Oral peroxidases: From antimicrobial agents to ecological actors (Review)

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Abstract. Sialoperoxidase and myeloperoxidase are the two main peroxidase enzymes found in the oral cavity. Sialoperoxidase is present in salivary secretions and in the biofilms that line the oral surfaces, while myeloperoxidase is abundant in the dento-gingival sulcus area. In the presence of hydrogen peroxide (H_2O_2), oral peroxidases catalyze the oxidation of the pseudohalide anion thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$), a strong oxidant that serves an antimicrobial role. Furthermore, oral peroxidases consume bacteria-produced H_2O_2 and could help inactivate toxic carcinogenic and genotoxic substances. Numerous *in vitro* studies have reported the antibacterial, antimycotic and antiviral role of peroxidases, suggesting possible applications in oral therapy. However, the use of oral hygiene products incorporating peroxidase systems has not yet been shown to be beneficial for the treatment or prevention of oral infections. This paradox reflects our incomplete knowledge of the physiological role of peroxidases in a complex environment, such as the oral region. While hygiene is crucial for restoring oral microbiota to a symbiotic state, there are no data to suggest that the addition of a peroxidase *per se* can create a dysbiotic state. Recent investigations have associated the presence of peroxidase activity with gram-positive cocci microbial flora, and its insufficiency with dysbiosis has been linked to pathologies, such as caries, periodontitis or infections of the oral mucosa. Therefore, oxidants generated by oral peroxidases appear to be an essential ecological determinant for oral health through the selection of a symbiotic microbiota capable of resisting oxidative stress. The objective of the present review was to update the current knowledge of the physiological aspects and applications of oral peroxidases in clinical practice.

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Contents

1. Introduction

Oral peroxidases are part of the innate non-immune defense mechanism of the saliva (1,2). In the oral medium in the presence of hydrogen peroxide (H_2O_2), these enzymes catalyze the oxidation of thiocyanate (SCN^-), a pseudohalide, into hypothiocyanite ($OSCN^-$), a powerful oxidant capable of inhibiting numerous bacterial species, mycoplasmas, fungi and viruses *in vitro* (Fig. 1). Sialoperoxidase and myeloperoxidase are the two main peroxidases found in the oral cavity. Sialoperoxidase is present in salivary secretions and in the biofilms that line the oral surfaces, while myeloperoxidase is abundant in the dento-gingival sulcus area (3,4). By regulating the commensal oral flora, as well as by consuming bacterial H_2O_2, which is toxic for the oral mucosa, oral peroxidases help protect the tissues bordering the oral cavity from microbial injuries. Although numerous studies have demonstrated an antimicrobial role *in vitro*, only a few studies have documented the use of oral hygiene products incorporating peroxidase systems for the treatment or prevention of oral infections. This paradox reflects our incomplete knowledge of the physiological role of these enzymes in a complex environment, such as the oral environment. The aim of this review was to update our current knowledge of the physiological aspects of oral peroxidases, as well as their potential applications in the oral clinic. The relevant articles published in English until August 2020 were retrieved from the literature indexed by PubMed (https://pubmed.ncbi. nlm.nih.gov). The MeSH terms were 'thiocyanate', 'hydrogen peroxide' and 'iodide' with 'oral peroxidases' in different combinations without exclusion criteria, provided they are related to the interested topic area. The systematic attention to the bibliographic section of the papers so-selected was a second source of references. The review data have been neither statically processed nor organized into flowcharts. The quality of evidence for clinical trials follows the recommendations of

the Grading of Recommendations Assessment, Development and Evaluation system.

2. Oral peroxidases: Enzymes and substrates

Peroxidase activity in the saliva was first suspected due to the restoration of an anti-*lactobacillus* effect following the addition of lactoperoxidase purified from bovine milk in heat-treated saliva (5,6). Salivary peroxidase was then considered to be similar, if not identical, to lactoperoxidase. Salivary peroxidase (or sialoperoxidase) was not considered to be an enzyme different from lactoperoxidase until later. Indeed, these two enzymes have the same amino acid composition (7) and exhibit immunological cross-reactivity (8). However, they differ in their carbohydrate composition and certain kinetic characteristics (7). The whole saliva also contains a significant amount of myeloperoxidase from neutrophils, which enter the oral cavity through the dento-gingival sulci (3). The coexistence of sialoperoxidase and myeloperoxidase in the oral environment may mask the activity of other peroxidases that may be present in much lower quantities (microbial peroxidases) or transiently (lactoperoxidase in dairy products).

