Antioxidants in Periodontal Diseases: A Review

S Lakshmi Sree*, R Mythili**

ABSTRACT

Periodontal disease is considered an inflammatory disorder that damages tissue through the complex interactions between periodontopathic bacteria and host defense systems. It is likely that the role of reactive oxygen species (ROS) is common to both bacterial- and host-mediated pathways of tissue damage. In recent years, there has been a tremendous expansion in the medical and dental research concerned with free radicals (FR), ROS and antioxidant defense mechanisms. This review is intended to provide a critical up-to-date summary of the field with particular emphasis on the evidence for oxidative damage and compromised antioxidant status in periodontal diseases.

Key words: Reactive oxygen species/free radicals, antioxidants, polymorphonuclear neutrophils, periodontal disease

Periodontitis, an inflammatory disease, is considered to be initiated and perpetuated by a small group of predominantly gram-negative, anaerobic or microaerophilic bacteria that colonize the subgingival area. Bacteria cause the observed tissue destruction directly by toxic products and indirectly by activating host defense systems (i.e. inflammation). Polymorphonuclear Leukocytes: A Key Role in Periodontitis

Polymorphonuclear leukocytes (PMNs) are the predominant leukocytes in blood and constitute the primary cellular host resistance factor against infection. In the oral cavity, following plaque accumulation and the development of clinical inflammation, 90% of leukocytes that enter the gingival crevicular fluid (GCF) and 50% of those that infiltrate junctional epithelium are PMNs. PMNs possess at least two main pathways for controlling micro-organisms (i.e., oxidative and nonoxidative) which either kill bacteria, influence bacterial growth or modify bacterial colonization in relation to the periodontium. Upon recognition of a phagocytic or soluble stimulus, both neutrophils and macrophages experience a ‘respiratory burst’, which is characterized by an increase in oxygen consumption, activation of the hexose-monophosphate (HMP) shunt and generation of free radicals (FR), reactive species and their metabolic products. At sites of chronic inflammation, there is considerable over production of FR and reactive species.

Free Radicals Definition and Formation

A FR may be defined as an atomic or molecular species capable of independent existence with one or more unpaired electrons in its structure. FR can be positively (NAD°+) or negatively charged (O2°¯) or electrically neutral (OH°). A feature of the reactions of FR is that they tend to proceed as chain reactions, one radical begets another one and so on. The reactive species including reactive oxygen species (ROS), reactive chlorine species (e.g., HOCl hypochlorous acid) and reactive nitrogen species (RNS) are produced in large quantities by activated neutrophils.

ROS Definition and Formation

In recent years the term ROS has been adopted to include molecules such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl) and singlet oxygen (1O2), which whilst not radicals in nature, are capable of radical formation in the extra- and intracellular environments.
The most important species implicated in inflammatory injuries to tissues are the hydroxyl (OH\(^{•}\)) radical, the superoxide anion (O\(_2^{•–}\)), the nitric oxide (NO\(^{•}\)) radical (where \(^{•}\) signifies an unpaired electron) and hypochlorous acid, hydrogen peroxide and \(\text{O}_3\), which are ROS.\(^3\)

**Potential Mechanisms for Periodontal Tissue Destruction by ROS (Fig. 1)**

Whilst most ROS have extremely short half-lives as \(10^{-9}\) to \(10^{-6}\) s (Pryor 1986), they can cause substantial tissue damage by initiating free radical chain reactions. Different mechanisms, which mediate tissue damage, include the following:\(^3\)

- DNA damage
- Lipid peroxidation (through activation of cyclooxygenases and lipo-oxygenases).
- Protein damage, including gingival hyaluronic acid and proteoglycans.\(^8\)
- Oxidation of important enzymes e.g. antiproteases such as \(\alpha\)-1-antitrypsin.
- Stimulation of pro-inflammatory cytokine release by monocytes and macrophages by depleting intracellular thiol compounds and activating nuclear factor kB (NFkB).

Recent reports\(^1\) have also suggested that ROS are produced by osteoclasts at the ruffled border/bone interface and may play a role in resorption. However, certain ROS, such as superoxide and hydrogen peroxide have been found to play a role in the activation of osteoclasts, rather than in the direct degradation of the bone matrix, whilst NO has been found to inhibit bone resorption.

---

**Figure 1.** Simplified diagram illustrating a central role of ROS in generating chronic inflammation and tissue damage in response to periodontal pathogens.

