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Smoking and inflammation: evidence for a synergistic role in chronic disease

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Tobacco smoking is the most important environmental risk factor for periodontitis, and the epidemiologic and clinical consequences of smoking on periodontal health, as well as the possible etiopathogenic mechanisms, have been extensively reviewed (15, 115, 132, 146). This review will provide perspective on the impact of smoking on periodontal and general health, focusing on possible commonalities of the pathogenic local and systemic effects of smoking. We will also look at periodontitis in parallel with other chronic inflammatory diseases and conditions for which smoking has a detrimental effect. Although such comparisons have to be performed with caution as a result of the obvious differences in etiology and pathogenesis, the findings from other fields of research may provide insights into new mechanisms of disease.

Burden of disease and epidemiology of smoking

Since the US Surgeon General's report in 1964, smoking has been recognized as one of the major risk factors for an ever-increasing number of diseases and conditions. Mounting epidemiologic, clinical, behavioral and biologic evidence has unequivocally implicated smoking in a substantial proportion of the global burden of cancers, and of cardiovascular and pulmonary diseases (46, 139, 170). Globally, tobacco use is related to more than 5 million deaths per year, and this number is expected to increase to 8 million deaths in 2030 (170). In the USA alone, approximately 450,000 deaths per year are attributable to cigarette smoking, and its annual health-care cost is approximately $200 billion (139). Unfortunately, global tobacco consumption is increasing despite the overwhelming scientific evidence gathered in the past 50 years and all the initiatives designed to curb tobacco use. It has been estimated that more than one billion people smoke daily worldwide, with China, India, Indonesia, Russia, USA, Japan, Brazil, Bangladesh, Germany and Turkey accounting for more than two-thirds of the smokers in the world (170).

In dentistry, it has been shown that smoking is an important risk factor for oral cancer and premalignant lesions (88, 168), tooth loss (169) and destructive periodontal diseases (1, 50). The role of smoking in dental/root caries remains controversial; nevertheless, it is clear that, at least in some populations, this relationship does, in fact, exist (169). In recent years, the widespread use of dental implants has been associated with an increased occurrence of peri-implant diseases. Whereas the epidemiology of peri-implantitis remains largely unknown, smoking has been associated with peri-implant bone loss and implant failure (62).

The burden of smoking on the periodontal health of large populations has been assessed in few studies. Population-attributable fractions for the USA indicate that approximately half of the cases of periodontitis may be attributable to smoking, depending on disease definition (65, 157). Susin et al. (152) and Do et al. (41) estimated that after accounting for other risk factors, approximately 12% and 32% of cases of periodontitis could be attributed to smoking in southern Brazilian and Australian adult populations, respectively. A large proportion of these cases of periodontitis among smokers could probably be prevented with smoking-prevention and cessation programs. Thomson et al. (155) estimated that in a representative birth cohort of New Zealand subjects, two-thirds of new cases of periodontitis in subjects at

32 years of age could be attributable to smoking. Therefore, smoking clearly represents a major part of the burden of destructive periodontal disease and there is a great potential for preventive measures.

Smoking has been clearly identified as a risk factor for periodontitis in young and adult subjects. Sufficient evidence exists to support the fact that smoking fulfils the following epidemiological principles for causality: biological plausibility; strength of association; temporal relationship; dose–response effect; and consistency of findings. Experimental and observational studies have explained, at least in part, the mechanisms underlying the local and systemic effects of smoking, and its consequences, on periodontal health (115). Cross-sectional studies have consistently observed a higher prevalence and a greater extent and severity of periodontal destruction in current smokers than in never or former smokers (1, 169). Similarly, longitudinal studies have observed a higher incidence and a faster progression rate of clinical attachment loss and radiographic bone loss among smokers (16, 67, 155). Conversely, stopping smoking has a beneficial effect on disease incidence and progression (16, 67, 155). A dose–response relationship has been consistently observed in these epidemiological studies, with heavy smokers and subjects with high lifetime smoking exposure having more periodontal destruction (1, 169). Clinical studies have also contributed to the understanding of the relationship between tobacco use and periodontal disease. Smoking has a deleterious effect on non-surgical and surgical periodontal therapy (59, 69, 78) as well as on reconstructive periodontal treatment (31, 102). In contrast, few clinical studies have shown that smoking cessation has a positive impact on periodontal health (123) and on the microflora (49). Smoking has also been identified as a important long-term predictor for tooth loss in patients treated for periodontitis (30).

Possible mechanisms

In contrast to the relatively well-described negative impact of smoking on the epidemiology of periodontitis, the underlying biologic mechanisms that explain the deleterious effects of smoking on periodontal health remain largely unclear.

Microbiota

Several studies have investigated the effects of tobacco smoking on the occurrence and composition of subgingival microflora, and contradictory results were obtained. Preber et al. (121) investigated deep pockets (probing depth ≥6 mm) for the presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* and found no significant differences in the prevalence of these microorganisms between smokers and non-smokers. Similar results were observed by Stoltenberg et al. (149) when a large sample of age-, gender-, plaque- and calculus-matched nonsmokers were compared with smokers regarding the presence of *A. actinomycetemcomitans*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *P. gingivalis* and *P. intermedia*. A more recent study (163) found no differences in the numbers of *A. actinomycetemcomitans*, *Tannerella forsythia* (*Bacteroides forsythus*), *F. nucleatum*, *P. gingivalis*, *P. intermedia* and *Parvimonas micra* (*Peptostreptococcus micros* or *Micromonas micros*) between smokers and nonsmokers before periodontal treatment. These findings were corroborated by other studies that could not observe significant differences between the subgingival microflora of smokers and nonsmokers (4, 21, 39).