Sialoperoxidase. The parotid and submaxillary salivary glands secrete human sialoperoxidase from birth at adult concentrations (9,10). Human sialoperoxidase occurs in different forms based on molecular weight and electrophoretic migration; sialoperoxidase may be free or linked to other salivary molecules, such as mucin (11-13). When unbound and monomeric, its molecular weight is ~78,000 (7), and its isoelectric point is 8-10 (11,14). This enzyme adsorbs different oral surfaces, while retaining its enzymatic activity (15-17), including enamel (15), dental plaque (18), salivary sediments (16) and bacteria such as streptococci (17). Other exocrine secretions, such as tears, sweat, airways or digestive secretions, also have a similar activity (usually referred to as lactoperoxidase and, at times, lacrimal peroxidase in tears). Salivary peroxidase, along with other proteins secreted by salivary glands (i.e. lysozyme and lactoferrin), participates in the antimicrobial protection of oral surfaces, such as mucosa and dental crowns.

Myeloperoxidase. Myeloperoxidase in saliva comes from the lysis of neutrophils entering the oral cavity by the dento-gingival sulci from the first dental eruptions (19-24). The leukocyte count in saliva decreases in edentulous patients and increases in oral inflammation processes (25). The number of neutrophils migrating into the oral cavity is ~10^6 cells/min in healthy subjects, and higher in patients with periodontal disease (3,26,27). In the saliva, neutrophils undergo hypo-osmotic shock and release myeloperoxidase along with other antimicrobial proteins, such as lysozyme and lactoferrin; in a similar manner, the salivary glands secrete the same two proteins, in addition to sialoperoxidase. Myeloperoxidase represents up to 20-25% of the total peroxidase activity in total centrifuged resting saliva, while it predominates in the salivary sediment and in the liquid of the dento-gingival sulci, where it participates in antimicrobial tissue protection (3,23). The primary substrate for myeloperoxidase is chloride (Cl⁻) in the tissues [with the production of hypochlorite (OCl⁻)] and SCN⁻ in the saliva (with the production of OSCN⁻).

Lactoperoxidase in dairy products. Lactoperoxidase, similarly to sialoperoxidase, catalyzes the oxidation of SCN⁻ *in vivo* and iodide (I⁻) *in vitro*, in the presence of H_2O_2. Easily purified from bovine milk, lactoperoxidase was first characterized and its enzymatic aspects were specifically documented by Aune and Thomas in 1977 and 1978 (28,29). Lactoperoxidase was commonly used to study the effects of a peroxidase-H_2O_2-SCN⁻ system on cariogenic bacteria, such as oral streptococci (30) or *Lactobacillus acidophilus* (31), on periodontopathogenic bacteria (32). The lactoperoxidase-SCN⁻ combination is used as a preservative for vegetables and as a supplement in several oral hygiene products. The lactoperoxidase-I⁻ combination allows for the radioactive labelling of proteins. Lactoperoxidase has the property of adsorbing different surfaces, including that of biomaterials, such as titanium (33).

H_2O_2. Oral H_2O_2 comes from the salivary glands [DUOX family of oxidases, reviewed in (34)], neutrophils [NOX family of oxidases, reviewed in (35)] and particularly certain bacterial species present in the mouth through the action of pyruvate oxidase (36). H_2O_2 is one of the reactive oxygen species (ROS) synthesized by neutrophils during phagocytosis and secreted by the salivary glands as a trigger for sialoperoxidase activity. Determination of H_2O_2 in the saliva is challenging, given its rapid consumption by oral peroxidases and bacterial catalases. Most of the oral H_2O_2 comes from bacteria of the genus *Streptococcus* (37-39) and, to a much lesser extent, from optional anaerobes (40,41). Based on the equilibrium constant of the SCN⁻ peroxidation reaction ($3.7x10^3$ M⁻¹) in the oral medium, the mean salivary H_2O_2 concentration has been estimated at 10 μM (42), the maximum dose tolerable by mammalian cells (43,44). At low concentrations (1-10 nM), H_2O_2 plays a role as a signaling molecule in bacteria-bacteria interactions (45,46). For example, H_2O_2 can promote the formation of *Streptococcus parasanguinis* biofilms co-cultured with *Actinobacillus actinomycetemcomitans in vitro* (47).