MMP = Matrix metalloproteinase; TIMP = Tissue inhibitor of matrix metalloproteinase; NF-\(\kappa\)B = Nuclear factor kappa B; AP-1 = Activating protein-1; PDL = Periodontal ligament; TNF = Tumor necrosis factor; IL = Interleukin; GM-CSF = Granulocyte-macrophage colony-stimulating factor; LPS = Lipopolysaccharide; ROS = Reactive oxygen species.
Garrett et al\textsuperscript{9} demonstrated both \textit{in vivo} and \textit{in vitro} that when free oxygen radicals were generated in the bone environment, osteoclasts were formed and bone resorption occurred. Few studies have addressed the degradation of the periodontal extracellular matrix by ROS. Earlier studies by Bartold et al\textsuperscript{8} demonstrated the \textit{in vitro} ability of ROS particularly the OH\textsuperscript{-} species, to degrade hyaluronan and proteoglycans extracted from porcine gingivae and within cryostat sections of tissue.

Proteoglycans and glycosaminoglycans (GAGs) when exposed to a broad-spectrum of ROS species of differing reactivity and over differing periods of time were found to undergo chain depolymerization and residue modification to varying degrees, particularly in the presence of the highly reactive OH\textsuperscript{-} species. Moreover, the nonsulfated GAG, hyaluronan was identified as being more susceptible to degradation by ROS than sulfated GAG.\textsuperscript{1}

The highly reactive OH\textsuperscript{-} species was also shown to exert the most detrimental degradative effects on the small chondroitin sulfate, proteoglycans from alveolar bone, compared to other ROS.\textsuperscript{1}

**Antioxidants: What are they and how do they Act?**

"An antioxidant is any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate".\textsuperscript{2}

Several biologically important compounds have been reported to have antioxidant functions. These include vitamin C (ascorbic acid), vitamin E (\(\alpha\)-tocopherol), vitamin A, \(\beta\)-carotene, metallothionein, polyamines, melatonin, nicotinamide adenine dinucleotide phosphate (NADPH), adenosine, co-enzyme Q-10, urate, ubiquinol, polyphenols, flavonoids, phytoestrogens, cysteine, homocysteine, taurine, methionine, S-adenosyl-L-methionine, resveratrol, nitrooxides, reduced glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), nitric oxide synthase (NOS), heme oxygenase-1 (HO-1) and eosinophil peroxidase (EPO).\textsuperscript{10}

A functional classification of antioxidant systems based on the way they act (Niki 1996) appears to be the most useful (Table 1).\textsuperscript{2}

<table>
<thead>
<tr>
<th>Types of defense system</th>
<th>Mode of action</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preventive antioxidants</td>
<td>Suppress the formation of FR: Nonradical decomposition of LOOH and H\textsubscript{2}O\textsubscript{2}</td>
<td>Catalase, GPX and serum-transferase</td>
</tr>
<tr>
<td></td>
<td>Sequestration of metal by chelation</td>
<td>Transferrin, ceruloplasmin, albumin, haptoglobin</td>
</tr>
<tr>
<td>Radical-scavenging antioxidants</td>
<td>Quenching of active O\textsubscript{2}</td>
<td>SOD, carotenoids</td>
</tr>
<tr>
<td></td>
<td>Scavenge radicals to inhibit chain initiation and break chain propagation</td>
<td>Lipophilic: Ubiquinol, vitamin A, vitamin E, carotenoids</td>
</tr>
<tr>
<td></td>
<td>DNA repair enzymes, protease, transferase, lipase</td>
<td></td>
</tr>
</tbody>
</table>

**Superoxide Dismutase**

Superoxide dismutase (SOD) is an antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O\textsubscript{2} and to the less reactive species H\textsubscript{2}O\textsubscript{2}, accelerating it upto 10,000 times.\textsuperscript{2}

\[
2\text{O}_2^{-} + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

In humans, there are three forms of SOD: Cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD and extracellular SOD (EC-SOD). Though, Cu/Zn-SOD is believed to play a major role in the first-line of antioxidant defense, recent reports have revealed that Mn-SOD is essential for life whereas Cu/Zn-SOD is not.\textsuperscript{10} SOD has been localized within human periodontal ligament and may represent an important defense mechanism with in gingival fibroblasts against excess superoxide release.\textsuperscript{11}

**Catalase**

Catalase (CAT) is an antioxidant enzyme, which contains heme bound iron and is mainly located in peroxisomes.\textsuperscript{2} It reacts very efficiently with H\textsubscript{2}O\textsubscript{2} to form water and molecular oxygen and with hydrogen donors (methanol, ethanol, formic acid or phenols) with peroxidase activity.\textsuperscript{10}
Thus, CAT protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells.

