

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

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ABSTRACT

Periodontal diseases are widely prevalent diseases and negatively affecting the quality of life of young and adult population. They are inflammatory conditions 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 neutrophils 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 utilised for killing of the invading pathogens. Prolonged release of ROS and increases matrix metallo-proteinases 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.

INTRODUCTION:

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.

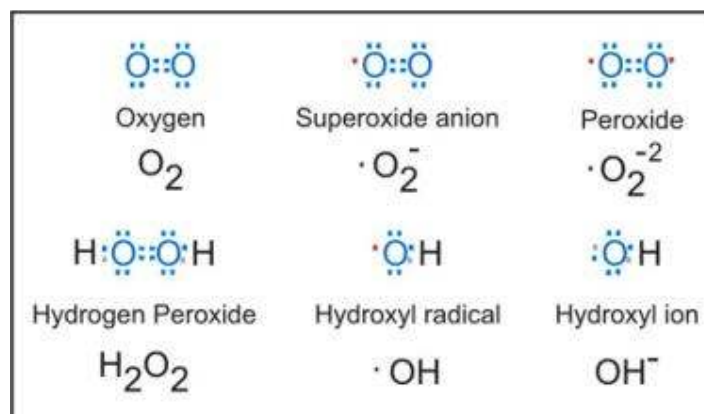


Fig 1: Types of reactive oxygen species (ROS). Electron structures of common reactive oxygen species.

EBSCO (dentistry and oral sciences), Medline and Pubmed databases were the sources for the articles collected. The databases were searched using certain key words as follows: Reactive oxygen species; periodontal disease; mitochondria; oxidation; free radicals. The significant articles were reviewed. In this paper, we highlighted ROS and tissue oxidation effects in periodontal structure destruction which in severe cases would lead to the loss of teeth.

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].

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 [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].

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 neutrophil 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 pro-inflammatory 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 non - enzymatic 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 behaviour 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].

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 ($\text{O}_2^{\cdot-}$, $\cdot\text{OH}$) 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

205 cartilage, inhibiting DNA, proteoglycan, hyaluronan and protein synthesis, and inhibiting the
206 post-translational incorporation of inorganic sulphate into biomolecules [58-61].

207 The basis of these alterations in collagen structure is the modification and loss of
208 functional groups of certain amino acids, such as methionine, histidine and tyrosine residues
209 [62, 63]. The ROS causes many other extracellular matrix proteins to undergo amino acid
210 modification and fragmentation to lower molecular weight [64], including fibronectin and
211 laminin. On the other hand, proline and histidine residues have been demonstrated to be
212 important sites for ROS damage in many proteins, due to their ability to chelate transition
213 metal ions, which promote localised $\cdot\text{OH}$ formation[65, 66]. Protein instability and
214 fragmentation follows modification of the functional groups within amino acids by ROS.
215 Amino acid modification can further cause changes in protein conformation, which can
216 increase or decrease protein susceptibility to proteolysis [67, 68].

221 **Degradation of periodontal tissues components by ROS**

222 The ability of ROS, particularly the $\cdot\text{OH}$ species, to degrade hyaluronan and
223 proteoglycans extracted from porcine gingivae and within cryostat sections of the tissue[69].
224 Exposure to ROS resulted in a reduction in the specific viscosity and molecular size of these
225 molecules. Degradation of the glycosaminoglycans and proteoglycans associated with
226 mineralised and non-mineralised periodontal tissue have been previously reported [11, 30,
227 70]. All glycosaminoglycans undergo chain depolymerisation and residue modification to
228 varying degrees, particularly in the presence of the highly reactive $\cdot\text{OH}$ species[30].The non-
229 sulphated glycosaminoglycan, hyaluronan is being more susceptible to degradation by ROS
230 than sulphated glycosaminoglycans. The highly reactive $\cdot\text{OH}$ species was also shown to
231 exert the most detrimental degradative effects on the small chondroitin sulphate
232 proteoglycans from alveolar bone, compared to other ROS [37]. The degradative effects
233 were manifested as modifications of amino acid functional groups, with a loss of proline,
234 leucine, tyrosine and phenylalanine residues most notable, in addition to peptide bond
235 cleavage and glycosaminoglycan chain depolymerisation. The proteoglycan core proteins
236 were demonstrated to be more susceptible to degradation in the presence of H_2O_2 compared
237 to the glycosaminoglycan chains, although both the core proteins and glycosaminoglycan
238 chains were extensively degraded in the presence of $\cdot\text{OH}$ species [71], the proteoglycans of

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

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 α_1 -antitrypsin.
- 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 [72], 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 [73]. 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 [74]. Indeed, it has been proved that ROS are capable of activating latent PMN collagenase in GCF [75].