SCN⁻. SCN⁻ is secreted by the parotid, submaxillary, sublingual and minor salivary glands (48,49) at a concentration that is dependent on exogenous intake, mainly brassica vegetables (50), as well as the smoking habits of individuals (51), and on endogenous production coupled with the detoxification of food cyanide, such as cassava (52). The salivary concentration of SCN⁻ is 0.5-2 mM in non-smokers and may be up to 10 mM in heavy smokers (12,51,53); its concentrations in the gingival crevicular fluid is ~40 μM, but may be higher in smokers (12). Of note, the optimal levels for peroxidase activity are in the order of mM. To the best of our knowledge, no study to date has documented the harmful effect of smoking on oral health through the inhibition of the natural peroxidase systems. SCN⁻ has already been proposed as a vasodilator to combat hypertension or as an antimicrobial agent in combination with a peroxidase system. In this context, various studies [reviewed in (54)] have suggest the absence of SCN⁻ toxicity up to concentrations sufficient to activate the enzymatic production of OSCN⁻. A more significant increase in plasma SCN⁻ concentration is linked to the inhibition of thyroid function, particularly in cases of iodine deficiency. Indeed, SCN⁻ is a competitive inhibitor of the Na⁺/I⁻ symport in thyroid follicular cells. This competition between a halogen
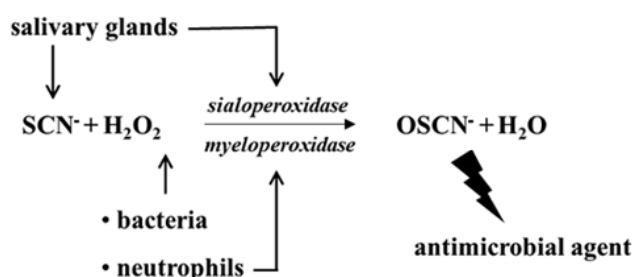
Figure 1. Peroxidase system in the oral cavity leading to the production of OSCN$^-$, which controls the oral microflora. SCN$^-$, thiocyanate; H$_2$O$_2$, hydrogen peroxide; OSCN$^-$, hypothiocyanite.

and SCN$^-$ for peroxidases explains the etymology of the word 'pseudohalogen' to qualify for SCN$^-$.

I$^-$. The ability of the salivary glands to concentrate I$^-$ into the salivary compartment (20-100 times higher than the plasma concentration) has long been known (55). This capacity for concentrating iodine in saliva before intestinal re-absorption contributes to the preservation of the iodine pool for optimal thyroid function (56). Iodine deficiency, which is marked by lower urinary and salivary rates, has been correlated with an increase in the incidence of dental caries (57), but no association between iodine deficiency and oral peroxidases has been reported in these studies. Other studies have implicated iodine deficiency in immune dysfunctions (56). Excess iodine has been reported to induce alterations of the salivary glands, including lymphocytic infiltrations (58), in an animal model. *In vitro*, I$^-$ is another substrate for sialoperoxidase, myeloperoxidase or lactoperoxidase in antimicrobial systems. The salivary concentration of I$^-$ in humans is estimated to be insufficient for antimicrobial action *in vivo*. Indeed, the salivary levels reported in the literature are in the order of μM (1,59,60), while those of SCN$^-$ are in the mM range. The ability of SCN$^-$ to inhibit the uptake of I$^-$ by the parotid cells in parallel with thyroid cells has long been known (61). The presence of SCN$^-$ at higher concentrations in the saliva should limit the metabolism of I$^-$ (oxidation, iodination of tyrosine and iodination of proteins) (12,62,63). However, certain studies have suggested a synergy between the two substrates (64), whereas others have documented the *in vitro* formation of iodine-SCN$^-$ complexes as a more active/stable antimicrobial component, as compared with OI$^-$ and OSCN$^-$ alone (65-68).

3. Oxidant production by oral peroxidases

Both peroxidases that present in the oral cavity transform SCN$^-$ into OSCN$^-$. *In vitro*, sialoperoxidase turns I$^-$ into hypoiodite (OI$^-$). In tissues, myeloperoxidase forms OCl$^-$ from Cl$^-$ but not in saliva due to the presence of SCN$^-$. *In vitro*, myeloperoxidase can also produce OI$^-$. In the oral environment, the two peroxidases oxidize SCN$^-$ more abundantly than I$^-$. H$_2$O$_2$ is a factor limiting the activity of oral peroxidases. These non-Michaelian enzymes are characterized by optimal concentrations, above which their substrates become their inhibitors (69).