Reduced Glutathione

Glutathione (GSH) is an essential tripeptide with many important functions. Glutathione’s three major roles in the body are summarized by the letters AID-Antioxidant, Immune booster and Detoxifier - three critical processes driven by GSH.

In its reduced form GSH is an important antioxidant (radical scavenger), a property bestowed upon it by its central thiol containing cysteine amino acid. It is also regarded as a pivotal molecule to the immune system especially for regulation of interleukin-2 (IL-2) dependent T-lymphocyte proliferation.

GSH has a dual role. It reacts directly with FR, but it also is alternatively a substrate or a co-factor of a transferase (GSH-tr), a peroxidase (GSH-PX) or a reductase (GSH-red). Oxidized glutathione (GSSG) is made by joining two GSH molecules by their -SH groups, losing the two hydrogens and forming a disulfide bridge. The reaction is catalyzed by a GSH-PX that detoxifies H$_2$O$_2$ very efficiently.

Glutathione Peroxidase

Glutathione peroxidase (GPX) is selenium containing peroxidase, which catalyses the reduction of a variety of hydroperoxides (ROOH and H$_2$O$_2$) using GSH, thereby protecting mammalian cells against oxidative damage. There are at least five GPX isoenzymes found in the mammals normally-GPX1, GPX2, GPX3, GPX4, and GPX5.

Although GPX shares the substrate, H$_2$O$_2$ with CAT, it alone can react effectively with lipid and other organic hydroperoxides. The glutathione redox cycle is a major source of protection against low levels of oxidant stress, whereas CAT becomes more significant in protecting against severe oxidant stress.

Plasma Antioxidant Status in Periodontal Diseases

In periodontal literature, early studies of individual antioxidant micronutrients were unconvincing in their associations between dietary antioxidant intake and periodontitis. Few studies (Nishada et al 2000, Amarasena et al 2005, Chapple et al 2007) that have explored individual antioxidant scavengers in serum or plasma have shown only mildly compromised levels in periodontitis subjects relative to healthy controls, except where smoking is a co-factor.

Sobaniec and S Lotowska (2000) reported lower serum antioxidant enzyme levels in ligature-induced periodontitis in a rat model. Abnormally high levels of hydroperoxide and compromised serum co-enzyme Q10 and vitamin E were observed in Papillon-Lefèvre syndrome subjects, suggesting substantial oxidative stress in these subjects and a potential role for specified antioxidant therapies.

An inverse relationship was found between serum vitamin C concentrations and antibody levels to porphyromonas gingivalis (Pussinen et al 2003). Panjamurthy et al observed lower plasma vitamin C, vitamin E and reduced GSH in periodontitis subjects. However, antioxidant enzyme levels were raised and the authors attributed this to a protective response to oxidative stress.

Total antioxidant capacity (TAOC) concentration was found to be reduced in serum and plasma of periodontitis patients. Tamaki et al observed a positive correlation between plasma oxidative status and clinical attachment loss in patients in the maintenance phase of periodontal treatment. They suggested that a systemic increase in oxidative stress may influence the rate of progression of periodontal disease.

Low levels of a number of carotenoids, in particular β-cryptoxanthin and β-carotene were found to be associated with an increased prevalence of periodontitis in the year 60-70 year old men.

Though, these studies reveal an antioxidant compromise in the plasma of periodontitis patients, the changes in
antioxidant status lack relevance or significance, given their low concentrations and rates of activity, relative to the antioxidant scavengers.

**Salivary Antioxidant Status in Periodontal Diseases**

Moore et al.\(^1\) who were the first to explore salivary total antioxidant activity found no difference in TAOC levels in periodontitis and nonperiodontitis subjects. The predominant antioxidant component of saliva was uric acid (>70% of antioxidant activity).

However, Chapple et al.\(^2\) found lower total antioxidant concentration in the saliva of periodontitis patients when compared to periodontally healthy controls.

Similar results were observed in a larger cohort study\(^2\) and in small case-control studies (Diab Ladki et al.\(^2\) and Brock et al.\(^3\)). Lower TAOC was reported in women than men. A higher level of protein carbonyls (oxidative stress) was found in periodontitis patients than in controls.\(^4\)

Salivary antioxidant levels (SOD, GPX, reduced GSH, ascorbic acid, α-tocopherol) were observed to be lower in periodontitis patients\(^5,6\) as well as in patients under antiepileptic therapy with gingival hyperplasia.\(^7\)

Markers of oxidative damage such as malondialdehyde\(^8,9\) 8-hydroxy-deoxyguanosine\(^10,11\) were found to be higher in saliva of patients with periodontitis which decreased following initial treatment approaching the mean control values.\(^12\)

Overall, the relevance of saliva as a medium for assessing surrogate markers of reactive oxygen and antioxidant species in periodontitis patients must be open to question. Moreover, saliva contains GCF and the contribution of GCF antioxidants to saliva will vary according to the degree of salivary stimulation.\(^13\)

**GCF Antioxidant Status in Periodontal Diseases**

GCF is the most appropriate fluid to sample when investigating biomarkers of tissue events in periodontium.

