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Review Article

Role of Oxidative Metabolism and Energy Production at Molecular Level in Oral Soft Tissues

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Abstract

There is a big misconception in general public that the hard tissues (Teeth) and soft tissues (gingival and periodontal tissues) are lifeless and dead. They lack vitality in contrast to other body tissues but when a patient develop toothache/pain or oral ulceration, does this belief needs the fact. So whether it is a belief or a fact that "Are oral tissues vital? If vital, how this vitality is maintained"???

Key-words: Teeth, Gingival tissue, periodontal tissue, Vitality.

Key Messages (Provide appropriate messages of about 35-50 words to be printed in centre box):

- Smoking, infection, UV light, high temperature, etc. play an important role in generation of free radicals, so one should avoid exposure to these agents.
- Consumption of nutrients with antioxidant ability like Vitamin-C, β-carotene, selenium, and manganese should be encouraged, as they help in fighting oxidative insults to the periodontal tissue.

CLINICAL RELEVANCE TO INTERDISCIPLINARY DENTISTRY:

- Dental professionals are looking for ways to fight inflammation by increasing the level of antioxidants in the oral cavity.
- PDT is a novel therapeutic approach and its applications in various fields of dentistry are bacterial/ fungal infection therapies, periodontal diseases and endodontic therapy.
- The LLLT opens the door for dentists to perform various dental procedures such as cavity preparations, composite curing and surgical procedures.

Introduction: The basic concept of life is cell. Cell itself speaks-"I am the unit of biological activity, contain into sub-cellular organelle, assigned to each are specific duties. Thus, I truly represent "LIFE"!!![1] Cells are the basic unit (Structural and functional unit) of life as well as building blocks of an organism. Life passes from one generation to the next generation or from one cell to its daughter cells in the form of

genes. Genes are the physical basis of cell and DNA is the chemical basis of cell.

THE IMPORTANCE/NEED OF OXYGEN:

Most people do not know how important oxygen is. Sure, we need it to breathe but there is more to it. All living things breathe, they require oxygen. Oxygen signifies life. Like all other living organism in this world, gingival, which is the part of periodontium also, breathes .On the molecular level, it utilizes oxygen for carrying out oxidative metabolism for energy production. Oxygen helps in the making of ATP which is the final acceptor of electron in the electron transport chain. Without it, the electron chain would come to a halt, therefore, ending cellular respiration.

Oxidative metabolism is carried out in the cell organelle named "mitochondria." Mitochondria are an "Aerobic Engine" or "Power House of cell" because they are the major centre of release of energy in aerobic respiration. Mitochondria play a central role in cell life and cell death. Without mitochondria, living organism have no need of oxygen in human system that delivers oxygen to the tissues.

In general, metabolism is broadly classified into two categories—

1. Anabolic reaction.
2. Catabolic reaction.

1. **ANABOLIC REACTION:** is a biosynthetic process that convert simple compound into complex ones with consumption of energy.
2. **CATABOLIC REACTION:** is a degradative process that converts complex compound into simpler ones coupled with release of energy.

These reactions take place at the tissue level that requires oxygen. Our body utilizes oxygen for carrying out metabolic purposes with the production of CO_2. Blood is a component that delivers oxygen at tissue level for various purposes[2].

BIOLOGICAL OXIDATION AND ENERGY PRODUCTION

Molecular oxygen (O_2) is essential for cellular respiration in all aerobic organisms. Oxygen is used as an electron acceptor in mitochondria to generate chemical energy. The energy is released by oxidation not transformed into heat but transformation consists of high energy rich compound ATP which is eventually produced on the inner surface of mitochondrial membrane and released in cellular respiration in discreet amount due to a series of enzymatic reactions. Cellular respiration is proceed through three stages- glycolysis, Krebs cycle, Oxidative phosphorylation. 95 % of the total ATP is produced during the terminal stages of catabolism including the major oxidative reactions. Around 60 %of energy produced during catabolism is heat and the remaining 40% is stored in the chemical bonds of ATP molecule. The oxidative process of cells used to degrade fuel molecule that yields NADH and $FADH_2$ which completely oxidizes the carbon atoms in acetyl-COA and conserves free energy[3].