It should be noted that the oral cavity is not a single homogeneous compartment, but rather a juxtaposition of sites, each characterized by its own ecosystem. The intraoral sites to

be considered are indeed numerous and can be grouped into the saliva, biofilms and dento-gingival sulci (70). The peroxidase activity varies across sites, depending on the concentrations of available substrates (H$_2$O$_2$ and SCN$^-$). The parotid production of H$_2$O$_2$ is already sufficient for generating OSCN$^-$ in the excretory Stenson's duct (71). The preferred method for determining salivary OSCN$^-$ is based on the oxidation of the 5,5'-dithiobis-2-nitrobenzoic acid (72). Other procedures are based on the oxidation of pyrogallol, guaiacol or ABTS (73). However, these methods, which are not specific to OSCN$^-$, do not make it possible to specifically analyze the products of the peroxidase activity in the presence of several substrates during the same test, in this case SCN$^-$ together with I$^-$. A colorimetric method in the presence of tetramethylbenzidine was recently developed to more specifically assess OI$^-$ (74). Fluorides have been described as inhibitors of lactoperoxidase and sialoperoxidase at acid pH regardless of the substrate, SCN$^-$ or I$^-$ (75).

OSCN$^-$. OSCN$^-$ is considered to be the major oxidant produced by oral peroxidases in the oral cavity. Concentrations of OSCN$^-$ up to 300 μM have been reported in the literature for the whole saliva (12). However, methodological problems when collecting samples may lead to default errors due to the instability of the molecule and the large number of oxidizable targets in saliva and oral biofilms. As SCN$^-$ is metabolized by both myeloperoxidase and sialoperoxidase, the production of OSCN$^-$ alone cannot be used to characterize the activity of one of these two enzymes (76). OSCN$^-$ at the doses required for an antibacterial effect does not appear to cause damage to the genetic material of the mucous membranes (77). However, selective oxidation of cell/tissue targets containing thiol groups could initiate significant cell damage, such as damage to gingival epithelial cells (78).

OI$^-$. *In vitro*, OI$^-$ decreases the survival rate of some microbial species at a lower concentration, as compared with that observed for OSCN$^-$, especially in *Candida albicans* (79). Some authors (80) have been able to demonstrate that ^{125}I only binds to a limited number of membrane molecules of *Candida albicans*, which is particularly sensitive to OI$^-$. However, the hypohalous ion present in the oral cavity is OSCN$^-$ rather than OI$^-$, as the substrate SCN$^-$ at the origin of the former is 1,000 times more abundant, as compared with I$^-$ at the source of the latter. The salivary SCN$^-$ concentration reaches the mM level, while OI$^-$ barely reaches concentrations in the μM range. At equal (pseudo)halide concentrations, lactoperoxidase has a higher affinity for SCN$^-$ than for I$^-$. However, experimentally, the substitution of SCN$^-$ by I$^-$ increases the innate antiviral defences of the respiratory mucosa against adenoviruses and the respiratory syncytial virus (81). Oral administration of KI vs. NaI increases iodemia, allowing for the accumulation of I$^-$ in the mucous secretions of the upper respiratory tract in humans (81) as well as animals (82,83). Although it has already been demonstrated that I$^-$ accumulates in submandibular glands in hamsters (84), no similar observations have been reported in humans. It would be interesting to be able to specifically assess salivary OI$^-$ in excretory ducts, such as the Stenson's duct, where the oral biofilms do not extend, except in the case of infectious parotitis. Finally, the

success of topical formulations based on povidone-iodine to reduce bacterial growth in the oral cavity in ventilated patients may be attributed to the increase in I⁻ available for the patient's peroxidases (85).

Iodine-thiocyanate complexes. The two substrates of peroxidase, SCN⁻ and I⁻, have been mostly tested independently in suspensions, and less frequently in combination (64). Moreover, few articles reporting that the activity of lactoperoxidase produces a mixture of OSCN/OI⁻ in the presence of a mixture of SCN⁻/I⁻ substrates have effectively analyzed the products generated under their experimental conditions (1,64,86). Mixtures of the two substrates, SCN⁻ and I⁻, however, appear to lead to the formation of a more stable oxidative complex over time (65,66,87-90). Under well-defined *in vitro* experimental conditions (high ionic strength; KI:KSCN ratio, ~4.5; neutral or acidic buffer; presence of H_2O_2), lactoperoxidase produces stable iodine-SCN⁻ complexes, such as $I_2(SCN)^-$, objectified by carbon-13 (C13) nuclear magnetic resonance spectroscopy (66) with antimicrobial properties against *Candida* (67). Oxidant concentrations are then less toxic to epithelial cells in the mouth, as compared with chlorhexidine (67). The resistance of epithelial cells may be explained by the presence of bacteria covering their surface and forming a protective biofilm, and/or by the detoxification of enzymatic activities in certain commensal species present in the oral environment (91). The use of these complexes for rinsing dentures *ex vivo* further limits the possible effect on epithelial cells *in vivo*. The efficacy of the iodine-SCN⁻ complexes prepared without peroxidase activity has already been demonstrated *in vitro* on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both in suspension and in biofilms (92). Other studies will have to analyze the interference between this solution of iodine-SCN⁻ complexes and oral microbial ecology.

