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Oxidative Stress and Osteoimmunology: The two Missing Pieces of the Oral Osseointegration Puzzle

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ABSTRACT

Oxidative stress arises when the level of oxidants exceeds that of antioxidants. It occurs either when oxidant production is excessive or when antioxidant release is insufficient. This physio-pathological state can cause two types of damage: first molecular, which involves alteration of DNA; and second, cellular, which involves genetic mutations and apoptosis leading to a wound healing issue. In the second case, all mechanisms of healing are slowed. Furthermore, the lack of antioxidants, which are mandatory for a proper running of the immune system, will lead to immune deficiency. Osteoimmunology, a new science recently proposed, is based on evidence of a large communication and connection pathway between the bone and immune systems. The immune system has a significant impact on bone health and diseases. Osteoimmunology is therefore an essential interdisciplinary field that will support the growth of understanding concerning bone regeneration.

In the oral surgery field, hard and soft tissue deficiencies should be regarded as immune diseases. The bone regeneration and its overtime stability is influenced by the oxidative stress level that the body experiences. It's now obvious that immune system and bone remodeling are affected by the level of oxidants.

INTRODUCTION

The term "oxidative stress" first appeared in the medical literature in 1985. Aerobic species, in contact with oxygen, physiologically produce numerous oxidants. To neutralise these oxidants, cells produce antioxidants. When the level of oxidants exceeds that of the antioxidants, the tissue is considered to be under oxidative stress. This situation occurs either when production of oxidants is excessive or when release of antioxidants is insufficient to counteract oxidation. This physio-pathological state can cause molecular or cellular damage, the first in the form of DNA alteration and the second in the form of genetic mutation and apoptosis. Furthermore, oxidative stress enhances the rates of occurrence of certain illnesses and accelerates cells ageing. Since 1594, the term "osteology" has been used to describe the different mechanisms of bone physiology, including those involved in bone synthesis. In 2000, the designation "osteoimmunology" was introduced by Arron and Choi. Evidence is increasing that the immune system and immune cells are involved in bone synthesis and control all phases of remodeling cascades. Oxidative stress has an immediate and constant effect on these bodily mechanisms and deteriorates the correct immune system functioning. A consequence of oxidative stress is the early or late failure of osseointegration. This is important in oral surgery, in which implants that might or might

not be combined with bone grafts are used to replace missing teeth. Bone loss could be prevented by managing immunity and oxidative stress before, during and after surgery.

OXIDATIVE STRESS

Oxidation-reduction (redox) reactions occur constantly in living creatures. Most take place in mitochondrial cells, which continuously produce endogenous free radicals, also called Reactive Oxygen Species (ROS) or oxidants. ROS are a highly reactive group of molecules that comprises several diverse chemical species. The major forms include the superoxide anion of oxygen (O_2-), hydrogen peroxide (H_2O_2) and free radicals such as hydroxyl radicals (•OH).

They are generated as by-products of aerobic metabolism, usually by leakage from the electron transport chain during oxidative phosphorylation in mitochondria [1,2]. At low concentrations, ROS serve as signalling molecules to activate specific physiological pathways that control several life processes [3,4]. However, they also interact with other molecules to generate "secondary" ROS, which are unstable and thus react more aggressively than primary ROS.

Free radicals are highly reactive species due to the presence in their outer shells of single unpaired electrons. They react with other molecules, mainly lipids, lipoproteins, proteins and nucleic acids, to regain electrons and therefore to regain atomic stability. These reactions lead to an alteration of both DNA and cell membranes and therefore favor mutagenesis and carcinogenesis. Free radicals are produced in quantity during immune reactions, repair of damaged tissues, and synthesis of adenosine triphosphate. Smoking, exposure to certain pollutants or pesticides and use of certain drugs can also induce significant formation of oxidants.

Elevated levels of ROS can damage proteins, lipids and DNA, trigger oxidative stress and kill cells [5,6]. Oxidative damage to bio-macromolecules has been shown to play an active role in the aetiology of a wide variety of acute and chronic diseases [7]. It plays a central role in the acceleration of the ageing process [8-10] and in osteoporosis [7]. An aberrant production of free radicals is also implicated in numerous and diverse pathologies, such as cancer, arthritis, cardiovascular conditions and neurodegenerative diseases, and may induce uncontrollable autoimmune illnesses [11-15]. Alzheimer's disease, other forms of dementia and Parkinson's disease may develop due to reaction of free radicals with brain neurons [16,17].