Nevertheless, there are a number of studies which indicate that smoking may have a detrimental effect on the subgingival microflora. Earlier studies found a higher proportion of *A. actinomycetemcomitans*, *T. forsythia* and *P. gingivalis* in smokers in comparison with nonsmokers (161, 175). A large study compared the microbial profile of 124 never smokers, 98 former smokers and 50 current smokers in shallow (probing depth ≤4 mm) and deep (probing depth >4 mm) periodontal pockets using checkerboard DNA–DNA hybridization (57). Current smokers had a significantly higher prevalence of *T. forsythia*, *Eubacterium nodatum*, *F. nucleatum* ss *vincentii*, *P. intermedia*, *P. micra*, *Prevotella nigrescens*, *P. gingivalis* and *Treponema denticola* than did former smokers or never smokers in shallow sites, whereas no significant differences could be observed among groups in deep pockets. According to the authors, the reasons for these differences in colonization patterns were unclear but might be associated with disease distribution in the study sample. Using culture methods, Winkelhoff et al. (165) assessed the likelihood of having clusters of periodontal pathogens among 468 treated and untreated periodontitis patients according to smoking status. Smokers had a 2.1 (95% CI = 1.4–3.1) and a 2.4 (95% CI = 1.6–3.8) higher chance than nonsmokers of having ≥10% and ≥20%, respectively, of the total anaerobic counts comprising *T. forsythia*, *P. micra* and *F. nucleatum*. Smoking also increased the risk of having clusters of periodontal pathogens after

periodontal treatment. A recent study of Gomes et al. (52) did not observe significant differences in the amounts of total bacteria, *P. gingivalis* or *A. actinomycetemcomitans* between smokers and never smokers after adjusting for demographics and probing depth using multivariable analysis. The counts of *Dialister pneumosintes* and *P. micra* were significantly higher in smokers than in never smokers (52).

Conflicting evidence of the impact of smoking on the periodontal microbiota might be explained by differences in the study design and in the sample population characteristics, definition of periodontitis, smoking status, population sampling methods and technique, and microbiological analysis. Most studies were cross-sectional in nature and used convenience samples, which hindered validity; nevertheless, they collectively suggest that smokers and nonsmokers exhibit no consistent differences in microflora. Longitudinal studies with well-defined samples using the latest microbiological techniques should be carried out to confirm differences in the microbiota between smokers and nonsmokers. In conclusion, it might be argued that the influence of smoking on periodontitis is certainly not extensively mediated by changes in the microflora and that immunological changes in the host play a critical role in disease occurrence.

Periodontitis and other types of chronic inflammatory diseases

The negative impact of tobacco smoking on several chronic tissue-destructive inflammatory diseases besides periodontitis has been well documented in the medical literature (Table 1).

Rheumatoid arthritis

Tobacco smoking is the most established environmental risk factor for rheumatoid arthritis. A large population-based case–control study in Sweden (148) found that smokers had a twofold higher risk of developing seropositive rheumatoid arthritis compared with never smokers. This association was dose-dependent on lifetime exposure to smoking (148). Population-attributable fractions calculated for Sweden indicate that approximately one-fifth of all and one-third of anti-citrullinated protein autoantibody-positive cases of rheumatoid arthritis can be attributed to smoking (75).

It is well known that rheumatoid arthritis has a strong genetic component with more than 50% heritability. Interestingly, recent studies have shown strong gene–environment interactions between tobacco smoking and human leukocyte antigen-shared epitopes, especially for heavy smokers (72). This gene–smoking interaction is associated with anti-cyclic-citrullinated peptide, and its presence clearly illustrates that smoking may have specific effects in different individuals. Citrullination involves the enzymatic conversion of arginine to citrulline in some proteins. This change may alter the three-dimensional conformation of the protein and lead to loss of tolerance. Citrullination has also been implicated in the pathogenesis of multiple sclerosis (100). Recently, antibodies against citrullinated peptides have been found in sera from patients with aggressive periodontitis (63).

Multiple sclerosis

A recently published meta-analysis (58), representing 3,052 cases and 457,619 controls, showed that smoking is associated with a 50% increase in susceptibility for multiple sclerosis (relative risk = 1.5; 95% CI = 1.4–1.6). The exact mechanism for the increased risk is unclear but it may be related to the fact that smoking generally increases susceptibility to autoimmune disease (8). Another plausible explanation could be an increased generation of reactive oxygen species (discussed later) (141, 171).

Similarly to rheumatoid arthritis, a gene–environment interaction has been found for multiple sclerosis. In a large population-based case–control study, Hedström et al. (61) found an interaction between smoking and genetic risk factors (two human leukocyte antigens). Compared with nonsmokers with neither genetic risk factor, smokers with both genetic risk factors were 13 times more likely to have multiple sclerosis (odds ratio = 13.5; 95% CI = 8.1–22.6). In contrast, the odds for multiple sclerosis decreased to 1.4 (95% CI = 0.9–2.1) for smokers without the genetic risk factors and to 4.9 (95% CI = 3.6–6.60) for nonsmokers with both genetic factors.