4. Biological roles of oral peroxidases

Over several decades, oral peroxidases have been recognized as being essential for oral health (93), despite the absence of clinical evidence. Indeed, no specific deficit in sialoperoxidase or its association with a particular pathology have been described. Although the decrease in salivary flow leads to carries or mucosa infections (for example, candidiasis), the deficiency of no particular salivary molecule (in this case, sialoperoxidase) has been definitively proven to cause these infections. Similarly, neutropenia leads to gingivitis/periodontitis, but it is not possible to definitively deduce that myeloperoxidase deficiency is individually responsible. The demonstration of the biological roles of peroxidases is based more on *in vitro* studies carried out under physiological conditions.

Antimicrobial effects. Oral peroxidases control the oral microbiota while detoxifying the environment of H_2O_2. By oxidizing thiol groups (29) and nicotinamide adenine dinucleotide phosphate (NAD(P)H) (94), OSCN⁻ exerts antimicrobial, antimycotic and antiviral effects (1,2). In particular, the bacterial hexokinase activity, which initiates the Emden-Meyerhoff pathway, decreases in the presence of OSCN⁻. Acid production by dental plaque is reduced in the presence of OSCN⁻ (95).

In vitro, OSCN⁻ slows the growth of cariogenic bacteria belonging to the genera *Streptococcus* or *Lactobacillus* (1). Periodontopathogenic bacteria are also inhibited (32,96-98), as are yeasts of the *Candida* genus, which are responsible for mucosal pathologies (99). Some viruses are sensitive to this oxidant, such as the herpes virus (100), HIV (101) or influenza virus (102,103). Antisepsis research suggests using peroxidase-generated OSCN⁻ as a putative prophylactic agent to prevent contamination with SARS-CoV-2 (104).

Some bacteria have developed various strategies to resist the ROS produced by neutrophils (105). Similarly, commensal oral surface bacteria, such as *Streptococcus sanguis* (one of the first colonizers of the dental plaque) have the biochemical equipment to protect themselves from OSCN⁻. By contrast, *Streptococcus mutans* (a cariogenic bacterial species) does not have this detoxifying system (91). Thus, peroxidases operate more like ecological selectors by allowing commensal bacteria capable of reducing OSCN⁻ to survive in the oral environment. Moreover, peroxidases coupled to a system detoxifying the environment of hypohalous compounds (of the thioredoxin type) participate in the protection of tissues against oxidants resulting from the metabolism of activated oxygen, such as the superoxide anions or H_2O_2 (106). There appears to be an interconnection between resistance to oxidative stress and the ability to form biofilms via quorum-sensing molecules, such as farnesol (107). On the other hand, non-active lactoperoxidase, after the depletion of the substrate, can promote the *in vitro* growth of certain anaerobes (98). At the same time, a clinical study (108) suggested a rebound in periodontitis after a phase of clinical improvement, possibly due to an accumulation of lactoperoxidase without substrate. The association of a peroxidase system with another innate non-immune defence factor further strengthens the inhibition of some bacteria *in vitro*. For example, lysozyme improves the inhibition of glucose metabolism by the peroxidase system in *Streptococcus mutans* (109).

Biofilm control. Sialoperoxidase, similar to lactoperoxidase *in vitro*, adsorbs enamel surfaces irreversibly (15) while retaining its activity. This property contributes to controlling the formation of dental plaque and regulating the bacterial microflora attached to the surfaces of the oral cavity. The activity of peroxidases present in dental plaque depends on the availability of its two substrates (SCN⁻ and H_2O_2) in the thickness of oral biofilms. SCN⁻ diffuses deeper into the biofilms from the salivary film that covers them. At the same time, H_2O_2 relies on the oxygen supply allowing certain bacteria to produce it *in situ*. Certain studies (12,106) have described a virtuous cycle summarizing the beneficial effect of sialoperoxidase on oral microflora control. After a supply of food sugars, commensal bacteria of the genus *Streptococcus* grow and produce more H_2O_2, which activates the production of OSCN⁻ in the presence of sialoperoxidase and SCN⁻, thereby limiting bacterial growth or even killing microorganisms. Theoretically, these mechanisms work less effectively deeper inside thick biofilms, given the consumption of peroxidase substrates in the most superficial layers. Similarly, mouth rinsing with solutions containing an OSCN⁻-generating system is likely to further impact the more superficial layers of biofilms. These remarks suggested that a peroxidase system

may act both on the formation of biofilms and on already formed biofilms.