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 signalling. Signalling 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 [76]. 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 antioxidants. In conclusion, ROS play an important role in the initiation and progression of periodontal disease.

References:

1. Waddington, R.J., R. Moseley, and G. Embery, *Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases*. Oral Dis, 2000. **6**(3): p. 138-51.
2. Chapple, I.L., *Reactive oxygen species and antioxidants in inflammatory diseases*. Journal of Clinical Periodontology, 1997. **24**(5): p. 287-296.
3. Squier, T.C., *Oxidative stress and protein aggregation during biological aging*. Exp Gerontol, 2001. **36**(9): p. 1539-50.
4. Bartosz, G., *Reactive oxygen species: destroyers or messengers?* Biochem Pharmacol, 2009. **77**(8): p. 1303-15.
5. Fleury, C., B. Mignotte, and J.L. Vayssiere, *Mitochondrial reactive oxygen species in cell death signaling*. Biochimie, 2002. **84**(2-3): p. 131-41.
6. Battino, M., et al., *Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species*. Crit Rev Oral Biol Med, 1999. **10**(4): p. 458-76.
7. Waddington, R.J., et al., *Relationship of sulphated glycosaminoglycans in human gingival crevicular fluid with active periodontal disease*. J Periodontal Res, 1996. **31**(3): p. 168-70.
8. Droge, W., *Free radicals in the physiological control of cell function*. Physiol Rev, 2002. **82**(1): p. 47-95.
9. Lobo, V., et al., *Free radicals, antioxidants and functional foods: Impact on human health*. Pharmacogn Rev, 2010. **4**(8): p. 118-26.

- 314 10. Chapple, I.L. and J.B. Matthews, *The role of reactive oxygen and antioxidant species in*
315 *periodontal tissue destruction*. Periodontol 2000, 2007. **43**: p. 160-232.
- 316 11. Moseley, R., R.J. Waddington, and G. Embery, *Degradation of glycosaminoglycans by*
317 *reactive oxygen species derived from stimulated polymorphonuclear leukocytes*. Biochim
318 Biophys Acta, 1997. **1362**(2-3): p. 221-31.
- 319 12. Passo, S.A., S.A. Syed, and J. Silva, Jr., *Neutrophil chemiluminescence in response to*
320 *Fusobacterium nucleatum*. J Periodontal Res, 1982. **17**(6): p. 604-13.
- 321 13. Miyasaki, K.T., et al., *Oxidative and nonoxidative killing of Actinobacillus*
322 *actinomycetemcomitans by human neutrophils*. Infect Immun, 1986. **53**(1): p. 154-60.
- 323 14. Miyasaki, K.T., M.E. Wilson, and R.J. Genco, *Killing of Actinobacillus actinomycetemcomitans*
324 *by the human neutrophil myeloperoxidase-hydrogen peroxide-chloride system*. Infect Immun,
325 1986. **53**(1): p. 161-5.
- 326 15. Thompson, H.L. and J.M. Wilton, *Effects of anaerobiosis and aerobiosis on interactions of*
327 *human polymorphonuclear leukocytes with the dental plaque bacteria Streptococcus*