Mitochondrial cell organelle is more numerous in deeper strata and decreased towards the surface of cell in the gingival epithelium. Mitochondrial activity are more

active in TCA cycle/Krebs cycle in basal and Para-basal cells where the proximity of blood supply i.e. underlying connective tissue facilitates energy production through aerobic glycolysis hence implies more oxygen consumption as compared to the upper layer of gingival epithelium. The number of mitochondrial cells varies depending upon the energy requirement i.e. tissue with high capacity to perform aerobic metabolic functions will have a larger number of mitochondria[2].

The inner and outer mitochondrial membranes provide compartmentalization through separation of matrix which is a central feature of the conversion of free energy derived from oxidisable substrates. The inner membrane carries the main enzymatic machinery of oxidative phosphorylation and complexes named complex I, II, III, IV and V. Complex V (f0-f1 ATP synthetase) is responsible for ATP production. Complex V acts as an ion channel that provides a proton flux back in to the mitochondrial matrix. This reflux releases free energy produced during the generation of the oxidizable forms of the electron carrier (NAD+ and Q coenzyme) .the free energy is used to drive ATP synthesis from ADP + Pi.[4] The mitochondrial matrix contains a range of enzymes and coenzymes which forms a part of major metabolic pathways such as TCA cycle, oxidative phosphorylation, mitochondrial ETC. TCA cycle is the central pathway and oxidizes the acetyl component of acetyl – COA to 2 molecule of CO_2 to conserve the liberated free energy in the form of NADH and $FADH_2$. It is amphibolic in nature i.e.it serves both catabolic as well as anabolic purposes.[5] The rate of respiration of mitochondria can be controlled by the availability of ADP. This is because oxidation and phosphorylation are tightly coupled i.e. oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP.[6]

ROLE OF FREE OXYGEN RADICALS AND ANTIOXIDANTS

Free oxygen radicals are the generation of unpaired electrons or free electrons produced during mitochondrial respiration. The interaction of these electrons with oxygen results in the generation of superoxide ions – highly reactive free radicals species – or ROS. Mitochondria represent the major source of free radicals species in all cells. Free oxygen radicals originate from exogenous and endogenous sources.

Exogenous sources include heat, trauma, UV light, smoking, exhaust fumes, radiation, infection, excessive exercise and therapeutic drugs.

Endogenous sources include:

- By products of metabolic pathways. Electron leakage from mitochondrial transport system forming superoxide.
- Functional generation by host defense cells (phagocytes) and cells of connective tissue (osteoclasts and fibroblasts).

These sources are directly or indirectly linked with generation of free radicals. The level of these radicals increases dramatically during environmental stress (UV or heat exposure) resulting

in significant damage to cell structure known as oxidative stress.[7]

Reactive oxygen species and periodontal disease

Periodontal disease is a result of inflammatory response resulting from the interaction between pathogenic bacteria and the host immune response. It affects the teeth's supportive structure (Gingiva, cementum, periodontal ligament, alveolar bone) in healthy tissues, the equilibrium or normal balance is maintained by ROS generation and antioxidant defense. When there is an excess of antioxidant defense and an overproduction of free radicals or drop in the level of antioxidants it will lead to an imbalance and cause deleterious effects.[8] Periodontitis is usually caused by disequilibrium between periodontal tissue destruction and repair involving the host response to bacterial challenge. PMN's are the primary host defense against periodontal pathogens. PMN's produce reactive oxygen species during phagocytosis as a part of the host response to infection. Reactive oxygen species play an important role directly or indirectly in tissue damage. PMN'S able to engulf bacteria by a process of phagocytosis. Engulfed bacteria are destroyed by oxidative production of toxic reduced oxygen metabolites such as superoxide anion. Non-oxidative lysosomal enzymes such as defensins and proteases found in lysosomal granules also kill bacteria. In the increasing anaerobosis of the pocket, oxidative killing is impaired.[9]

During direct damage, lipopolysaccarides (LPS) Endotoxins are principally released during cell breakdown and contribute to surrounding tissue destruction by a multitude of mechanisms. These include:

