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Original Research Paper

Dental Science

EFFECT OF OXIDATIVE STRESS AND ROLE OF ANTIOXIDANTS IN CONSERVATIVE DENTISTRY AND ENDODONTICS

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ABSTRACT Oxygen is an ubiquitous element and an important substance for life on earth especially for human life and it can both be beneficial and harmful to life. High and soaring concentration of oxygen are found to be toxic, and can damage tissues. When cells use oxygen to generate energy in the form of ATP in the mitochondria, free radicals are created. Excessive free radicals formation that is not counteracted by the removal, creates 'oxidation stress' which is associated with various oral and systemic destructive diseases. Dental treatment with the use of composites, amalgams, glass-ionomers, materials for root canal filling or irrigation, or whitening agents can disturb oral redox homeostasis by affecting the antioxidant barrier and increasing oxidative damage to salivary proteins, lipids, and DNA. It is suggested that the addition of antioxidants to dental materials or antioxidant therapy applied during dental treatment could protect the patient against harmful effects of oxidative stress in the oral cavity.

KEYWORDS : Antioxidants ; oxidative stress ; reactive oxygen species

INTRODUCTION

For the majority of organisms, oxygen is indispensable for life. However, when there occurs in the environment an excess of reactive oxygen species (ROS) in the form of oxidative stress, cells are destroyed, which leads to the development of many pathological processes.

The poisonous effects of oxygen were unknown until Gershman's free radical theory of oxygen toxicity in 1954, which states that the toxicity of oxygen is due to partially reduced forms of oxygen.[1] When cells use oxygen to generate energy in the form of ATP in the mitochondria, free radicals are created. [2, 3] Free radicals are chemically active atoms that have a charge due to an excess or deficient number of electrons. They can be reactive oxygen species (ROS) or reactive nitrogen species (RNS).

Organisms have developed protective mechanisms against the toxic effect of oxygen, represented by discoveries established by the 2019 Nobel Prize winners Kaelin Jr., Sir Peter J. Ratcliffe and Gregg L. Semenza. Studies by these Nobel laureates allowed an understanding of the way in which the levels of oxygen exert an effect on cell metabolism and physiological functions. These findings also paved the way for new strategies in control of diseases of the cardiovascular, nervous, skeletal systems, kidney damage, as well as in the pathogenesis of oral diseases [1][2][3]

Oxidative Stress

Oxidative stress (OS) is defined as an imbalance between the production of oxygen (ROS) and nitrogen (RNS) free radicals and their neutralization by compounds called antioxidants [4]. Interestingly, ROS also act as signaling molecules involved in cell growth, proliferation and survival [5]

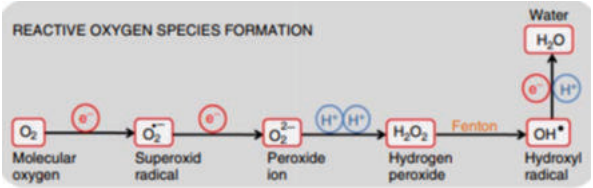

Figure: 1- Generation Of Different Oxygen Species [41]

There are two important sources of free radical formation.

1) internal factors i.e. normal cellular metabolisms like mitochondrial ETC, endoplasmic reticulum oxidation and many enzymatic activities.
2) external factors- radiation, oxidation of engine exhaust, carbon tetrachloride, cigarette smoke and oxygen itself [32],[33],[34]

Table: 1- Various Free Radicals [31]

TRUE RADICALS	RADICAL SYMBOL	ROS	ROS SYMBOL
superoxide	$O_2^{\bullet-}$	Hydrogen peroxide	H_2O_2
hydroxyl	$^{\bullet}OH$	Hypochlorous acid	HOCl
perhydroxyl	$HO_2^{\bullet}$		
hydroperoxyl	$RO^{\bullet}$	Singlet oxygen	O_2
Alkoxyl	$ArO^{\bullet}$	ozone	O_3
Aryloxyl	$ArOO^{\bullet}$		
Peroxyl	$(ROO^{\bullet})$		
Acyloxyl	$(RCOO^{\bullet})$		
acylperoxyl	$(RCOOO^{\bullet})$		