328 *mutans, Capnocytophaga ochracea, and Bacteroides gingivalis*. Infect Immun, 1991. **59**(3): p.
329 932-40.
- 330 16. Shapira, L., et al., *Porphyromonas gingivalis lipopolysaccharide stimulation of human*
331 *monocytes: dependence on serum and CD14 receptor*. Oral Microbiol Immunol, 1994. **9**(2): p.
332 112-7.
- 333 17. Lynch, M.C. and H.K. Kuramitsu, *Role of superoxide dismutase activity in the physiology of*
334 *Porphyromonas gingivalis*. Infect Immun, 1999. **67**(7): p. 3367-75.
- 335 18. Miyasaki, K.T., *The neutrophil: mechanisms of controlling periodontal bacteria*. J Periodontol,
336 1991. **62**(12): p. 761-74.
- 337 19. Dean, R.T. and J.K. Pollak, *Endogenous free radical generation may influence proteolysis in*
338 *mitochondria*. Biochem Biophys Res Commun, 1985. **126**(3): p. 1082-9.
- 339 20. Knowles, R.G. and S. Moncada, *Nitric oxide synthases in mammals*. Biochem J, 1994. **298** (Pt
340 **2**): p. 249-58.
- 341 21. Kimura, S., T. Yonemura, and H. Kaya, *Increased oxidative product formation by peripheral*
342 *blood polymorphonuclear leukocytes in human periodontal diseases*. J Periodontal Res, 1993.
343 **28**(3): p. 197-203.
- 344 22. Katsuragi, H., et al., *Intracellular production and extracellular release of oxygen radicals by*
345 *PMNs and oxidative stress on PMNs during phagocytosis of periodontopathic bacteria*.
346 Odontology, 2003. **91**(1): p. 13-8.
- 347 23. Kantarci, A., K. Oyaizu, and T.E. Van Dyke, *Neutrophil-mediated tissue injury in periodontal*
348 *disease pathogenesis: findings from localized aggressive periodontitis*. J Periodontol, 2003.
349 **74**(1): p. 66-75.
- 350 24. Guarnieri, C., et al., *Enhanced superoxide production with no change of the antioxidant*
351 *activity in gingival fluid of patients with chronic adult periodontitis*. Free Radic Res Commun,
352 1991. **15**(1): p. 11-6.
- 353 25. Garrett, I.R., et al., *Oxygen-derived free radicals stimulate osteoclastic bone resorption in*
354 *rodent bone in vitro and in vivo*. J Clin Invest, 1990. **85**(3): p. 632-9.
- 355 26. Steinbeck, M.J., et al., *NADPH-oxidase expression and in situ production of superoxide by*
356 *osteoclasts actively resorbing bone*. J Cell Biol, 1994. **126**(3): p. 765-72.
- 357 27. Silverton, S.F., et al., *Osteoclast radical interactions: NADPH causes pulsatile release of NO*
358 *and stimulates superoxide production*. Endocrinology, 1995. **136**(11): p. 5244-7.
- 359 28. Silverton, S.F., et al., *Direct microsensor measurement of nitric oxide production by the*
360 *osteoclast*. Biochem Biophys Res Commun, 1999. **259**(1): p. 73-7.
- 361 29. Bax, B.E., et al., *Stimulation of osteoclastic bone resorption by hydrogen peroxide*. Biochem
362 Biophys Res Commun, 1992. **183**(3): p. 1153-8.