Cardiovascular disease

Inflammation plays a pivotal role in the progression of atherosclerosis and thus in the progression of cardiovascular disease (131). Atherosclerosis is a multifactorial disease and tobacco smoking is a major risk factor. The INTERHEART study, comprising 12,438 subjects and 14,605 controls, estimated that compared with never smokers, current smokers had a higher risk (odds ratio = 2.3; 95% CI = 2.1–2.6) for myocardial infarction after adjusting for age, region and gender (91). Similarly, former smokers had a 30%

Table 1. Association between smoking and various chronic inflammatory diseases

Disease	Study design	Sample characteristics	Estimates* (95% CI)
Periodontitis	National cross-sectional survey (157)	USA (NHANES III) 6,460 men / 7,190 women	Odds ratio = 4.0 (3.2–4.9)
	Regional cross-sectional survey (151, 152)	South Brazil 676 men / 784 women	Odds ratio = 3.5 (2.1–5.7)
Multiple sclerosis	Meta-analysis (58)	Data pooled from 14 studies 3,052 cases / 457,619 controls	Relative risk = 1.5 (1.4–1.7)
Rheumatoid arthritis	Meta-analysis (150)	Data pooled from 16 studies 13,885 cases / 579,691 controls	Odds ratio$_{males}$ = 1.9 (1.5–2.3) Odds ratio$_{females}$ = 1.3 (1.1–1.5)
Systemic lupus erythematosus	Meta-analysis (36)	Data pooled from nine studies 1,458 cases / 162,189 controls	Odds ratio = 1.5 (1.1–2.1)
Ulcerative colitis	Meta-analysis (89)	Data pooled from 13 studies 1,770 cases / 9,971 controls	Odds ratio = 0.6 (0.5–0.8)
Crohn's disease	Meta-analysis (89)	Data pooled from nine studies 1,471 cases / 9,139 controls	Odds ratio = 1.8 (1.4–2.2)
Myocardial infarction	Case–control (91)	Data pooled from 252 centers in 52 countries 12,438 cases / 14,605 controls	Odds ratio = 2.3 (2.1–2.6)
	Longitudinal (mean follow-up 12.3 years) (122)	Copenhagen, Denmark 13,191 men / 11,472 women	Relative risk$_{male}$ = 1.4 (1.3–1.6) Relative risk$_{female}$ = 2.2 (1.9–2.7)

*Odds ratio or relative risk estimate for current smokers (irrespective of exposure) compared with never smokers.

higher possibility (odds ratio = 1.3; 95% CI = 1.2–1.5), and passive smokers had a 60% higher possibility (odds ratio = 1.6; 95% CI = 1.4–1.6), of having myocardial infarction compared with never smokers (91). The Systematic Coronary Risk Evaluation (SCORE) system, based on the follow up of more than 205,000 subjects derived from 12 European cohort studies, showed that smoking had a major impact on the 10-year risk of fatal cardiovascular disease, even after adjusting for age, blood pressure and cholesterol level (33). A large number of possible mechanisms has been suggested as being responsible, but decreased production of nitrous oxide and increased release of reactive oxygen species are often mentioned (44). Smoking has also been shown to be associated with increased markers of systemic vascular inflammation, including C-reactive protein, endothelial-selectin and soluble intercellular adhesion molecule-1 (18).

Inflammatory bowel disease

Smoking seems to have a contradictory effect on inflammatory bowel disease. A meta-analysis of 15 studies, comprising more than 20,000 patients with inflammatory bowel disease, found that current smoking was associated with an increased risk for Crohn's disease (odds ratio = 1.76; 95% CI = 1.40–2.22), whereas it was a protective factor for ulcerative colitis (odds ratio = 0.58; 95% CI = 0.45–0.75) (89). This apparent contradictory effect of smoking on inflammatory bowel diseases can only be speculated on, but might be related to smoking having an organ-specific, rather than a disease-specific, effect (71). The anti-inflammatory effect of nicotine could have a positive influence on ulcerative colitis, while the negative effect of smoking on Crohn's disease could be mediated by non-nicotine substances. Whereas no specific mechanism for the negative effect of smoking on Crohn's disease has been pointed out, it is reasonable to believe that it could be mediated by humoral and cell immunity (80). Interestingly, a gene–environment interaction has also been found regarding the influence of smoking on susceptibility for Crohn's disease (162).

Influence of tobacco smoking host response in periodontitis

Influence on leukocyte activity

Exposure of leukocytes, and other cells, to cigarette smoke condensate has resulted in a variety of effects, some of which could be of importance for suscepti-

bility and pathogenesis in periodontitis. Chronic cigarette smoking has been shown to increase the total white blood cell count, and to have a greater effect on neutrophilic granulocytes than on other types of white blood cells (142, 167). Smoking also activates inflammatory cells (86), increasing the systemic levels of several inflammatory markers, including C-reactive protein, fibrinogen, interleukin-6 and haptoglobin (174). Many of these substances and mediators have been associated with periodontitis (27, 85).

Neutrophilic granulocytes are the most abundant type of leukocytes in humans and play a pivotal role in our defence against bacterial invasion (38). Aberrant neutrophil responses have been associated with an increased susceptibility to periodontitis (133). Neutrophils from patients with aggressive periodontitis have been shown to have both impaired and hyperactive functions. A large number of studies have shown differences in neutrophil activity between smokers and nonsmokers that are somewhat comparable with those seen between healthy individuals and periodontitis patients. This suggests that smoking may have detrimental effects that are comparable with other important etiological factors for periodontitis.