In addition to protecting the walls of the oral cavity, sialoperoxidase may also protect the excretory ducts of the salivary glands, which are rarely infected when they open into an oral cavity abundantly colonized by microbial germs; however, to the best of our knowledge, no scientific study has yet considered this physiological aspect.

Protection against oxidative stress. The uncontrolled production of activated oxygen species (ROS: Superoxides, H_2O_2, free radicals, hypohalous compounds) or reactive nitrogen species (RNS: Compounds derived from nitric oxide and superoxides, such as peroxynitrite, nitrogen dioxide and dinitrogen trioxide) is implicated in various oral pathologies (110). These oxidative derivatives damage cells and are considered to be mutagenic and carcinogenic. They are controlled in the salivary environment by scavengers (mainly uric acid) and antioxidant enzymes (bacterial catalase and oral peroxidases). Lactoperoxidase transforms cytotoxic H_2O_2 into a less toxic product, as suggested by *in vitro* experiments on fibroblast cultures (44). The coupling of peroxidases to a bacterial thioredoxin system helps protect tissues from oxidants produced from peroxidase activities. In tissues (including gingival), catalase and glutathione peroxidase help remove H_2O_2.

Certain studies [reviewed in (12)] considered that the enzymatic peroxidation of mutagenic and carcinogenic molecules bound to diet as another function of oral peroxidases. However, the related data are currently insufficient to assess its actual significance in the detoxification of toxic molecules introduced into the oral cavity, through food or other means.

5. Mimicking biological systems

Manufacturers have attempted to mimic the peroxidase activity of saliva by incorporating lactoperoxidase into oral hygiene products [reviewed in (111)]. A 'peroxidase system' is a galenic formulation associating a peroxidase with one of its substrates of the (pseudo)halide type plus H_2O_2 or an H_2O_2 donor. The formulation of such a system requires a methodical step-by-step approach to optimize the volume and rinsing time, the concentration of H_2O_2 and SCN⁻, as well as the pH, in order to achieve the production of an adequate antimicrobial level in the oral cavity (112). Even before the launch of oral hygiene products with a peroxidase system, Food and Agriculture Organization (FAO)/World Health Organization (WHO) recommended its use for the safe preservation of raw milk or vegetables (113). Articles on proteins derived from milk list the oral benefits of lactoperoxidase, sometimes associated with other proteins, such as lactoferrin or lysozyme (114). Numerous preclinical *in vitro* tests (through saliva sample collection, solid or liquid cultures, biofilm models) have evaluated peroxidase systems for pharmacokinetics (115), antimicrobial effects on the oral microflora (116), as well as toxicity on epithelial cells (117). Some *ex vivo* studies have reported the decontamination of materials that have remained in an oral environment, while others describe the advantage of their use *in vivo*. The SCN⁻/lactoperoxidase system is the most extensively investigated, but there have been few clinical studies on the I⁻/lactoperoxidase system (118).

Unfortunately, these investigations often test a peroxidase system coupled with other milk antimicrobial proteins also found in the saliva (119). Such systems have been introduced into oral hygiene products with a curative intent (periodontitis, halitosis) or with a preventive perspective (dry mouth, caries). However, the efficacy of these products has not been sufficiently documented (117), and there are only a limited number of small-scale phase I/II clinical trials. Investigators face the difficulty of finding adequate biological markers to demonstrate the effects of a peroxidase system on the regulation of oral microflora in the oral environment. Another dilemma could be to choose between quantitative (bacterial count) or qualitative (metabolic markers) markers. For example, a mouth-rinse/tooth-wash with an *in vivo* peroxidase system increases the concentration of OSCN⁻ in the saliva (120) but does not change the total number of salivary bacteria, while it could reduce bacterial ATP (an indicator of their metabolic potential). Several studies have thoroughly evaluated peroxidase systems by following clinical indices (gingival index, plaque index, etc.) and some others, as dry mouth, settle for satisfaction surveys. The determination of an appropriate control is challenging. Moreover, the short half-life of the oxidants produced and the bacterial resistance constitute other limitations to their use. Recently, the SCN⁻/I⁻ combination has made it possible to obtain more stable products. Molecular techniques (16SrRNA gene sequencing analysis) demonstrated that the use of lactoperoxidase coupled with lactoferrin promotes supragingival and lingual biofilms with more gram-positive and fewer gram-negative bacteria (119).

