The role of Reactive Oxygen Species and Antioxidants in Periodontal Tissue destruction

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Periodontitis is a term used to describe an inflammatory process, initiated by the plaque biofilm, that leads to loss of periodontal attachment to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. The inflammatory and immune responses to the bacteria that colonize the periodontal and associated tissues involve the systemic circulation and ultimately the peripheral systems of the body. This creates a complex bi-directional series of host– microbial interactions involving cellular and humoral factors and networks of cytokines, chemokines, and growth factors. It is believed that while the primary etiological agent is specific, predominantly gram negative anaerobic or facultative bacteria within the sub gingival biofilm, the majority of periodontal tissue destruction are caused by an inappropriate host response to those microorganisms and their products. More specifically, a loss of homeostatic balance between proteolytic enzymes (e.g. neutrophil elastase) and their inhibitors (e.g. a1-antitrypsin) and reactive oxygen species (ROS) and the antioxidant defence systems that protect and repair vital tissue, cell, and molecular components is believed to be responsible. The larger upward shifts in the pro-oxidant/antioxidant ratio intracellularly bring about direct damage to vital biomolecules and structures, cell membrane damage and dysfunction, and cell death (by necrosis or accelerated apoptosis), and extracellularly cause direct connective tissue damage (both mineralized and unmineralized) and damage to extracellular matrices and their components.

Definition -

Free radicals have been defined as any species capable of independent existence that contain one or more unpaired electrons. They are, by nature, highly reactive and diverse species, capable of extracting electrons and thereby oxidizing a variety of biomolecules vital to cell and tissue function. (Halliwell 1991)

ROS is a term that has become more popular because it encompasses other reactive species which are not true radicals but are nevertheless capable of radical formation in the intra- and extracellular environments. (Battino et al.1999)

Antioxidants are defined as those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate. In normal physiology there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when that equilibrium shifts in favour of ROS, either by a reduction in antioxidant defenses or an increase in ROS production or activity, oxidative stress results. (Halliwell 1989).

Oxidative stress was defined as a disturbance in the pro-oxidant–antioxidant balance in favor of the former, leading to potential damage. (Sies 1991).

The redox potential is a measure (in volts) of the affinity of a substance for electrons, relative to hydrogen.

Within the gingival crevice/pocket a low redox potential is regarded as essential for the growth and survival of subgingival anaerobes, whereas within cells and tissues a reducing environment (low redox potential) is protective against oxidative stress. There is therefore an apparent conflict in developing future therapeutic strategies for periodontitis which are based on redox biology, because maintaining a low redox status to protect host cells and tissues from oxidative stress is conducive to encouraging growth and survival of anaerobes. However, it is vital to understand that the body is compartmentalized. Bacteria are not intracellular pathogens (unlike viruses) and therefore maintaining a low redox state within a cell may not have relevance to a high redox state within the periodontal pocket/gingival crevice. A key example of compartmentalized differences in antioxidant composition was highlighted by (Brock et al.) who demonstrated that the antioxidant composition of gingival crevicular fluid differed substantially from the plasma and saliva compartments, with reduced glutathione (GSH) being a major antioxidant within gingival crevicular fluid, whereas uric acid predominates in saliva and plasma

Exogenous sources of oxygen radicals include heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise, and therapeutic drugs.

Endogenous sources are primarily:

Bi-products of metabolic pathways – electron leakage from mitochondrial electron transport systems forming superoxide;

Functional generation by host defense cells (phagocytes) and cells of the connective tissues (osteoclasts and fibroblasts).

Free Radicals And Reactive Oxygen Species

Free Radicals	Reactive Oxygen Species
Superoxide	Hydrogen Peroxide
Hydroxyl	Hypochlorous Acid
Perhydroxyl	Singlet Oxygen
Hydroperoxyl	Ozone
Alkoxyl	
Aryloxyl	
Peroxyl	
Arylperoxyl	
Acyloxyl	
Acylperoxyl	

Types Of Tissue Damage By Reactive Oxygen Species.