The free radicals have a special affinity for lipids, proteins and nucleic acid (DNA) [35] Reactive oxygen species can cause tissue damage by a variety of different mechanism which include

- DNA damage- mutations
- Lipid peroxidation (through activation of cyclogenase and lipo oxygenase pathway)
- Protein damage including gingival hyaluronic acid and proteoglycans (Bartold et al 1984)
- Oxidation of important enzymes
- Eg Antiprotease such as ; 1 antitrypsin (Varani et al 1990)
- Stimulation of pro inflammatory cytokine release by monocytes and macrophages (Staal et al 1990)

Various Dental Materials And Procedures And Their Oxidation Characteristics

1. Tooth Whitening / Bleaching

Tooth whitening is one of the most frequently chosen procedures to restore the esthetics of discolored teeth.[6,7] Hydrogen peroxide (H2O2) is the most commonly used substance for this purpose and is characterized by high

reactivity, although it is not a free radical. It participates in oxidation reactions, the most biologically significant of which is the oxidation of sulfhydryl groups.[8]. It leads to the formation of disulfide bridges, which changes the conformations as well as biological functions of proteins. H2O2 also has the ability to oxidize unsaturated fatty acids during the process of lipid peroxidation.[9]

The redox reaction under the influence of H2O2 results in the formation of: superoxide anion, hydroxyl radical and singlet oxygen, all of which have an oxidizing effect on the organic components of the enamel, leading to the whitening of tissues [11,12]. ROS generated during the whitening process induce a sequence of reactions that increase the expression of heme oxygenase 1 (HO-1) in fibroblasts and osteoblasts [10]. Considering the antioxidant and anti-inflammatory properties of this enzyme, it can be concluded that increased expression of HO-1 in cells exposed to H2O2 is a defensive mechanism against the adverse effects of whitening

2) Glass-ionomer Cement

Little is known about glass- ionomer-induced oxidative stress. It was demonstrated that metal ions released from glass-ionomer fillings are not toxic to cells and do not induce ROS formation, whereas polyacrylic acid [15] and fluorine ions released during the first phase of GI filling [15,16] have cytotoxic effects. During the first seven days, ROS production enhances, increasing TAC levels

The observed increase in TAC is an adaptive reaction of the cells that effectively prevents OS development, shifting the redox balance towards antioxidant reactions [15]. After 21 days, the level of TAC in the cell culture exposed to glass-ionomer filling did not differ from the control culture level, which most likely indicated that glass-ionomer filling was no longer cytotoxic to cells, and it was a sign of achieving redox balance

3) Dental Resin Composite

Dental resin composite materials are currently the most commonly applied materials for tooth reconstruction in conservative dentistry. The most common component released into the saliva of them are bisphenol-A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and 2-hydroxyethyl methacrylate (HEMA). It is believed that HEMA is released in the largest amount from composite materials, and Bis-GMA—in the smallest, which is related to the size of the molecules as well as molecular weight of these monomers [17].

It is known that monomers released from composites contribute to genetic changes at the cellular level, and show cytotoxic effects [18]. It is generally believed that the cytotoxicity of these monomers might be ranked in the decreasing order: Bis-GMA, UDMA, TEGDMA and HEMA [17]. Moreover, studies showed that OS is responsible for monomer cytotoxicity [20,19], whereas silorane-based dental resin composites Hermes III, free of TEGDMA, HEMA and other monomers, does not lead to significant ROS/RNS production, and its cytotoxicity towards pulp cells is low compared to TEGDMA and HEMA-based dental resin composites [19].

4) AMALGAM

The harmful effects of amalgam fillings are related to mercury contained in them. Studies demonstrated that mercury interferes with the metabolism of porphyrins which actively participate in numerous metabolic processes, including cellular respiration. The disruption of porphyrin metabolism may result in metabolic diseases, cancer or blood disorders, i.e., anemia or porphyrias [21,22]. Organic mercury, found in the form of methylmercury (MeHg), is considered particularly harmful [22].

In proteins and enzymes containing thiol or selenol groups, the formation of S-Hg or Se-Hg bonds leads to the impairment of a given protein function [25,26] or entails the production of protein deposits rich in cysteine residues [23,24].