- Indirect damage by the stimulation of collagenase (MMP-1) release from macrophages.
- Activation of complement via the alternative pathway. It can occur directly due to Ag – Abs complexes or indirectly by Endotoxins, polysaccharides and other bacterial products.
- Stimulation of bone resorption by interleukin -1

Endotoxins directly destroy gingival cells. They enhance tissue destruction due to stimulation of the immune response associated with gingival inflammation. During indirect damage, there is a binding of bacteria by the surface receptors [Toll like receptors (TLRs) AND Fcg- receptors (Fcg –Rs]) of neutrophil which trigger phagocytosis and superoxide radical formation. This may be subsequently converted to hydrogen peroxide and the highly reactive hydroxyl radical. The generation of free radicals activates bactericidal enzymes within the phagosomes, causing indirect damage to the bacteria.[10,11]

During periodontitis, there is an increase in number and activity of PMN's. This proliferation result in high degree of reactive oxygen species release culminating in heightened oxidative damage to gingival tissue, periodontal ligament and alveolar bone. These reactive oxygen species contribute to tissue

destruction by damaging DNA, causing lipid peroxidation and stimulating proinflammatory cytokines release. Majority of tissue destruction in periodontitis is considered to be result of an aberrant inflammatory/immune response to microbial plaque and involve prolonged release of reactive oxygen species and neutrophil enzymes. Reactive oxygen species generation in periodontal disease causes bone resorption; degrade connective tissue, increases matrix, metalloproteinase activity causing an imbalance.

Oxidation reaction can produce free radicals which start chain reaction that damage cells and extra cellular matrix directly. 8-Hydroxydeoxy-guanosine (8-OHDG) level which is a marker for oxidative damage in chronic inflammatory disease generated from guanosine by the action of reagents that generate oxygen radicals. Reactive oxygen species has a variety of effect on type-1 collagen including direct fragmentation and polymerization. The molecule is more prone to proteolysis. In connective tissue, collagen is particularly susceptible to glycation which modifies its structural properties and alter its interactions with surrounding extra cellular matrix molecule. Once damage has occurred, oxidative stress within the cell can be amplified because of decreased expression of proteins critical for ETC leading to cell death. Oxidative stress lies at the heart of periodontal tissue damage that result from host microbial interactions either

- As a direct result of excessive ROS activity
- Antioxidant deficiency results in per oxidative damage at the tissue level and can provoke a “deterioration” of the periodontium. Deficiency of dietary vitamins such as vita. A, C, E, B-complex, green tea catechins are vital for the maintenance of healthy gingival tissues. Such nutritional deficiencies render patients less able to defend against the cycle of bacterial growth, acid production and plaque formation. Topical antioxidants such as folic acid in a mouth rinse binds to plaque derived Endotoxin and reduce antigenic stimulation with a reduction in the level of gingival inflammation. Presence of vitamin A in toothpaste useful in treating periodontitis with fewer deeper pockets and reduces gingival bleeding.[7,9,12]
- Activation of transcription factor such as AP-1 (activating protein- 1 or “heat shock proteins” or “stress protein”) and NFkB (nuclear factor kappa B) bind at the promoter regions of a large variety of genes and their role in the expression of various proteins such as TNF, IL-1, IL-2, Collagenase, MMP etc and the creation of proinflammatory state such as release of cytokines by monocytes and macrophages.[7,10]

ANTIOXIDANT DEFENSES (POWERFUL NEW WEAPONS IN THE FIGHT AGAINST PERIODONTAL DISEASE)

Antioxidant means "against oxidation." Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant, it no longer needs to attack the cell and chain reaction of oxidation is broken. Extracellular antioxidants likely to be of importance in periodontal disease are the chain breaking or radical scavenging antioxidants such as ascorbate, α-tocopherol, Carotenoids, metal binding proteins and compounds with oxidisable – SH (Thiol) groups.

Decrease antioxidant capacity may indicate either inherently low basal antioxidant defense or status or may result from an increase in oxidative stress.[13] Various forms of antioxidants have been introduced as an approach to fight dental diseases and improve general gingival health. The body's antioxidant defense system plays a crucial role in fighting inflammatory chronic disease such as periodontal disease. Antioxidants are manufactured within the body and can also be extracted from the food such as fruits, vegetables, seeds, nuts, meats and oil. There are two lines of antioxidant defense within the cell.

