

The Role of Reactive Oxygen Species in Initiation and Progression of Periodontal Diseases

ABSTRACT

Periodontal diseases are widely prevalent diseases and negatively affecting the quality of life of young and adult population. They are inflammatory conditions that result in destruction of the supporting structure of the tooth. Periodontal diseases are associated with phagocytosis and increased oxidative stress which could generate oxidative burst during the process of killing and phagocytosis. Plaque bacteria and their by-products could initiate neutrophil recruitment to the area of bacterial invasion in the periodontal tissues that would result in stimulation of free radical generation. Usually, reactive oxygen species (ROS) produced by phagocytes will be used for killing of the invading pathogens. Prolonged release of ROS and increased matrix metallo-proteinase activity causes bone resorption and degradation of connective tissue surrounding the teeth. In this paper, we review the oxidation and its effect in periodontal tissue destruction which in severe cases would lead to the loss of teeth.

Keywords: Periodontal disease; Reactive oxygen species; Mitochondria; Oxidation; Free radicals.

1.0 INTRODUCTION:

DEFINITION AND TYPES OF REACTIVE OXYGEN SPECIES

ROS includes oxygen derived free radicals (ODFR), such as the hydroxyl, superoxide and nitric oxide radical species, and non-radical oxygen by-products, such as hypochlorous acid and hydrogen peroxide [1]. The existence of un-paired electrons in the ODFR's external orbitals will result in the production of such species, especially the hydroxyl radical type which is highly reactive in nature. The active hydroxyl radical can degrade a number of important macromolecules in an attempt to equilibrate its un-paired electronic form. Macromolecules which can be degraded involve proteins (free and conjugated), carbohydrate and lipids, thus leading to cellular disturbance and damage [2, 3]. Free radicals have also essential roles in cell homeostasis and signalling [4].

In mitochondria, by-products will be generated in form of reactive oxygen species due to mitochondrial electron transport [5]. ROS are also generated as required intermediates of metal-catalyzed redox reactions. Due to the presence of two un-paired electrons in isolated orbits in the external shell of atomic oxygen [6]; this electron distribution encourages oxygen to form

radicals. The progressive oxygen reduction by the adding of electrons enhances the production of ROS including superoxide; hydrogen peroxide; hydroxyl ion; nitric oxide and hydroxyl radical.

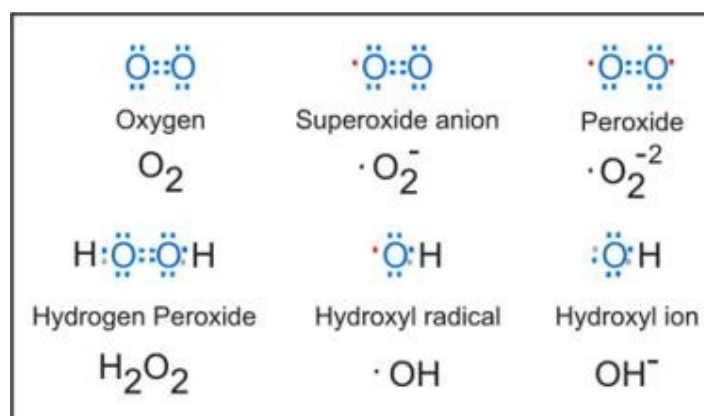


Fig 1: Types of reactive oxygen species (ROS). Electron structures of common reactive oxygen species.

2.0 REACTIVE OXYGEN SPECIES SOURCES

Reactive oxygen species can be produced from several sources. In the human cells, the function of the respiratory chain in mitochondria results in formation of a by-product in the form of superoxide. This is known as endogenous sources of free radicals. The controlled production of ROS in mammalian cells is achieved through the phagocytes respiratory burst by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[7]. This enzyme utilize intracellular NADPH electrons to produce superoxide anion, which can be processed to many other free radicals-by-products enhancing the host immune systems against the microbial invasion[8]. The activated oxygen radicals are interacting with a large diversity of easily oxidizable components, including NADH, NADPH, nucleic acids, glutathione, tryptophan, ascorbic acid, histidine, cysteine, tyrosine, and proteins[4]. Free radical also comes from exogenous sources which are radiation, ozone, pathogenic microorganisms, air pollutants, toxins and chemicals[9].

2.1 Production of ROS in the host defense system

Periodontal disease occurs due to the accumulation of microbial plaque on the dentition of susceptible individuals with an abnormal host immune and inflammatory response to[10]. Host defense system against bacterial pathogens is responsible for the production and recruiting the polymorphonuclear leukocytes (PMN) during various pathological conditions, as in the periodontal structures inflammation[11]. Previous studies reported that periodontal pathogens and the by-products are responsible for the production of free radicals by leukocytes [12-17].