Considering the fact that both thiol and selenol groups are critical for the catalytic activity of numerous enzymes involved in antioxidant mechanisms and that MeHg reduces the activity of, inter alia, such enzymes (i.e., glucose-6- phosphate dehydrogenase [69], creatine kinase [60], glutathione reductase [70], glutathione peroxidase [59,71], thioredoxin reductase [10,72] there are grounds to postulate that this interaction disturbs the redox balance and results in increased production of ROS and RNS.

5) COMPOSITE RESIN

Low-viscosity resins are commonly used in restorative dentistry as binding agents connecting dental materials with dental tissue. The inclusion of iodine salts in dental bonding systems was found to be very interesting because they can act as catalysts, reducing activation energy. Due to their ionic nature, they participate in the polymerization of hydrophilic monomers and thus increase the polymerization reaction capacity and percentage of monomer conversion rate [27]

Initiators are an essential component of light-hardened composites and dental adhesives. Currently, visible-light photoinitiators, such as phenylbis(2,4,6-trimethylbenzoyl) phosphine oxide (BAPO) and diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (TPO), are used. Both of these photoinitiators belong to the group of Norrish type I photoinitiators and unlike camphorquinone (CQ) they do not generate ROS. Popal et al. [28] found that both BAPO and TPO used in micromole concentrations do not increase ROS/RNS production in human keratinocytes and V79 fibroblasts during 90 min of exposure. Interestingly, the concentration of ROS/RNS in cells treated with the said photoinitiators was lower compared to the control cells. It is probable that aromatic and phosphine oxide groups serve as electron donors that compete with the fluorescent dye DCFH2 used in the ROS detection method.

6) ENDODONTIC THERAPY

The complicated morphology of the root canal system of teeth makes instrumentation challenging. One of the most important elements of root canal treatment is the use of irrigating solutions which are designed to neutralize microorganisms as well as dissolve and remove tissue residues, including the smear layer

The most commonly used rinsing solutions include the main ones: sodium hypochlorite (NaOCl) and chlorhexidine (CHX) as well as auxiliary solutions: ethylenediaminetetraacetic acid (EDTA) and citric acid. Botton et al. [36] observed that 2% CHX and 6% citric acid did not cause lipid peroxidation, regardless of the exposure time of human peripheral blood mononuclear cells (PBMCs). The 72-h exposure of PBMCs to both 1% and 2.5% NaOCl resulted in increased lipid peroxidation, which suggests that prolonged contact of the cells with the rinsing solution may result in OS and, consequently, disturbed integrity of cell membranes due to the destruction of the lipid bilayer [37]. The solution of 17% EDTA boosted the process of lipid peroxidation only after 24 h of exposure of the cells, which was not maintained after 72 h (Botton et al. [36], co Saghiri et al. [37] is the adaptation of cells to environmental conditions. The genetic material appears to be more susceptible to the damaging effects of the rinsing agents, as increased oxidative DNA modifications were observed after both 24 and 72 h of exposure of PBMCs to all the flushing solutions.

Antioxidants

ROS scavengers/ antioxidants are of prime importance for preventing and controlling human diseases by counteracting

ROS formation[38].

Mechanism of Action [29]

Antioxidants end the electron stealing reaction of free radicals by donating one of their electrons. The antioxidant does not become a free radical by donating an electron because they are stable in either form. Antioxidants act by scavenging or chain breaking, such as Vitamin E (alpha tocopherol), Vitamin C (ascorbic acid), or Vitamin A (beta carotene) and preventative antioxidants that function basically by sequestering transition metal ions and preventing fenton reactions and are therefore predominantly proteins by nature (e.g., albumin, transferring, or lactoferrin).

Classification of anti-oxidants [29]

1. Enzymatic: Superoxide dismutase, Glutathione Peroxidase, Selenium, Catalase, Glutathione reductase, Glutathione transferase
2. Non-enzymatic: They are further subdivided into two.

i. Nutrient: Alpha tocopherol, □- Carotene Ascorbate, Glutathione, Selenium, Proanthocyanidin, Lycopene, Green tea

ii. Non-Nutrient: Ceruloplasmin, Transferrin, uric acid, Peptides Camosine Anserine

Vitamin E, flavonoids, catechins, gallic acid derivatives, salicylic acid derivatives, cinnamic acid derivatives chlorogenic acid, resveratrol, folate, curcumin, caffeine, anthocyanins and tannins are examples of polyphenolic natural antioxidants derived from plant sources.