1. The first line found in fat/lipid soluble cellular membrane consists of vitamin-E, β- carotene and coenzymeQ10. Vitamin-E is the most potent chain breaking antioxidant within the membrane of the cell. It protects the cell membrane from lipid peroxidation. Scavenging peroxyl radicals are much faster than the radicals that can react with adjacent fatty acid side chains or with membrane proteins.
2. The second line found inside the cell i.e. water soluble antioxidant scavengers are present. These are vitamin-C, superoxide dismutase, and catalase and glutathione peroxidase. Vitamin-C is very effectively scavenges a wide array of ROS and free radicals. Thiol containing antioxidants in particular reduced glutathione are present in high concentration in GCF and high level in oral epithelial cells.

Antioxidant deficiency both local (saliva and GCF) and peripheral (plasma and serum) have been directly linked to periodontal disease. Supplementation with high grade antioxidant has been shown to improve periodontal pocket depth up to three times as compared to scaling and root planing procedure alone. Nutrients that can act as antioxidant may modulate gingival inflammation. Deficiency of dietary vitamins such as vitamin A, C, E, B-complex, green tea catechins may contribute to tissue damage in many chronic human diseases [atherosclerosis, ageing, neurodegenerative disorders (Alzheimer's disease, Parkinson's disease), cancer including periodontitis and diabetes. Vitamin C deficiency (scurvy) generally present with periodontal disease, gingival bleeding, tooth mobility and loss of gingival attachment.[8,14,15] Nutritional supplement (perio therapy) could be beneficial

in improving gingival and periodontal health and nourishes the tissues of the oral cavity. Coenzyme Q10 is added to enhance the production of ATP in the gingiva itself to promote health, reduced periodontal disease, decreased pocket depth and improved healing after gingivectomy.[14] Antioxidant Vitamins supplements that work systemically to support the immune system may help to fight the disease and aid in the healing process. When antioxidants are depleted, the ability of gum tissue to overcome oxidative stress, maintain normal tissue and control the bacterial damage appears to be compromised.

CLINICAL APPLICATIONS

PHOTODYNAMIC THERAPY ("THE ENERGY OF GOD")

It is the novel non-invasive photochemical approach for infection control and now a day's receiving much attention in the treatment of oral diseases. It is the method which combines the application of non-toxic chemical agent (photo sensitizer) with low level light energy. During inflammation, there is venous stagnation and reduced oxygen consumption by tissues. This decrease in oxygen level and change in PH may enhance the growth of anaerobic species. Photodynamic therapy may improve tissue blood flow in the microcirculatory system and reduce venous congestion in gingival tissue. Photodynamic therapy may increase oxygenation of gingival tissue by 21-47%. This in turn decreases the time and speed of oxygen delivery and utilization. Thus normalizing oxygen metabolism in periodontal tissue.[16,17]

PDT involves three components –light, a photo sensitizer (photofrin) and oxygen. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid and selective destruction of the target tissue. The triplet state photo sensitizer reacts with biomolecules by two mechanisms.

1. The type I reaction involves electron /hydrogen transfer directly from the photo sensitizer, producing ions or electron/ hydrogen removal from a substance molecules to form free radicals. These radicals react rapidly with oxygen resulting in the production of highly reactive oxygen species.
2. The type II reaction produces electronically excited and singlet oxygen.

   These two reactions indicate the mechanisms of tissue/cell damage which is dependent on both oxygen tension and photo sensitizer concentration. PDT produces cytotoxic effects through photo damage to sub cellular organelles and molecules. Mitochondria, lysosomes, cell membranes, and nuclei of tumor cells are considered potential targets, along with tumor vasculature. During light exposure, sensitizers that localize in mitochondria may induce apoptosis, while sensitizers localized in lysosomes and cell membranes may cause necrosis.