Neutrophils are the main defense cells in inflamed gingival tissues[18]. During phagocytosis, microbial antigen stimulates PMN to produce superoxide radical through the metabolic process of the 'respiratory burst', enhanced by NADPH oxidase[19]. Other free radicals as nitric oxide can be generated in the macrophages through nitric oxide synthase in acute inflammation of vascular endothelium[20]. During phagocytic degranulation, the azurophilic enzyme, myeloperoxidase, facilitates the generation of the non-radical species, HOCl, which has antibacterial activity [18]. Previous studies have proven that stimulated neutrophils in the gingival crevicular fluid and blood of adult periodontitis patients led to increased level of O_2^- production in the as compared to the healthy control group[21-23]. It was also shown that spontaneous low level of O_2^- was produced by PMN of adult periodontitis group, whereas the control group appeared to be unable to produce the O_2^- spontaneously [24].

ROS has been reported to have a role in bone resorption as the free radicals being generated at the ruffle border/bone interface by osteoclasts[25-28]. Conversely, few studies on resorption of bone indicated that free radicals, such as hydrogen peroxide and superoxide, are only activating the osteoclasts, and these radicals are not the main cause for resorption of bone matrix[29, 30], whereas nitric oxide suppresses bone destruction[28, 31]. The generation of ROS during periodontal inflammation by phagocytes would consequently enhance osteoclast formation and activation[2].

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82 **3.0 ROLE OF ROS IN PERIODONTAL DISEASE**

Electron reduction of oxygen in the mitochondria was reported to be the main cellular source of superoxide. The possibility that cellular oxygen is reduced to superoxide more than to water is increased if the concentration gradient of proton at the inner membrane of mitochondria is elevated and the sufficient electrons flux is diminished[4].

Tissue microenvironment could build-up high concentrations of radicals due to 1) overproduction by increased numbers of activated and 2) diminished catalase and SOD concentrations [32, 33]. Individuals with periodontal disease have an imbalance between oxidants and antioxidants. ROS produce significant direct and indirect tissue damage, and could initiate much of the tissue destruction coincident to periodontal disease[2].

Periodontal disease is caused by disequilibrium between periodontal tissue destruction and repair involving the host response to bacterial challenge. Polymorphonuclear leukocytes (PMNs) are the primary host defence against periodontal pathogens[1]. It has been found that the *Fusobacterium nucleatum* (FN) is associated with periodontal disease. FN strains stimulated PMN to produce a large amount of reactive oxygen species (ROS) or free radicals[34]. Following bacterial antigen stimulation, ROS or free radical molecular species are generated by PMNs, as a result of the inflammatory tissue response mechanisms. These mechanisms have been implicated in ROS

involvement in periodontal disease, including the possible interactions of PMNs with respect to the level of oxidation products and transition metal ions, neutrophil dysfunction, and antioxidant levels[7]. The phagocyte nicotinamide adenine dinucleotide phosphate oxidase (NOX2) is most likely one of the key sources of ROS in periodontal tissues[35]. The strongest case for involvement of NOX2 in periodontal diseases is aggressive periodontitis. Increased ROS generation by leukocytes from patients with aggressive periodontitis has clearly been documented. Altered neutrophils functions, such as abnormalities in adherence, chemotaxis, superoxide generation, phagocytosis, and bactericidal activity are known to play a role in the prevalence, progression, and severity of aggressive periodontitis.

Numerous diseases (which have connection with periodontal diseases) are also known to involve oxidative stress, including atherosclerosis, diabetes, hypertension, AIDS, cancer and also chronic inflammatory condition as well as aging process[36]. Many vascular proinflammatory states are associated with elevated expression of NOX2 and possibly NOX1 in the vessel wall. Expression of NOX1 increases considerably following balloon injury of the vessels. In addition, amyloid- β peptide was demonstrated to activate ASK-1 in neurons and NOX2 of microglia, increasing ROS generation. Both effects lead to apoptotic neuronal death. Oxidative stress in diabetes contributes to the generation of ROS by glycoxidation of sugars. Therefore, it has been pointed out that overexpression or over activation of NOX enzymes can lead to pathologies[37].

Tobacco use may be one of the most significant risk factors in the development and progression of periodontal disease because it promotes a high degree of ROS release which causes oxidative damage to gingival tissue, periodontal ligament, and alveolar bone[38]. Some of the effects of continued smoking are persistent gingival bleeding[39], vertical bone loss[40], and poor treatment outcomes[41]. Nicotine inhibits the attachment and growth of gingival and periodontal ligament fibroblasts[42] and decreases fibroblast migration[43]. At the cellular level, protein content was significantly decreased and cell membranes were damaged in the presence of nicotine. Other tobacco products can harm periodontal health. Smokeless tobacco can cause gingival recession and worsen periodontal disease. All tobacco products cause a higher oral cancer risk, halitosis, stained teeth, bone loss, loss of taste, less successful periodontal treatment, less success with dental implants, gingival recession, mouth sores, and facial wrinkling[41, 44].