1. Protein Damage
2. Lipid Peroxidation
3. Dna Damage

Superoxide	**Hydrogen Peroxide**	**Hydroxyl Radical**
Functional production of superoxide involves activation of the HMP (or NADPH-oxidase) shunt.). This process comprises the so-called respiratory burst within polymorphonuclear leukocytes. Superoxide forms initially and then spontaneously dismutates to hydrogen peroxide. Hydrogen peroxide may also undergo Fenton reactions in the presence of Fe2+ or Cu2+ ions, forming the most potent of all oxygen radicals, the hydroxyl radical (•OH).	Hydrogen peroxide is a weak ROS, the potential of which to cause tissue damage is limited to its interaction with transition metal ions via Fenton chemistry or ultraviolet light, when it forms the more potent hydroxyl radical. The principal enzymes charged with removal of hydrogen peroxide are the antioxidant enzymes catalase, which predominantly acts intracellularly and glutathione peroxidase, which operates within mitochondria and extracellularly.	The hydroxyl (•OH) radical and the related perhydroxyl radical (HO2) are the most potent species known to cause damage and destruction to an array of cellular and tissue components. Specifically, damage may affect cellular and extracellular targets. Targets include: • Lipids • Proteins • Carbohydrates • DNA

Antioxidant Defence Systems

The preventative antioxidants function by enzymatic removal of superoxide and hydrogen peroxide or by sequestration of divalent metal ions, preventing Fenton reactions and subsequent hydroxyl radical formation. The chain-breaking antioxidants are the most important within extracellular fluids. The lipid soluble antioxidants (a-tocopherol and the carotenoids) act at the cell membrane level and protect against lipid peroxidation, whereas the water-soluble scavengers are more important within the extracellular tissue fluids. It is important to recognize however, that several antioxidants have dual and sometimes triple actions. For example, ascorbate acts as a chainbreaking or scavenging antioxidant as well as a preventative antioxidant by virtue of its ability to recycle a-tocopherol (vitamin E) from its oxidized form and by its ability to bind metal ions, respectively. Similarly, the intracellular enzymes (superoxide dismutase, catalase, glutathione peroxidase) are regarded by some as preventative antioxidants. The efficacy of an antioxidant depends upon:

- Its location (intra- vs. extracellular or cell membrane bound);
- The nature of the ROS-challenge;
- Other antioxidant species important in co-operative interactions
- Other environmental conditions (e.g. pH, oxygen tension).

Superoxide Dismutase	Different types of superoxide exist, • Superoxide dismutase 1 – a Cu2+/Zn2+-dependent enzyme found within the cytosol; • Superoxide dismutase 2 – the Mn2+-dependent enzyme located within the mitochondria; • Superoxide dismutase 3 – extracellular enzyme, found at low levels extracellularly. O_2 + O_2 + 2H+_SOD O2 + H2O2 →
Catalase	Catalase contains heme- bound iron and is mainly located in perioxisomes. It removes H_2O_2 with great efficacy. It dismutates H_2O_2 to form water and O_2.
Ascorbic Acid (Vitamin C)	The role of vitamin C as an antioxidant in inflammatory diseases is summarized as: • scavenging water-soluble peroxyl radicals; • scavenging superoxide and hypochlorous acid; • prevention of damage mediated by hydroxyl radicals • decreases heme breakdown and Fe2+ release thereby preventing Fenton reactions; • re-forms a-tocopherol from its radical; • protects against ROS-release from cigarette smoke.
A-Tocopherol (Vitamin E)	Vitamin E is generally regarded as the most important and effective lipid-soluble antioxidant in vivo, vital to maintaining cell membrane integrity against lipid peroxidation by peroxyl radical scavenging. It requires other antioxidant species to re-constitute vitamin E, the most effective being the reduced form of co enzyme Q10 (ubiquinol) in the lipid environment and ascorbic acid in the aqueous phase.
Carotenoids	Carotenoids include lycopene, a-carotene, b-carotene, lutein, cryptoxanthine, retinol (vitamin A1), dehydroretinol (vitamin A2). Carotenoids are lipophilic and higher plasma concentrations have been shown to protect against various inflammatory and malignant diseases.
Coenzyme Q10	Co-enzyme Q10 exists in an oxidized form (ubiquinone or CoQ) and a reduced Co-enzyme Q10 exists in an oxidized form (ubiquinone or CoQ) and a reduced form (ubiquinol or CoQH2), both of which possess antioxidant activity. Co-enzyme Q10 deficiency has been demonstrated in the gingival tissues of periodontitis subjects.
Uric Acid	Uric acid is one of the major radical scavengers within plasma, urine, and saliva. Its antioxidant activities include: • scavenger of singlet oxygen, hydroxyl radicals • binding of divalent metal ions preventing Fenton chemistry.
Polyphenols	The most researched polyphenols are the water-soluble catechin, epigallocatechin gallate, and the polyphenol, Quercetin. Polyphenols function by: • Radical scavenging, terminating lipid peroxidation, iron chelation, sparing vitamin E, restoration of vitamin C.
Glutathione	Glutathione is a non-essential tri-peptide in that it can be synthesized within the cell. Glutathione exists in oxidized (GSSG) and reduced (GSH) forms and GSH is a ubiquitous thiol that plays a major role in human physiology and pathology.