- 363 30. Moseley, R., et al., *The chemical modification of glycosaminoglycan structure by oxygen-*
364 *derived species in vitro*. Biochim Biophys Acta, 1995. **1244**(2-3): p. 245-52.
- 365 31. Rausch-Fan, X. and M. Matejka, *From plaque formation to periodontal disease, is there a role*
366 *for nitric oxide?* Eur J Clin Invest, 2001. **31**(10): p. 833-5.
- 367 32. Sies, H., *Oxidative stress: introductory remarks*, in *Oxidative stress*, H. Sies, Editor. 1985,
368 Academic Press; London. p. 1-8.
- 369 33. Ellis, S.D., et al., *Factors for progression of periodontal diseases*. J Oral Pathol Med, 1998.
370 **27**(3): p. 101-5.
- 371 34. Sheikhi, M., et al., *Lipid peroxidation caused by oxygen radicals from Fusobacterium-*
372 *stimulated neutrophils as a possible model for the emergence of periodontitis*. Oral Dis, 2001.
373 **7**(1): p. 41-6.
- 374 35. Giannopoulou, C., K.H. Krause, and F. Muller, *The NADPH oxidase NOX2 plays a role in*
375 *periodontal pathologies*. Semin Immunopathol, 2008. **30**(3): p. 273-8.
- 376 36. Stocker, R. and J.F. Keaney, Jr., *Role of oxidative modifications in atherosclerosis*. Physiol Rev,
377 2004. **84**(4): p. 1381-478.
- 378 37. Lambeth, J.D., *Nox enzymes, ROS, and chronic disease: An example of antagonistic*
379 *pleiotropy*. Free Radical Biology and Medicine, 2007. **43**(3): p. 332-347.
- 380 38. Morishige, T., et al., *Titanium dioxide induces different levels of IL-1beta production*
381 *dependent on its particle characteristics through caspase-1 activation mediated by reactive*
382 *oxygen species and cathepsin B*. Biochem Biophys Res Commun, 2010. **392**(2): p. 160-5.
- 383 39. Dietrich, T., J.P. Bernimoulin, and R.J. Glynn, *The effect of cigarette smoking on gingival*
384 *bleeding*. J Periodontol, 2004. **75**(1): p. 16-22.
- 385 40. Baljoon, M., *Tobacco smoking and vertical periodontal bone loss*. Swed Dent J Suppl,
386 2005(174): p. 1-62.
- 387 41. Johnson, G.K. and M. Hill, *Cigarette smoking and the periodontal patient*. J Periodontol,
388 2004. **75**(2): p. 196-209.
- 389 42. James, J.A., et al., *Effects of tobacco products on the attachment and growth of periodontal*
390 *ligament fibroblasts*. J Periodontol, 1999. **70**(5): p. 518-25.
- 391 43. Fang, Y. and K.K. Svoboda, *Nicotine inhibits human gingival fibroblast migration via*
392 *modulation of Rac signalling pathways*. J Clin Periodontol, 2005. **32**(12): p. 1200-7.
- 393 44. Albandar, J.M., et al., *Cigar, pipe, and cigarette smoking as risk factors for periodontal*
394 *disease and tooth loss*. J Periodontol, 2000. **71**(12): p. 1874-81.
- 395 45. Agnihotri, R., et al., *Association of cigarette smoking with superoxide dismutase enzyme*
396 *levels in subjects with chronic periodontitis*. J Periodontol, 2009. **80**(4): p. 657-62.
- 397 46. Xu, Y., et al., *[Effects of tobacco on proliferation and attachment of human periodontal*
398 *ligament fibroblast]*. Zhonghua Kou Qiang Yi Xue Za Zhi, 2003. **38**(5): p. 367-9.
- 399 47. Gamal, A.Y. and M.M. Bayomy, *Effect of cigarette smoking on human PDL fibroblasts*
400 *attachment to periodontally involved root surfaces in vitro*. J Clin Periodontol, 2002. **29**(8): p.
401 763-70.
- 402 48. San Miguel, S.M., et al., *Antioxidants counteract nicotine and promote migration via RacGTP*
403 *in oral fibroblast cells*. J Periodontol, 2010. **81**(11): p. 1675-90.
- 404 49. DuPont, G.A., *Understanding dental plaque; biofilm dynamics*. J Vet Dent, 1997. **14**(3): p. 91-
405 4.
- 406 50. Wood, Z.A., L.B. Poole, and P.A. Karplus, *Peroxisredoxin evolution and the regulation of*
407 *hydrogen peroxide signaling*. Science, 2003. **300**(5619): p. 650-3.
- 408 51. Snyderman R, M.G., *Analogous mechanisms of tissue destruction in rheumatoid arthritis and*
409 *periodontal disease*, in *Host-Parasite Interactions in Periodontal Disease*, M.S. Genco RJ,
410 Editor. 1982, American Society of Microbiology: Washington, DC. p. 254-362.