Reduced chemotaxis was the first neutrophil impairment shown and is the most studied in periodontitis. A large number of studies have shown a decreased chemotactic response to various stimuli, both *in vitro* and *in vivo* (2, 114). Furthermore, neutrophils from patients with aggressive periodontitis have been shown to have impaired phagocytosis (9, 164). In this context, impaired chemotaxis of neutrophils from smokers has also been shown after *in-vitro* exposure to smoke extract (42, 135). Noble & Penny (106) showed decreased chemotaxis of neutrophils from smokers. In contrast, other *ex-vivo* studies, investigating migration of neutrophils from smokers, demonstrated similar or increased chemotaxis of neutrophils from smokers compared with neutrophils from nonsmokers (77, 145). A recent study investigating neutrophil chemotaxis in chronic obstructive pulmonary disease showed a slight increase in chemotaxis in smokers (10). These contradictory findings illustrate the perils of transferring results from *in vitro* models using smoke extract stimulation to chronic smoking in humans.

Neutrophils are the primary phagocytes in the periodontium and are crucial for the defence against bacterial invasion. Both *in-vitro* and *ex-vivo* studies have shown that smoking can impair neutrophil phagocytosis (34, 55, 73, 176). Some studies have also demonstrated changes in the membrane expression of cytoplasmic domain 18 integrin and cytoplasmic domain 62L-selectin on neutrophils exposed to cigarette smoke (134, 176). These changes could be one explanation for impaired neutrophil migration and phagocytosis.

Collectively, these studies contribute to the mounting evidence demonstrating that smoking leads to impairment of various leukocyte functions, thus indicating that smokers could have a defective defence against bacteria colonizing the gingival crevice, contributing to the increased prevalence and severity of periodontitis found in smokers. Nevertheless, it is important to acknowledge that the validity of this 'acute' exposure compared with chronic smoking exposure is still not fully understood (136).

Humoral immunity

The negative effects of smoking on the humoral immune response have been extensively studied and have been considered as one of the main mechanisms for the increased occurrence of periodontitis in smokers. Several studies have shown decreased levels of IgG, especially of the IgG2 subclass, in smokers (51, 101, 126). IgE, on the other hand, has been shown to be elevated in smokers (28, 68). In contrast, smokeless tobacco or nicotine-replacement therapy seem to have a marginal effect on serum immunoglobulin levels (56).

In general, studies have not observed significant differences in the concentrations of immunoglobulins between nonsmoker and smoker patients with periodontitis (27, 48, 54). However, smoking has been inversely correlated with the expression of antibodies specific for some periodontal pathogens. Corroborating these findings, Graswinckel et al. (54) did not find a difference in total plasma levels of IgG, IgA and IgM between patients with periodontitis and healthy controls. Interestingly, the plasma levels of IgG1 and IgG2 were significantly higher in patients with moderate and severe periodontitis compared with controls, whereas no significant differences were observed for IgG3 and IgG4. In contrast, lower levels of IgG were consistently found in smokers than in nonsmokers, and smoking was associated with decreased total IgG levels in patients with periodontitis and IgG2 levels in patients with severe periodontitis.

Vlachojannis et al. (166) assessed the distribution of antibodies specific for a number of periodontal

bacteria, including the red-complex species, using data derived from 8,153 subjects from the National Health and Nutrition Examination Survey (NHANES) III. Compared with never smokers, current smokers were 40–50% less likely to have high antibody titers for *P. gingivalis*, *Campylobacter rectus*, *E. nodatum*, *P. nigrescens*, *Prevotella melaninogenica*, *Veillonella parvula* and *Actinomyces naeslundii* after adjusting for important cofactors. Singer et al. (140), using data from 4,717 subjects participating in the Atherosclerosis Risk in Communities longitudinal study, observed that systemic oxidative stress was associated with a decrease of total IgG and smoking was found to be an effect modifier of this association.

The humoral immune response in aggressive periodontitis has been widely described in the literature; however, the impact of smoking on this relationship has not been fully explored. Mooney et al. (97) assessed the IgG levels to periodontal pathogens in 65 treated and untreated subjects with generalized aggressive periodontitis. No significant differences were observed between nonsmokers and smokers among untreated subjects, whereas treated nonsmokers had significantly lower titers of IgG to *A. actinomycetemcomitans*, *P. intermedia* and *T. denticola* than did treated smokers. Following the assessment of serum immunoglobulin levels in race-matched patients with generalized aggressive periodontitis, Quinn et al. (125) observed lower levels of IgG2 among smokers than among nonsmokers. No other significant differences were observed for IgG1, IgG3 and IgG4. Likewise, no significant differences were observed between smokers and nonsmokers in individuals with localized aggressive periodontitis. Similar findings were observed for serum levels of IgG2 to *A. actinomycetemcomitans* among African–American patients with generalized aggressive periodontitis (154).

Cytokines

The possibility that smoking causes systemic and local disturbances in cytokine levels, which could explain increased disease susceptibility, has been investigated. Published studies are inconclusive regarding the overall net effect of proinflammatory or anti-inflammatory cytokines.