Tobacco smoking can induce the cellular mechanisms that negatively influence oral health. In addition to stimulating production of ROS, smoking may reduce antioxidant levels. A dose-related reduction of salivary and GCF superoxide dismutase levels was found in both light and heavy smokers compared to non-smokers [45]. Smokers also had significantly lower serum levels of vitamin C than other levels of nonenzymatic antioxidants, such as vitamins A and E and coenzyme Q10. Human periodontal ligament cells respond to nicotine and tobacco extracts nearly the same way as

gingival fibroblasts by changing morphology and structure, with decreased growth and attachment through cytoskeletal disruption[46]. PDL cells were flattened in the control groups but rounded in the smoking groups, indicating a change in cytoskeletal structure. The investigators suggested cigarette smoking compromises PDL cell adhesion to root surfaces, which might affect periodontal regeneration following therapy[47]. Recent studies have also shown that nicotine decreases PDL and gingival fibroblast migration but treatment with antioxidants reverse the cellular behavior and increase migration rates[48].

Bacterial plaque is the most important substrate in periodontal disease development. Dental plaque bacteria involved in inducing "oxygen shock" to activate free radicals and the collagen-destroying enzymes. The process of collagen matrix degradation affects not only the amount of bone destruction and rate of inflammation but also the free radical damage, mechanical trauma, and tissue destruction[49].

3.1 Degradation of extracellular matrix components by ROS

The extracellular matrix comprises predominantly of a fibrous collagenous and non-collagenous network surrounding cells, which provide connective tissues with mechanical strength and physical support. Degradation of the connective tissue components during periodontal disease will lead to a loss of structural integrity of the periodontal tissues[7].

In considering the direct action of ROS upon periodontal connective tissue components, much information has been obtained from the study of other inflammatory conditions, particularly rheumatoid arthritis[50]. This is because since rheumatoid arthritis and chronic periodontal diseases are two examples of inflammatory diseases where the mechanisms of soft and mineralised tissue destruction have some similarities [51]. However, although inflammation in periodontal disease is likely to result from the reaction of the host response to factors within the bacterial plaque, the antigenic factor in rheumatoid arthritis is less clear[7].

The ROS has degradative effects on aggrecan which is the predominant extracellular matrix constituent in cartilage. Aggrecan is a large aggregating proteoglycan with a molecular weight of 1000–4000 kDa and containing chondroitin sulphate or keratan sulphate glycosaminoglycan chains, which represent approximately 93% of the total molecular mass[52]. As many as 200 aggrecan molecules may noncovalently bind to a single hyaluronan chain via a hyaluronan binding region, which is stabilised by link proteins [53]. Both nonradical species (H_2O_2 , $HOCl$) and radical species (O_2^- , $\cdot OH$) capable of aggrecan degradation *in vitro*, with the proteoglycan core proteins and the link protein regions of the proteoglycan aggregate being the most susceptible regions to degradation, compared to the glycosaminoglycan chains[19, 54, 55]. The explanation of the precise mechanism and oxidative products of ROS attack on glycosaminoglycans has been achieved using hyaluronan.

The depolymerisation of hyaluronan is believed to occur by $\cdot\text{OH}$ attack at multiple sites within the hyaluronan structure, resulting in the random destruction of unit monosaccharides formation of unstable radicals, followed by hydrolytic cleavage of the $\beta 1-3$ bond between the D-glucuronic acid or N-acetylglucosamine rings[56, 57]. However, the D-glucuronic acid regions being more vulnerable to ROS attack than the N-acetylglucosamine regions. Besides that, the degradative effects of ROS on extracellular matrix molecules can also influence the metabolism and proliferation of the resident connective tissue cells in cartilage, inhibiting DNA, proteoglycan, hyaluronan and protein synthesis, and inhibiting the post-translational incorporation of inorganic sulphate into biomolecules[58-61].

The basis of these alterations in collagen structure is the modification and loss of functional groups of certain amino acids, such as methionine, histidine and tyrosine residues[62, 63]. The ROS causes many other extracellular matrix proteins to undergo amino acid modification and fragmentation to lower molecular weight [64], including fibronectin and laminin. On the other hand, proline and histidine residues have been demonstrated to be important sites for ROS damage in many proteins, due to their ability to chelate transition metal ions, which promote localised $\cdot\text{OH}$ formation[65, 66]. Protein instability and fragmentation follows modification of the functional groups within amino acids by ROS. Amino acid modification can further cause changes in protein conformation, which can increase or decrease protein susceptibility to proteolysis[67, 68].