Non-phenolic secondary metabolites such as melatonin, carotenoids, retinal, thiols, jasmonic acid, ei-cosapentaenoic acid, ascopyrones and allicin that show excellent antioxidant activity [30].

Antioxidants Relevant To Dentistry [31,39]

Name of the antioxidant	About the antioxidant
ASCORBIC ACID(VIT.C)	Decrese nitrosation Effect leukocytes and macrophages
β CAROTENE	increased activity of TNF- αincreases cell mediated immune response
αTOCOPHEROL	reduce oxidative damage caused by hydroxyl radicals.
PROANTHROCYANIDIN	1.reduce cell proliferation in human oral cancer cells induce apoptosis 2. inhibit glucan synthesis, 3.decrease adhesion of S. mutans to tooth
TANNINS	Effective against Streptococcus mutans , Streptoco ccus salivarius , Strepto coccus mitis, and Streptococcus sanguis
EGCG epigallocatechin3-gallate (catechins)	1.Bactericidal to S. mutans 2. decrease acid production in dental plaque 3.inhibit the production of important metalloproteases 4. increase acid resistence of teeth
FLAVANOIDS	Regulate nitric oxide production of LPS-stimulated human gingival fibroblasts.
LYCOPENE	Inhibit cancer cell proliferation Effective against candida albicans
Propolis (propolis resin, bee glue	Pharmacologically active components in propolis are phenolics, aromatics, and flavonoids. The compounds in propolis are resin and balsam (50-70%), essential oil, sand wax (30-50%), and pollen (5-10%). The other components found in propolis are amino acids, minerals, vitamin A, B complex, and E. A highly active biochemical substance known as bioflavonoid (vitamin P) is also found.
Azadirachta indica (Indian neem, Indian lilac or margosa tree)	Active ingredients isolated are nimbolide, nimbin, nimbidinin, nimbinin, and nimbidic acid.[12] Neem extract alters bacterial adhesion and prevents microbial colonization.[5]
Acacia nilotica (babool, gum Arabic tree)	antibacterial against Streptococcus mutans and E. faecalis. Active ingredients which have antimicrobial action are phenolics compounds, tannins, essential oil, and flavonoids.[3]
Acacia catechu wild	Active ingredients are catechin, eoicatechin, epigallocatechin, gallate, phloroglucin, protocatechuic acid, quercetin, procyanide, kaempferol, D-galactose, and epicatechin
Arctium lappa Linn (greater burdock)	Roots, seeds, and immature flower stalks may be used for medicinal purposes. The active ingredients found are mucilage, polyacetylenes, guaianolide, arctin, and lignans.
Triphala	The citric acid present in the fruit helps in removal of smear layer and thus can be used as a chelating agent
Rhus lancea (Searsia lancea, L.f.)	The active ingredients found are gallic tannins and gallic acid. It has been found that gallic acid reduces periapical inflammation. The water extract of the plant is seen to help in opening up of blocked dentinal tubules.

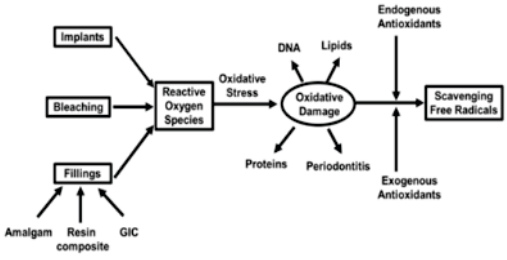

Figure 2: Schematic Diagram Of Ros Production And Clearance During Dental Procedure [42]

CONCLUSION

Oxidative stress is associated with the pathogenesis of numerous systemic diseases, including those related to the oral cavity. Oxidative stress of any form leads to damage of cell components, mainly proteins, lipids and nucleic acids, which leads not only to structural changes of the cell, but also to its death through apoptosis and necrosis

The interdependence of systemic and oral health is well known, thus its probable that reducing oxidative stress in oral environment with antioxidants has the potential of not only prolonging the life of the treatment provided but also improving well-being of an individual(31)

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