The gingival crevice fluid levels of 22 cytokines / chemokines were investigated in a recent study, conducted by Tymkiw et al. (159), that included 12 periodontally healthy controls and 40 periodontitis patients equally divided according to smoking. Periodontitis patients had significantly

higher concentrations of 14 out of 22 cytokines / chemokines compared with controls. Among periodontitis patients, smokers had decreased amounts of several proinflammatory cytokines (interleukin-1α, interleukin-6 and interleukin-12 [p40]), chemokines (interleukin-8, monocyte chemotactic protein-1, macrophage inflammatory protein-1 and regulated upon activation, normal T-cell expressed, and secreted [RANTES]) and the T-cell regulators (interleukin-7 and interleukin-15). These findings seem to indicate that smoking may have an immunosuppressant effect contributing to an increased susceptibility to periodontitis. Similarly, significantly lower gingival crevice fluid concentrations of interleukin-1β / interleukin-1ra (127) and interleukin-1α (120) were observed among smokers than among nonsmokers with chronic periodontitis. Kamma et al. (70) compared the gingival crevice fluid levels of four cytokines between smokers and nonsmokers using a large sample of 65 patients with aggressive periodontitis and 35 periodontally healthy controls. Among patients with aggressive periodontitis, no significant differences were observed between smokers and nonsmokers for interleukin-1β, whereas the concentration of interleukin-4 was significantly increased in smokers. Interestingly, contrasting results were observed among healthy controls for these cytokines. Moreover, the concentration of interleukin-6 was significantly higher among smokers, whereas that of interleukin-8 was significantly higher among nonsmokers, regardless of the periodontal status.

In contrast to these findings, Boström et al. (22, 23) observed, in a series of studies, higher gingival crevice fluid levels of tumor necrosis factor-α among smokers, and no significant differences in interleukin-1β / interleukin-1ra (24) and interleukin-6 (23) between smokers and nonsmokers. Collectively, most studies seem to indicate that smokers have lower gingival crevice fluid levels of cytokines than do nonsmokers.

In contrast to gingival crevice fluid findings, the cytokine levels in serum seem to be elevated among smokers. The concentration of tumor necrosis factor-α in serum has been shown to have a dose-dependent relationship with smoking exposure (119). This finding is supported by a recent study (11) showing significantly elevated serum levels of interleukin-1β and tumor necrosis factor-α in healthy smokers compared with nonsmokers. In an *ex-vivo* experiment, Torres de Heens et al. (158) showed that stimulated whole-blood-cell cultures from smokers release more interferon-γ and interleukin-13. This could indicate that smoking stimulates T-helper-cell 2 activity,

which has been associated with periodontal disease progression.

Collagen synthesis and fibroblast attachment and proliferation

Decreased fibroblast migration, attachment and collagen synthesis has been suggested to contribute to the negative effects of smoking on the periodontium. This effect of smoking has been associated with the impaired outcome of periodontal treatment in smokers (74). However, this view is primarily based on *in-vitro* studies conducted with nicotine concentrations that greatly exceed those normally found in plasma. The nicotine concentration in blood is approximately 14 ng/ml after tobacco smoking and smokeless tobacco use and 9 ng/ml after use of nicotine substitutes (14).

James et al. (66) showed that very high concentrations of nicotine (>0.5 mg/ml) inhibited attachment and growth of periodontal ligament fibroblasts, while a concentration comparable to that found in plasma had a limited effect. Similarly, Tipton & Dabbous (156) observed that high concentrations of nicotine inhibited fibroblast proliferation as well as fibronectin and collagen production. A recent study, using a rat model (95), found that a daily systemic administration of nicotine decreased the number and proliferation of gingival fibroblasts. In contrast to most studies, Peacock et al. (118) showed that nicotine enhanced human gingival fibroblast attachment in a concentration-dependent manner and that low concentrations stimulated fibroblast proliferation. Fang & Svoboda (47) showed that nicotine decreased gingival fibroblast migration by 50%.

Although most studies have focused on the effect of high concentrations of nicotine, it is probable that smoke residues accumulating on root surfaces will also have a detrimental effect on periodontal healing. However, it remains to be shown if plasma levels of nicotine can inhibit periodontal tissue turnover, leading to disease initiation and progression.

Tissue-degrading enzymes

Increased release and diminished inhibition of tissue-degrading enzymes, such as collagenases and serine proteases, are essential for the destruction of periodontal tissues. Smoking causes an elevation in the circulating levels of several enzymes, including myeloperoxidase, lysozyme, human neutrophil lipocalin and matrix metalloproteinases (3, 5, 174). Ozçaka et al. (110) demon-

strated that smokers with chronic periodontitis had significantly higher serum concentrations of myeloperoxidase and elastase than did nonsmokers, while the concentration of tissue inhibitor of matrix metalloproteinases-1 was significantly reduced. These differences were not seen in periodontally healthy individuals, regardless of smoking status. The matrix metalloproteinase-9/tissue inhibitor of matrix metalloproteinase-1 ratio was also higher in smokers with periodontitis but not in periodontally healthy smokers.

Tissue-degrading enzymes have also been shown to be elevated in gingival crevice fluid and in periodontal tissues. Söder (143) and Söder et al. (144) showed higher elastase activity in the gingival crevice fluid of smokers than in the gingival crevice fluid of nonsmokers. Liu et al. (84) found more matrix metalloproteinase-8 in periodontal connective tissues from smokers with periodontal disease than in periodontal connective tissues from nonsmokers. Zang et al. (177) showed *in vitro* that cigarette smoke condensate increased collagen degradation from human fibroblasts while nicotine *per se* had a limited effect.

In-vitro studies have shown that smoke and smoke components have an activating effect on leukocytes. Seow et al. (138) demonstrated that nicotine decreased chemotaxis and phagocytosis on neutrophils in a dose-dependent manner, whereas it increased degranulation and generation of eicosanoids on these cells. A recent study showed a decreased respiratory burst in neutrophils, indicating a decreased ability to kill bacteria by the production of reactive oxygen species, but increased degranulation, which contributes to an elevated release of tissue-degrading enzymes and consequently tissue destruction (176). The influence of tobacco smoking on the pathogenesis of periodontitis is illustrated in Fig. 1.