Low level laser therapy (LLLT)

LLLT are semiconductor diode lasers at low power intensities and with a wavelength in

the range of 540nm – 830nm. The effects are thought to be mediated by photochemical reactions that alter cell membrane permeability, leading to increased mRNA synthesis and cell proliferation.

These lasers emit biological effects on immune cells, bone cells, fibroblasts and epithelial cells with an extended input on blood vascular and nervous system. They offer promising result on destruction of cariogenic bacteria, microorganisms in root canal and periodontal pockets. Initially He- Ne gas lasers are used but now a day's most LLLT clinical procedures are undertaken using semiconductor diode laser (for e.g. Aluminium: Gallium: Arsenide- based diode laser). When a light is applied to the tissue, the absorption of light occurs by the respiratory chain components causes' short term activation of respiratory chain and oxidation of NADH pool. This stimulation of oxidative phosphorylation leads to changes in the redox status of both the mitochondria and the cytoplasm of the cell. The electron transport chain is able to provide increased levels of promotive force to the cell through increased supply of ATP, synthesize DNA, RNA, protein enzymes and other biological materials needed to repair or regenerate cells and tissue components. This leads to cell proliferation results in tissue repair or pain control and restore homeostasis.[18,19]

When low level laser therapy is applied to soft tissues, there is stimulation of specific metabolic processes in wound healing. LLLT may increase the motility of epithelial cells and are able to migrate across wound sites with accelerated closure of defects. Endothelium forms granulation tissue more quickly. LLLT may also enhance the phagocytic activity of macrophages during initial phases of repair response (6hrs after trauma). Macrophages resorb fibrin as a part of the demolition phase of wound healing more quickly with LLLT.

Major changes are seen in wound treated with LLLT include:

- Increased granulation tissue
- Early epithelization
- Increased fibroblasts proliferation and matrix synthesis
- Enhanced neo-vascularization

LLLT in dentistry include the promotion of wound healing in a range of sites including:

1. Surgical wounds to oral tissue
2. Gingival incisions
3. Extraction sites (bone fill and soft tissue healing)
4. Lesion of recurrent aphthous stomatitis (canker sores)
5. The dental pulp , with secondary dentin formation after pulpotomy
6. Oral ulceration (mucositis)induced by cancer chemotherapy
7. TMJ injury
8. Neuronal tissue which has been injured or transected to accelerate regeneration

LLLT showed the greatest wound area reduction between one and third day after treatment with higher number of myofibroblasts. LLLT has been found to accelerate wound healing and reduce pain by

stimulating oxidative phosphorylation in mitochondria and modulating inflammatory responses. LLLT have marked effects upon cells in all phases of wound healing but particularly during the proliferative phase.[20]

CONCLUSION

Oxygen plays a vital role in the breathing processes as well as in the energy metabolism of living organisms. The metabolic pathways such as TCA cycle, mitochondrial ETC, oxidative phosphorylation occur in gingival tissue similar to that of body's metabolism. The mitochondria lie at the heart of cell life and cell death. Mitochondria are essential in maintaining the battle against entropy necessary to sustain life. They provide the energy required for almost all cellular process. Without adequate oxygen the chemical reactions involved in aerobic respiration are impaired. “THE BIGGER THE AEROBIC ENGINE, THE FASTER ONE CAN GO!”

We are constantly exposing to ROS whether it is exogenous or endogenous in origin. Oxidation of cells by free radicals makes the human body “rust” like, oxidation of metal makes it rust and you know what rust does to the strength and natural beauty of the metal. Our bodies need the help of antioxidant to neutralize the oxidation properties of those invading free radicals. It can block the production of ROS or obstruct its effect. Without radicals, there would be no life. Antioxidants either enzymatic or non-enzymatic limit oxidative damage to biological molecules by various mechanisms. Cellular rejuvenations, life extensions and improved vitality have been achieved using antioxidant that can actually keep cells looking and acting younger.

Oxygen is one of the most important elements required to sustain life. Without it our health begins to suffer or we die. Unhealthy or week cells due to improper metabolism loose their immunity and are thus susceptible to viruses and lead the way to all kinds of serious health problems. Oxygen not only gives us life but also destroys the harmful bacteria present in our bodies without affecting the beneficial bacteria that we need.

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