Tables I and II list the clinical studies evaluating a peroxidase system used alone (108,121-130) or in combination (131-140) with other exocrine proteins.

Curative vs. preventive. The use of peroxidase systems may be considered as topical administration to protect oral surfaces *in vivo* as a prophylactic measure. Recommending these galenic preparations may not be of value as a curative approach, as the infection itself indicates that a deregulated oral flora has overtaken physiological systems, including peroxidase. Reserving or even developing these galenic preparations as prophylactic treatment would avoid the appearance of strains resistant to antimicrobials used continuously to prevent the occurrence of certain infections (for example, oral candidiasis in patients with AIDS).

In vitro vs. in vivo. Peroxidase systems present a paradox: Although they have been shown to be active *in vitro* for a long time, the definitive evidence of their effects *in vivo* is still lacking. A possible explanation is the actual availability of H_2O_2 in the oral environment, which is rich in bacterial catalase. In this case, the increased availability of H_2O_2 can compensate for its consumption by other enzymatic activities (141,142), thus allowing the exogenous lactoperoxidase to be active. Another explanation would be to consider a transient metabolic suppression demonstrated, for example, by assaying ATP, but not observed on culture media.

In vivo vs. ex vivo. Peroxidases adsorb different biomaterials used in dentistry: Titanium and resin are two examples. *Ex vivo* use may be employed to disinfect removable prostheses

Table I. Bibliographic references for clinical trial reports evaluating the efficiency of a peroxidase system in oral medicine when used alone (references cited until August 2020 in the PubMed database).

First author/s, year	Field	Pathology	Peroxidase system tested	N	Marker	Effect	(Refs.)
Koch et al, 1973	H	Plaque accumulation	AGC, GOD	15	PI	Yes	(121)
Hugoson et al, 1974	H	Caries, gingivitis	AGC, GOD	22	CI, GI, PI	Yes	(122)
Koch and Strand, 1979	H	Caries	AGC, GOD	750	CI, dental X-rays	Yes	(123)
Rotgans and Hoogendoorn, 1979	A	Caries	AGC, GOD	48	Histology	Yes	(124)
Afseth and Rølla, 1983	H	Plaque accumulation	AGC, GOD	8	PI, plaque pH	No	(125)
Midda and Cooksey, 1986	H	Plaque accumulation, gingivitis	AGC, GOD, SCN⁻	135	GI, PI	Yes	(126)
van Steenberghe et al, 1994	H	Post-irradiation xerostomia	G/GOD/SCN⁻/LPO	12	PI, SBI	Yes	(108)
Bánóczy et al, 1994	H	Xerostomia	G/GOD/SCN⁻/LPO	41	Self-rating survey/bacteria monitoring/cytological tests	Yes/no/yes	(127)
Toljanic et al, 1996	H	Post-irradiation xerostomia	G/GOD/SCN⁻/LPO	60	GI, PI	Yes	(128)
Epstein et al, 1999	H	Post-irradiation xerostomia	G/GOD/SCN⁻/LPO	19	Self-rating survey/bacteria monitoring	Yes/no	(129)
Warde et al, 2000	H	Post-irradiation xerostomia	G/GOD/SCN⁻/LPO	28	Self-rating survey	Yes	(130)

A, animal; AGC, amyloglucosidase; CI, caries index; H, human; GI, gingival index; GOD, glucose oxidase; SCN⁻, thiocyanate; LPO, lactoperoxidase; N, number of cases; PI, plaque index; SBI, sulcus blood index.

Table II. Bibliographic references for clinical trial reports evaluating the efficiency of a peroxidase system in oral medicine when used in combination with other exocrine proteins (references cited until August 2020 in the PubMed database).

