharma Corporation. Non-Surgical Treatment of Chronic Periodontitis Using Photoactivated Disinfection. International Association of Dental Research, March 2006.
☐ 2. N.G. Loebel, R. Andersen, Y. Li, R. Shu, X. Zhang., Meta-Analysis of Three Chronic Periodontitis Trials with Periowave™ Photodisinfection, International Association of Dental Research, March 2008
☐ 3. A Multi-Center, Randomized, Examiner-blinded Study of Photodisinfection in the Treatment of Chronic Periodontitis.

Legal Aspects of this Scientific Review: Periowave™ has been approved for sale and use by Health Canada since March of 2006. Ondine Biopharma Corp. is currently seeking FDA approval to distribute this product in the United States.

To date there are no known drug interactions with the use of the Periowave™ system. Theoretical there may be a concern for patients with severe Glucose-6-phosphate dehydrogenase deficiency but since the Periowave™ photosensitizer is not ingested during a normal treatment procedure, this outcome would be extremely unlikely and should therefore not be at risk to cause a reaction.
Periowave™ is not contraindicated in pregnancy or lactating women.

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