Osteoclast differentiation and activation

RANKL and osteoprotegerin are important modifiers of alveolar bone resorption. RANKL initiates osteoclast differentiation by activating osteoclast progenitors and regulates the activity of mature osteclasts. Osteoprotegerin inhibits osteoclast differentiation by binding to RANKL and blocking the RANK/RANKL interaction (79). Several studies have shown an increased RANKL/osteoprotegerin ratio in periodontitis (12). Smokers have an increased RANKL/osteoprotegerin ratio in saliva and in serum, mainly because of decreased levels of osteoprotegerin (26, 82, 111, 112). As there are contradicting reports on osteoprotegerin
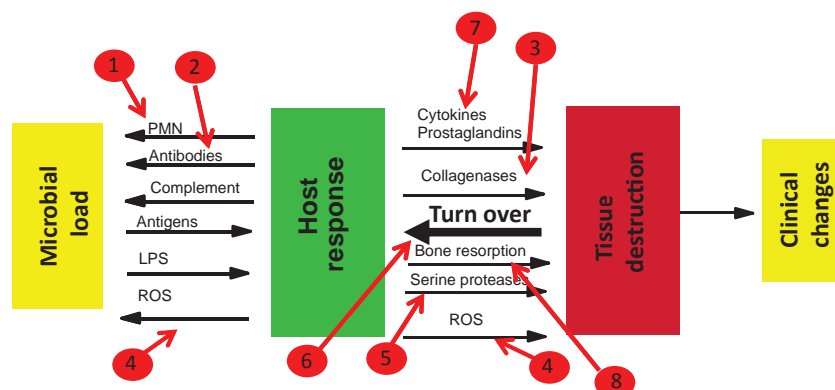
Fig. 1. Effect of smoking on pathogenesis of periodontitis (113). Numbers indicate the following effects: 1, impaired chemotaxis, impaired phagocytosis (2, 9, 114, 164); 2, decreased levels of immunoglobulins (51, 101, 126); 3, increased release of collagenase (84, 176); 4, decreased or increased generation of reactive oxygen species (ROS) (90, 137, 145, 176); 5, increased release of serine proteases, such as elastase (144, 176); 6, impaired fibroblast attachment and decreased collagen synthesis (95, 156); 7, increased or decreased release of cytokines and chemokines (23, 127, 159); 8, increased RANKL∕osteoprotegerin ratio (26, 82, 112). LPS, lipopolysaccharide; PNM, polymorphonuclear neutrophil.

levels in serum from smokers, this potential mechanism for the detrimental effect of smoking needs further investigation (83).

Reactive oxygen species

One puff of cigarette smoke contains up to 10^{17} oxidant molecules (124). Reactive oxygen species play an important role in periodontal pathogenesis. They are important for intracellular bacterial killing but can also cause destruction of extracellular tissues. This tissue destruction can be direct, via increased oxidative stress, or indirect, by inducing a proinflammatory state (32). The literature regarding the effect of tobacco smoking on the generation of reactive oxygen species is inconclusive. Most studies investigating the effect of smoking on the generation of reactive oxygen species have shown that generation is reduced (134, 145, 176). However, increased generation of reactive oxygen species has also been shown in animal and clinical studies. Talukder et al. (153) demonstrated that leukocytes from mice exposed to cigarette smoke for 32 weeks had increased generation of reactive oxygen species. In a recent *in-vitro* study on human neutrophils, Matthews et al. (90) showed that smoke extract increased the extracelluar release of reactive oxygen species, while preincubation with the extract decreased the response to subsequent stimuli. Sela et al. (137) observed increased generation of reactive oxygen species in neutrophils from healthy male smokers, after activation of the neutrophils with phorbol myristate acetate. Moreover, several studies have shown a systemic imbalance on oxidant–antioxidant levels as a result of decreased plasma levels of circulating antioxidants (174). Such reduction in antioxidants, in combination with increased numbers of activated leukocytes, could contribute to periodontal tissue destruction.

Gene–smoking interaction

Susceptibility to periodontal disease has a strong genetic component (94, 146). The possibility of a gene–environment interaction between a specific genotype and smoking has not been explored for periodontitis. Few studies have shown an additive effect of a specific genotype and smoking. The interleukin-1B C(3953∕4)T polymorphism is probably the most investigated in regard to periodontitis. A recent meta-analysis (103) showed a significant association between this polymorphism and chronic periodontitis. Other studies have investigated a possible composite effect of the polymorphism and smoking. The original study on the interleukin-1B polymorphism (76) indicated that smoking attenuated the effect of the genotype, while later studies showed an additive effect (37, 92). McGuire & Nunn (92) followed 42 patients with periodontitis for 14 years and showed that the risk of tooth loss as a result of periodontal disease was increased by 2.7-fold for patients positive for the composite genotype (interleukin-1A −889 and interleukin-1B +3953) and by 2.9-fold for heavy smokers. The combined effect of being genotype positive and smoking increased the risk of tooth loss by 7.7-fold. Meisel et al. (93), using a population-based sample of 1,085 individuals, observed a significant interaction between smoking and the interleukin-1 polymorphism. Compared with genotype-negative nonsmokers, the likelihood of having periodontitis among smokers increased from 2.4 (95% CI = 2.0–2.9) to 4.5 (95% CI = 2.3–8.8) if

they were genotype positive. No increase in risk was observed among nonsmokers who were genotype positive. Similarly, smokers positive for the high-ligand-binding genotype of the FCγ-receptor (Fcγ-RIIa-H ∕ H131) have been shown to have more periodontitis than smokers not positive for this genotype and nonsmokers positive for the genotype (172).