First author/s, year	Field	Pathology	Peroxidase system tested	N	Marker	Effect	(Refs.)
Kirstilä et al, 1996	H	Xerostomia	G/GOD/SCN⁻/LPO	20	Chemical monitoring/bacteria monitoring/self-rating survey	No/no/no	(131)
Matear and Barbaro, 2005	H	Xerostomia	G/GOD/SCN⁻/LPO + Lf + Lz	20	Self-rating survey	Yes	(132)
Hatti et al, 2007	H	Plaque accumulation	G/GOD/SCN⁻/LPO + Lf + Lz	20	PI/bacteria monitoring	Yes/yes	(133)
Gil-Montoya et al, 2008	H	Xerostomia	G/GOD/SCN⁻/LPO + Lf + Lz	20	Candida monitoring/self-rating survey	No/yes	(134)
Shin et al, 2011	H	Halitosis	G/GOD/LPO + Lf	15	Chemical monitoring/bacteria monitoring	Yes/yes	(135)
Jose et al, 2016	H	Xerostomia	LPO + Lf + Lz	396	Self-rating survey	Yes	(136)
Nakano et al, 2016	H	Halitosis	GOD/LPO + Lf	39	Chemical monitoring	Yes	(137)
Morita et al, 2017	H	Oral hygiene in the elderly	LPO + Lf	37	Bacteria monitoring	Yes	(138)
Barbe et al, 2018	H	Xerostomia	G/GOD/SCN⁻/LPO + Lf + Lz	40	PI/organoleptic score	Yes/yes	(139)
Nakano et al, 2019	H	Gingivitis	LPO + Lf	150	GI, PI, self-rating survey	Yes	(140)

H, human; G, glucose; GI, gingival index; GOD, glucose oxidase; SCN⁻, thiocyanate; LPO, lactoperoxidase; Lf, lactoferrin; Lz, lysozyme; N, number of cases; PI, plaque index.

by short-circuiting *in vivo* use; the intra-oral administration is indeed considered by some to be dangerous, due to cytotoxicity and potential immunogenicity. The toxicity of peroxidase systems on the oral mucosa appears to be limited by the oral biofilms covering them. However, the accumulation of inactive lactoperoxidase in biofilms may favor certain anaerobic bacteria, such as *Porphyromonas gingivalis* (98). On the other hand, the accumulation of I$^-$ during regular use would not be advised for some patients. With regards to health and safety, it should be noted that the FAO recommends the SCN$^-$/lactoperoxidase system for the decontamination of milk and vegetables for food sales (113).

Toxicity. Few reports have analyzed the oral toxicity of peroxidase systems when recommended in oral care products. Indirectly, these products may be beneficial for patients who have aphthous ulcers secondary to the toxicity of lauryl sulfate, often used as a detergent in toothpaste (143). Indeed, the incorporation of such enzymes in oral hygiene products implies the no-use of detergent foaming agents, which have the ability to denature proteins, and therefore enzymes.

Perspective in oral hygiene. Numerous investigations have analyzed the effects of peroxidase systems on the various pathogens present in the oral cavity, including viruses. *In vivo*, oral peroxidases defend the gingival sulcus against microbial invasion (myeloperoxidase) and help regulate the microflora adhering to the surfaces of the oral cavity (sialoperoxidase). Incorporating lactoperoxidase with other salivary antimicrobial proteins in salivary substitutes may mimic the antimicrobial effect of the salivary exocrine fluid (144). The beneficial effect of oral sprays with such peroxidase preparations in combating dryness of the oral mucosa in patients on respirator treatment has already been proposed. However, their benefit in daily oral hygiene has not yet been quantified or even demonstrated by clinical studies. Certain studies have suggested considering peroxidases as ecological selectors directing the oral microflora towards a Gram-positive cocci microflora poor in cariogenic or periodontopathogenic germs, thereby preserving the mucosa and protecting it from yeast overgrowth, among others. Thus, the administration of an efficient lactoperoxidase system in a toothpaste also containing lactoferrin and lysozyme may prevent oral dysbiosis (145). A few studies have demonstrated an inverse association between the activity of sialoperoxidase and the depth of the periodontal pockets (146). On the other hand, the administration of inactive peroxidase without substrate seems to disturb *in vitro* the balance between commensals and periodontopathogens to the latter's advantage (98,147). The peroxidase systems' clinical benefit in oral hygiene must still be demonstrated on a large scale through evidence-based practice.

6. Conclusion

Oral peroxidases are involved in the control of the oral microflora as ecological selectors favoring a commensal flora, which can develop or survive in the presence of OSCN$^-$ (148,149). The use of peroxidase systems may be beneficial for preventing topical administration to protect the oral surfaces *in vivo*. By contrast, they may be used *ex vivo* to disinfect removable prostheses. The efficacy of formulations with peroxidase activity is not yet sufficiently documented, and there are no large-scale clinical trials to date. The short half-life of the produced oxidants and bacterial resistance constitute limiting factors for their use. However, the SCN$^-$/I$^-$ combination has recently made it possible to obtain more stable products.

Availability of data and materials

Not applicable.

Author's contributions

PC conceived and designed the study, and drafted the manuscript. Data authentication is not applicable. The author read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The author declares that he has no competing interests.

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