Role Of Reactive Oxygen Species In Periodontal Tissue Damage

Chronic inflammatory conditions are generally thought to be associated with increased oxidative stress, with phagocytes (particularly the neutrophil) being implicated in disease pathogenesis because of the generation of the oxidative burst during phagocytosis and killing. Originally, ROS were thought to be directly microbicidal but recent evidence indicates that their role is to establish an environment in the phagocytic vacuole suitable for killing and digestion by enzymes released into the vacuole from cytoplasmic granules. Significant ROS generation by neutrophils requires a minimum oxygen tension of about 1% and a pH of 7.0–7.5. Both these conditions are found within periodontal pockets, indicating that chronic or excess ROS production is possible at this important site of periodontal tissue damage.

Plaque bacteria and their products are an obvious source of factors that could stimulate neutrophils infiltrating the periodontal tissues. Enhanced ROS generation by peripheral neutrophils from patients with both chronic and aggressive disease can be stimulated with opsonized bacteria associated with periodontal disease

(Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans). Further studies have shown that several isolates of two strains of fusobacteria (F. nucleatum and F. necrophorum) can stimulate significant ROS generation, cytokine [interleukin-1b (IL-1b), TNF-a, IL-8] and elastase production by neutrophils isolated from healthy individuals. This group has also shown that F. nucleatum, in the absence of plasma, could also stimulate large amounts of ROS production and induce lipid peroxidation in vitro. That F. nucleatum might be pivotal in neutrophil-dependent, ROS induced tissue damage within the periodontium is also supported by the finding that phagocytosis of F. nucleatum induces significantly greater ROS generation than phagocytosis of Porphyromonas gingivalis or A. actinomycetemcomitans.

A variety of pro-inflammatory cytokines (TNF-a, granulocyte–macrophage colony stimulating factor, granulocyte colony-stimulating factor, IL-8, IL-1, IL-6), growth factors (e.g. platelet activating factor), and lipopolysaccharide have been shown to have a priming effect on the human neutrophil oxidative burst both in vitro and in vivo. TNF-α can prime for ROS generation by neutrophils from patients with chronic and aggressive periodontitis as well as periodontally healthy individuals. Cytokines can modulate the respiratory burst activity of neutrophils and have a role in determining oxidative stress locally within the tissues.

Conclusion

Oxidative stress lies at the heart of the periodontal tissue damage that results from host–microbial interactions, either as a direct result of excess ROS activity/antioxidar deficiency or indirectly as a result of the activation c redox-sensitive transcription factors and the creation of pro-inflammatory state. A body of literature support peripheral blood neutrophil hyper reactivity in chroni and aggressive forms of periodontitis, with respect to tota Fcc-receptor-mediated ROS generation. This, togethe with the evidence for compromised plasma antioxidar capacity, independent of smoking, suggests an underlyin environment of oxidative stress within periodontiti patients. Considerable evidence has emerged over the las 2 years that oxidative stress and depressed antioxidar function are features of periodontal tissues and fluids i periodontitis subjects. This concept has led to search fc appropriate antioxidant therapy in inflammatory disease It may become necessary to deliver antioxidant selectively to cell types in inflammatory diseases lik periodontitis to achieve normal cell function.

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