- 411 52. Hardingham, T.E., Beardmore-Gray, M., Dunham, D.G., & Ratcliffe, A.). *Cartilage*
412 *proteoglycans*. Ciba Found Symp, 1986. **124**: p. 30-46.
- 413 53. Carney, S.L. and H. Muir, *The structure and function of cartilage proteoglycans*. Physiol Rev,
414 1988. **68**(3): p. 858-910.
- 415 54. Katrantzis, M., et al., *The oxidant hypochlorite (OCl-), a product of the myeloperoxidase*
416 *system, degrades articular cartilage proteoglycan aggregate*. Free Radic Biol Med, 1991.
417 **10**(2): p. 101-9.
- 418 55. Panasyuk, A., et al., *Effect of reactive oxygen species on the biosynthesis and structure of*
419 *newly synthesized proteoglycans*. Free Radic Biol Med, 1994. **16**(2): p. 157-67.
- 420 56. Uchiyama, H., et al., *Chemical change involved in the oxidative reductive depolymerization of*
421 *hyaluronic acid*. J Biol Chem, 1990. **265**(14): p. 7753-9.
- 422 57. Hawkins, C.L. and M.J. Davies, *Direct detection and identification of radicals generated*
423 *during the hydroxyl radical-induced degradation of hyaluronic acid and related materials*.
424 Free Radic Biol Med, 1996. **21**(3): p. 275-90.
- 425 58. Bates, E.J., C.C. Johnson, and D.A. Lowther, *Inhibition of proteoglycan synthesis by hydrogen*
426 *peroxide in cultured bovine articular cartilage*. Biochim Biophys Acta, 1985. **838**(2): p. 221-8.
- 427 59. Murrell, G.A., M.J. Francis, and L. Bromley, *Modulation of fibroblast proliferation by oxygen*
428 *free radicals*. Biochem J, 1990. **265**(3): p. 659-65.
- 429 60. Hutadilok, N., M.M. Smith, and P. Ghosh, *Effects of hydrogen peroxide on the metabolism of*
430 *human rheumatoid and osteoarthritic synovial fibroblasts in vitro*. Ann Rheum Dis, 1991.
431 **50**(4): p. 219-26.
- 432 61. Hickery, M.S. and M.T. Bayliss, *Interleukin-1 induced nitric oxide inhibits sulphation of*
433 *glycosaminoglycan chains in human articular chondrocytes*. Biochim Biophys Acta, 1998.
434 **1425**(2): p. 282-90.
- 435 62. Venkatasubramanian, K. and K.T. Joseph, *Action of singlet oxygen on collagen*. Indian J
436 Biochem Biophys, 1977. **14**(3): p. 217-20.
- 437 63. Heinecke, J.W., et al., *Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative*
438 *cross-linking of proteins*. J Clin Invest, 1993. **91**(6): p. 2866-72.
- 439 64. Dean, R.T., et al., *Biochemistry and pathology of radical-mediated protein oxidation*.
440 Biochem J, 1997. **324** (Pt 1): p. 1-18.
- 441 65. Creeth, J.M., Cooper, B., Donald, A.S., & Clamp, J.R., *Studies of the limited degradation of*
442 *mucus glycoproteins. The effect of dilute hydrogen peroxide*. Biochemistry Journal, 1983.
443 **211**(2): p. 323-332.
- 444 66. Dean, R.T., S.P. Wolff, and M.A. McElligott, *Histidine and proline are important sites of free*
445 *radical damage to proteins*. Free Radic Res Commun, 1989. **7**(2): p. 97-103.
- 446 67. Trachootham, D., et al., *Redox regulation of cell survival*. Antioxid Redox Signal, 2008. **10**(8):
447 p. 1343-74.
- 448 68. Wang, C.H., et al., *Oxidative stress response elicited by mitochondrial dysfunction:*
449 *Implication in the pathophysiology of aging*. Exp Biol Med (Maywood), 2013. **238**(5): p. 450-
450 60.
- 451 69. Bartold, P.M., O.W. Wiebkin, and J.C. Thonard, *The effect of oxygen-derived free radicals on*
452 *gingival proteoglycans and hyaluronic acid*. J Periodontal Res, 1984. **19**(4): p. 390-400.
- 453 70. Moseley, R., et al., *The modification of alveolar bone proteoglycans by reactive oxygen*
454 *species in vitro*. Connect Tissue Res, 1998. **37**(1-2): p. 13-28.
- 455 71. Snyderman, R. and G.A. McCarty, *Analogous mechanisms of tissue destruction in rheumatoid*
456 *arthritis and periodontal disease*, in *Host-Parasite Interactions in Periodontal Diseases*, R.J.
457 Genco and S.E. Mergenhagen, Editors. 1982, American Society for Microbiology: Washington
458 DC. p. 354-362.
- 459 72. Rahemtulla, F., *Proteoglycans of oral tissues*. Crit Rev Oral Biol Med, 1992. **3**(1-2): p. 135-62.

460 73. Heath, J.K., et al., *Bacterial antigens induce collagenase and prostaglandin E2 synthesis in*
461 *human gingival fibroblasts through a primary effect on circulating mononuclear cells.* Infect
462 Immun, 1987. **55**(9): p. 2148-54.

463 74. Birkedal-Hansen, H., et al., *Matrix metalloproteinases: a review.* Crit Rev Oral Biol Med,
464 1993. **4**(2): p. 197-250.

465 75. Loesche, W.J. and N.S. Grossman, *Periodontal disease as a specific, albeit chronic, infection:*
466 *diagnosis and treatment.* Clin Microbiol Rev, 2001. **14**(4): p. 727-52, table of contents.

467 76. Dahiya, P., et al., *Reactive oxygen species in periodontitis.* . J Indian Soc Periodontol, 2013.
468 **17**: p. 411-6.

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470