Human leukocyte antigen play an important role in the regulation of the T-cell response and could have an influence on the susceptibility for periodontitis. A meta-analysis including 12 studies (147) investigated the association between human leukocyte antibodies and aggressive ∕ chronic periodontitis. Human leukocyte antibody-A9 and human leukocyte antibody-B15 increased the probability of having aggressive periodontitis, respectively, by 2.6-fold (95% CI = 1.4–4.8) and 1.9-fold (95% CI = 1.2–3.2). On the other hand, human leukocyte antibody-A2 and human leukocyte antibody-B5 were associated with a 28% (95% CI = 0.6–0.9) and a 51% (95% CI = 0.3–0.8) decrease in the likelihood of having aggressive periodontitis, respectively. No association between human leukocyte antibodies and chronic periodontitis could be observed. A gene–environment interaction like that between smoking and the human leukocyte antigen-shared epitope in rheumatoid arthritis has not been reported for periodontitis and smoking.

Role of nicotine

Tobacco smoke contains more than 4,000 different chemical compounds, and nicotine is the most studied of these components. The role of nicotine on periodontal inflammation is unclear. Several *in-vitro* studies show that application of nicotine to various types of cultured cells induces an inflammatory response that could be of importance for the initiation and progression of periodontitis (115). Similarly, animal models have shown that systemic administration of nicotine will increase alveolar bone loss in rats with ligature-induced periodontal inflammation (107, 108). In contrast, Matthews et al. (90) showed that smoke extract increased the respiratory burst in neutrophils, whereas nicotine and cotinine alone had no effect. Nicotine may have an anti-inflammatory effect owing to its effect on the α7-nicotinic-acetylcholine receptors (160).

Fewer clinical and epidemiological studies have been carried out to investigate the effect of moist snuff or other smokeless tobacco products. Earlier cross-sectional studies, on the effect of tobacco chewing on the periodontal health of baseball players, observed localized attachment loss and bone loss in sites where the tobacco was placed (45, 129). In

contrast to these early findings, recent cross-sectional studies including Swedish adolescents (96, 130) and Navy servicemen (17) failed to show a major detrimental effect of moist snuff (also called snus) on periodontal health other than localized gingival recession. It is important to acknowledge that these studies had a cross-sectional design and included healthy, young subjects. A recent increase in the use of moist snuff in Sweden offers an interesting opportunity to compare the effect of nicotine with and without the other non-nicotinic components in tobacco smoke. Snuff use leads to a similar or higher exposure to nicotine compared with tobacco smoking (64). In a recent study, Carlens et al. (29) compared the effect of smoking and snuff on rheumatoid arthritis, ulcerative colitis, Crohn's disease, sarcoidosis and multiple sclerosis in a large group of construction workers. The study showed that tobacco smoking significantly increased the risk for rheumatoid arthritis, ulcerative colitis, Crohn's disease and multiple sclerosis, while it decreased the risk for sarcoidosis. In contrast, moist snuff was not associated with any of these diseases and conditions. These contradictory findings underscore the importance of airway exposure to non-nicotine components. A recent population-based case–control study (60), including 902 incident cases of multiple sclerosis and 1,855 controls, assessed the effect of tobacco smoking and the use of moist snuff on the risk of developing multiple sclerosis. Tobacco smoking was associated with a significantly increased risk for multiple sclerosis (women: odds ratio = 1.4, 95% CI = 1.2–1.7; men: odds ratio = 1.8, 95% CI = 1.3–2.5), whereas long-term use of snuff was associated with a decreased risk (odds ratio = 0.3, 95% CI = 0.1–0.8). Collectively, studies comparing tobacco smoking with the use of other tobacco products indicate that there are other components in cigarette smoke that contribute to the negative effects of smoking.

Passive smoking

Passive smoking, also known as second-hand smoking or environmental tobacco smoking, is defined as the involuntary inhalation of tobacco smoking and it has been widely associated with several diseases and conditions, including respiratory diseases, cancer and cardiovascular diseases. In contrast, the effect of passive smoking on periodontal health has not been extensively studied. An earlier study, using data derived from the NHANES III, reported that the odds of having periodontitis was 1.6 (95% CI = 1.2–2.2) higher among individuals exposed to passive smoking after

adjusting for sociodemographic factors, diabetes and dental care (6). Former and current smokers were excluded from the analysis, and passive smoking was assessed using a self-reported questionnaire.

A series of observational studies was carried out using the salivary levels of cotinine (a major metabolite of nicotine in body fluids) to classify a sample of Japanese factory workers into active (cotinine ≥8 ng/ml) or passive (cotinine 1–7 ng/ml) smokers. Yamamoto et al. (173) showed that after adjustment for other lifestyle factors, passive and active smoking increased the likelihood of having periodontitis by 2.9-fold (95% CI = 1.1–7.8) and 4.9-fold (95% CI = 1.8–13.6), respectively. Exposure to passive smoke was associated with elevation of interleukin-1β, albumin and aspartate aminotransferase levels in saliva (105). Furthermore, the study reported no increased proportion of periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *P. intermedia* and *P. nigrescens*) in saliva in passive smokers, despite periodontal destruction. A 2-year follow-up of this sample showed a similarly higher risk for clinical attachment loss in passive (odds ratio = 2.2; 95% CI = 1.0–4.8) and active (odds ratio = 2.3; 95% CI = 1.0–5.0) smokers compared with nonsmokers (104). The concentrations of salivary proteins such as albumin, aspartate aminotransferase and lactoferrin, were significantly elevated in passive smokers relative to nonsmokers. Regarding periodontal pathogens there were no significant differences between groups, except for *P. nigrescens*, which was present at lower levels in passive smokers.