3.2 Degradation of periodontal tissues components by ROS

The ability of ROS, particularly the $\cdot\text{OH}$ species, to degrade hyaluronan and proteoglycans extracted from porcine gingivae and within cryostat sections of the tissue[69]. Exposure to ROS resulted in a reduction in the specific viscosity and molecular size of these molecules. Degradation of the glycosaminoglycans and proteoglycans associated with mineralised and non-mineralised periodontal tissues have been previously reported[11, 70, 71]. All glycosaminoglycans undergo chain depolymerisation and residue modification to varying degrees, particularly in the presence of the highly reactive $\cdot\text{OH}$ species[70]. The non-sulphated glycosaminoglycan, hyaluronan is being more susceptible to degradation by ROS than sulphated glycosaminoglycans. The highly reactive $\cdot\text{OH}$ species was also shown to exert the most detrimental degradative effects on the small chondroitin sulphate proteoglycans from alveolar bone, compared to other ROS[37]. The degradative effects were manifested as modifications of amino acid functional groups, with a loss of proline, leucine, tyrosine and phenylalanine residues most notable, in addition to peptide bond cleavage and glycosaminoglycan chain depolymerisation. The proteoglycan core proteins were demonstrated to be more susceptible to degradation in the presence of H_2O_2 compared to the glycosaminoglycan chains, although both the core proteins and glycosaminoglycan chains were extensively degraded in the presence of $\cdot\text{OH}$

species[72], the proteoglycans of alveolar bone and gingival tissues undergo a similar mechanism of destruction by ROS as the aggrecan proteoglycan species of cartilage.

3.3 Mechanisms of periodontal tissue destruction by ROS

Reactive oxygen species cause tissue damage by a variety of different mechanisms:

- lipid peroxidation (through activation of cyclooxygenases and lipoxygenases)
- DNA damage (base hydroxylations and strand breaks);
- protein damage, including gingival hyaluronic acid and proteoglycans;
- oxidation of important enzymes for example, anti-proteases such as oct-1-antitrypsin"; and
- stimulation of pro-inflammatory cytokine released by monocytes and macrophages, by depleting intracellular thiol compounds and activating nuclear factor KB (NF-KB).

There is a significant role for ROS in the complex pathological events occurring during periodontal diseases. In the advanced periodontitis, the proteoglycan metabolites are likely to originate from alveolar bone and be released into the GCF following their partial degradation. The metabolites have loss in their functional ability to interact with other matrix components and of a size sufficiently small enough to pass through the connective tissue into the GCF[7]. In the inflamed gingival tissue, it has been also identified that the core proteins of gingival proteoglycans present in inflamed tissues undergo extensive degradation[73], while the sulphated glycosaminoglycan chains remain relatively intact. In addition, as PMN contain no hyaluronidase activity and is unlikely to play a major role in the initial degradation of hyaluronan in inflamed gingival tissues it therefore follows that the only potential mechanism for hyaluronan depolymerisation is via ROS. This coincides with studies demonstrating that hyaluronan is more susceptible to ROS breakdown than sulphated glycosaminoglycans[11].

In consideration of other mechanisms of periodontal tissue destruction, bacterial antigens have been proposed to stimulate cytokine production by circulating mononuclear cells. These in turn stimulate the resident connective tissue cells and inflammatory cells to produce proteolytic enzymes, such as matrix metalloproteinases, resulting in an imbalance in the normal metabolism and leading to the degradation of both the collagenous and non-collagenous components within connective tissues[74]. However, ROS may further have an indirect role in potentiating extracellular matrix degradation by matrix metalloproteinases, via the activation of latent enzymes, such as collagenases and gelatinases[75]. Indeed, it has been proved that ROS are capable of activating latent PMN collagenase in GCF[76].

4.0 CONCLUSION

The human periodontal diseases are inflammatory disorders that give rise to tissue damage and loss, as a result of the complex interactions between pathogenic bacteria and the host's immune response. ROS include not only oxygen free radical but also non-radical oxygen derivatives involved in oxygen radical production. ROS are not only inevitable by-products of oxygen metabolism but also play a role in cellular signaling. Signaling via ROS is dangerous as overproduction of reactive signal molecules may be destructive especially in periodontal disease. ROS may play a part in the direct degradation of connective tissue components and cause modifications to the structures of connective tissue components, which are likely to lead to a loss in function of the periodontal tissues. ROS may also lead to altered metabolic activity of the connective tissues, by enhancing or deactivating proteolytic activity and by altering cellular activity. This problem can be prevented by consuming antioxidants which present in the fruits and vegetables that can neutralize free radicals by donating an electron without becoming unstable themselves. As many antioxidants can be found, they can be divided into enzymatic and non-enzymatic anti-oxidants. In conclusion, ROS play an important role in the initiation and progression of periodontal disease.

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