Guarnieri et al.\(^14\) observed spontaneous generation of superoxide in the GCF of periodontitis subjects, with no differences in antioxidant scavenging capacity between cases and controls. However, no difference was reported in the SOD activity in GCF of periodontitis subjects.\(^15\)

Reduced GSH was the most important antioxidant in GCF with levels 1,000-fold higher than paired plasma samples\(^16\) and was significantly lower in periodontitis relative to matched control subjects.\(^17\)

The antioxidant enzyme GPX correlated negatively with pocket depth and attachment loss and increased post-therapy (Hung et al. 2000).\(^18\) However, significantly greater levels of GPX, lactoferrin, myeloperoxidase and IL-1β in the GCF were in periodontally diseased sites when compared to healthy sites.\(^19,20\)

Studies investigating oxidative stress and antioxidant status both locally and peripherally (in serum, saliva and GCF) in periodontitis patients reported higher levels of malondialdehyde and total oxidant status, which decreased following Phase I therapy.\(^12,21\)

Tsai et al.\(^22\) reported a positive correlation between GCF lipid peroxidation and periodontopathogens and a negative correlation between GCF GPX and periodontopathogens. They concluded that the increased levels of lipid peroxidation with decreased level of antioxidants provided the evidence that oxidative stress, after the stimulation of periodontopathogens might play a role in the pathogenesis of periodontitis.

A negative correlation between serum and GCF TAOC and gingival inflammation was reported in miniature poodle dogs (Pavlica et al. 2004).\(^12\) Similarly, TAOC in the GCF of periodontitis subjects was significantly lower.\(^23\) Based on GCF studies, it can be concluded that local antioxidant scavenging defenses are compromised in periodontitis, but whether this represents a predisposition to disease or results from the inflammatory lesion is not clear.

**Periodontal Tissue Antioxidant Status in Periodontal Diseases**

Gingivitis subjects exhibited higher levels of GSH in gingival tissue samples when compared to controls (Giorgi et al. 1992).\(^12\) Tissue levels of CAT and SOD decreased with increasing pocket depth in periodontitis patients scheduled for extractions.\(^24\)

On the contrary, higher levels of SOD activity was
observed in the GCF and gingival tissue samples of periodontitis patients.\textsuperscript{29}

Smokers with periodontitis exhibited increased levels of metallothionein (a radical scavenging and preventive antioxidant) in the gingival tissue indicating a protective response to the increased inflammation in these patients.\textsuperscript{36} Another study in smokers\textsuperscript{37} observed higher levels of HO-1 antioxidant enzyme levels in smokers with periodontitis than in nonsmoker periodontitis patients. Higher levels of thiobarbituric acid reactive substances (TBARS), a marker of oxidative stress was found in the gingival tissue obtained from unresolved pockets following Phase I therapy in patients with chronic periodontitis.\textsuperscript{38} In a similar study, Panjamurthy et al\textsuperscript{14} also observed higher levels of TBARS and enzyme antioxidants with lower levels of scavenging antioxidants in the gingival tissue of periodontitis subjects when compared to controls.

Recently, Borges et al\textsuperscript{39} reported increased activities of myeloperoxidase, GPX, glutathione-S-transferase, oxidized GSH and higher levels of TBARS in gingival tissue of chronic periodontitis patients when compared to controls, suggesting a correlation between oxidative stress biomarkers and periodontal diseases. Biopsy studies are difficult to implement for ethical and technical reasons, but the limited data so far confirm the presence of more significant oxidative stress in the periodontal tissues of diseased periodontium relative to control tissue and the apparent upregulation of antioxidant enzyme systems.

**Conclusion**

Whilst a myriad of possible mechanisms leading to the destruction of periodontal tissues exist, ROS would appear to play a significant role in the pathology of periodontal diseases. Oxidative stress observed in a diseased periodontium could result directly from excess ROS activity or antioxidant deficiency or indirectly by creating a pro-inflammatory state. Novel adjunctive antioxidant and anti-inflammatory strategies to the traditional periodontal therapy can help us in achieving good clinical results.

**References**