Among the limitations of studies investigating the association between passive smoking and periodontal disease is the validity of self-reported exposure to smoking. It is well established in the literature that insomuch as smokers consistently underestimate tobacco use (116), nonsmokers also underestimate exposure to environmental smoke (109). Therefore, cotinine assessment should be used in future investigations as a result of its validity in estimating current smoking and exposure to smoke (7, 13, 53). Overall, the literature seems to indicate a relationship between passive smoking and destructive periodontal diseases. Larger, longitudinal studies are necessary to investigate the role of passive smoking on immunological changes in the periodontium.

Tobacco cessation

Epidemiological studies suggest that stopping smoking may decrease the risk of having periodontitis and may slow down or arrest periodontal destruction (1, 169). Cross-sectional studies usually observed a lower occurrence of periodontitis among former smokers than among current smokers, and a time-dependent relationship seems to exist of a lower risk of periodontitis among subjects with longer periods of abstinence (20, 65, 117). Using data from the NHANES III, Hyman & Reid (65) observed a decrease in the odds ratio from 4.6 (95% CI = 1.6–13.3) to 2.7 (95% CI = 1.2–5.9) for severe attachment loss among subjects, 20–49 years of age, who had stopped smoking for ≤5 and >5 years, respectively. Also using data from the NHANES III, Tomar & Asma (157) observed a decrease from 3.2 (95% CI = 2.2–4.8) to 1.7 (95% CI = 1.1–2.2) in the likelihood of having periodontitis among former smokers who had stopped smoking ≤2 and >10 years previously, respectively. An early, 10-year longitudinal study showed significantly lower alveolar bone loss in individuals who stopped smoking than among those who continued smoking (19). Recent longitudinal studies have also observed that stopping smoking decreases the progression of periodontal destruction (16, 67, 155).

An intervention study by Preshaw et al. (123) assessed the effect of smoking cessation on nonsurgical periodontal treatment outcome after 1 year. Those individuals who successfully stopped smoking showed a better response to periodontal treatment (123) and an improved microbial profile (49) compared with individuals who continued to smoke. Further investigation of the subgingival biofilm using molecular microbiological analysis revealed that 1 year post-treatment, individuals who stopped smoking had consistently lower counts of periodontal pathogens compared with smokers, with significant differences between groups for *T. denticola*, *P. micra* and *Filifactor alocis* counts (40). A recent study investigated the short-term effect of smoking cessation on periodontal tissue physiology and the host response in 16 smokers. Gingival crevice fluid and gingival blood flow increased after smoking cessation (98), possibly as a result of improvement in the gingival microcirculation, which could enhance gingival tissue metabolism and local immune responses. Moreover, stopping smoking significantly increased peripheral neutrophil mRNA expression levels for matrix metalloproteinase-8 after 8 weeks (99). The levels of mRNA for interleukin-1β, interleukin-8, vascular endothelial growth factor, tumor necrosis factor-α and matrix metalloproteinase increased over 8 weeks but did not reach statistical significance. These results may indicate a positive interaction between microflora and the host response, which might contribute to improvement in periodontal health.

In patients with other inflammatory diseases, such as chronic obstructive pulmonary disease, Crohn's disease and cardiovascular disease, several biochemical markers have been investigated in regard to tobacco cessation (35, 43, 81, 128, 167). These studies indicate that tobacco cessation may reduce the symptoms in CD patients and also reduce the levels of inflammatory markers that had been associated with higher risk for CVD, such as interleukin-6 and C-reactive protein. A longitudinal study reported that for those who had stopped smoking for longer than 10 years the C-reactive protein levels were still higher compared with those of nonsmokers; nevertheless, the levels of C-reactive protein decreased over time (87). In animal models, tobacco cessation appears to reduce the levels of interleukin-α and tumor necrosis factor-α, while those of interleukin-12 do not change (25). From a clinical point of view it is obvious that tobacco cessation plays an important role in the treatment of several diseases and conditions, including periodontitis; however, the scientific evidence is still scarce.

Conclusions

In conclusion, tobacco smoking seems to induce changes such as decreased leukocyte chemotaxis and decreased production of immunoglobulins, and impaired phagocytosis, but the findings of only minor differences in the microflora between clinically similar sites in smokers and nonsmokers suggest that these changes are not the main reason for the elevated prevalence of periodontitis in smokers. Smoking also appears to cause a stronger inflammatory reaction with an increased release of tissue destructive substances (e.g. reactive oxygen species, collagenase, serine proteases and proinflammatory cytokines). An increased inflammatory response is in line with the overall hypothesis that periodontitis is a hyperinflammatory condition rather than a hypo-inflammatory condition.

A gene–environment interaction, as described for other chronic inflammatory diseases and smoking has not been reported for periodontitis and smoking. This line of research could generate new important knowledge, not only about smoking but also about periodontal pathogenesis in general.

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