Diabetes and the effects of smoking are redox diseases and high levels of oxidants build up in these conditions. These oxidants lead to the development of many complications and failures of physiological mechanisms. For example, smoke, in contact with tissues such as skin, lung or oral tissues, breaks down antioxidants [18]. Oxidative stress is also observed when patients are vitamin D deficient or have an excess of Low-Density Lipoprotein (LDL) cholesterol [19].

High levels of oxidants are produced during chronic hypoxia and inflammation. Tissues or bone become hypoxic by losing their vasculature when they are exposed to overpressure [20]. Inversely, when insufficient pressure is placed on bone because of lack of mechanical activity, production of oxidants also increases, leading to bone loss. Astronauts who spend long periods in space also risk bone loss due to a lack of pressure [21].

Clinicians should look out for seven well-known signs of oxidative stress: increased fatigue, memory loss and/or brain fog, muscle and/or joint pain, wrinkles and grey hair, impaired eyesight, headaches and sensitivity to noise, and susceptibility to infections [23].

The human body counteracts oxidants production by synthesizing antioxidants. Antioxidants are low weight molecules such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) [23].There are various natural antioxidants, which are either thiol-based (mainly glutathione) or are non-thiol compounds such as polyphenols, vitamins and various enzymes.

OXIDATIVE STRESS AND BONE

One of the major bone diseases that has been linked with oxidative stress is osteoporosis. Physiological redox changes occur during bone remodeling through the coordinated action of the bone cells (osteoclasts, osteoblasts and osteocytes). Therefore, changes in levels of ROS and/or in antioxidant systems affect the pathogenesis of bone loss [24].

Increased amounts of free radicals in osteoblasts inhibit their functions [25-27] and cause apoptosis of both osteoblasts and osteocytes [28]. At a molecular level, the heightened levels of oxidants increase the production of Receptor Activator of Nuclear-Factor-Kappa-B Ligand (RANKL) and activate the

extracellular receptor kinase/nuclear factor B/tumour necrosis factor/interleukin 6 molecular processes. This activation promotes osteoblast apoptosis and osteoclastogenesis [29].

Excessive apoptosis of osteocytes also causes an imbalance in favour of osteoclastogenesis and inhibits osteogenesis and mineralization. This process increases bone remodelling turnover and bone loss [30,31].

However, it has been reported that increased levels of oxidants have opposite effects on osteoclast cells, as ROS play crucial roles in osteoclast differentiation and function [32,33].

OXIDATIVE STRESS AND BONE REMODELLING

Bone remodelling follows a complex cycle that lasts for approximately six months and involves three main groups of cells: osteoclasts, osteoblasts and osteocytes. Under normal circumstances, the cycle is regulated by cytokines, growth factors and hormones [34].

Oxidative stress activates the differentiation of pre-osteoclasts into mature osteoclasts while inducing apoptosis of osteoblasts and osteocytes and thus increasing bone resorption. In contrast, antioxidants enhance mineralization processes and reduce the activity of osteoclasts, either directly or by counteracting the oxidant effects [35-37].

Melatonin has antioxidant, anti-inflammatory and bone-preserving effects. It shows promise as a preventer of the inhibitory effects of oxidative stress on osteogenesis [38]. The presence of melatonin also reverses the loss of stemness in human bone marrow, which is induced by the presence of Tumour Necrosis Factor (TNF)-α. It achieves this through two interventions: it enhances the osteogenic differentiation of human mesenchymal stem cells (MSCs); and it restores osteogenesis that was inhibited by oxidative stress through activation of Adenosine-Monophosphate-Activated Protein Kinase (AMPK) in human MSCs. This finding suggests that activation of AMPK by melatonin may represent a promising therapeutic strategy for the treatment of metabolic bone diseases such as osteoporosis [39].

OSTEOIMMUNOLOGY

Arron and Choi introduced the concept of osteoimmunology in 2000 [40], based on the strong links that have been found between the bone and immune systems. Researchers hope that the interdisciplinary nature of osteoimmunology will lead to major discoveries in bone regeneration and the development of targeted therapies for bone diseases [41]. The immune system and bone cells have a conjoined heritage in stem cells: they share signalling pathways and influence each other permanently [42].

After a bone fracture, immune cells, especially macrophages, are present throughout the healing process, organizing the body's defense against pathogens and discharging a complex variety of effectors to regulate bone remodeling [43].

Fracture healing starts through an inflammatory reaction, which is commonly the response of living vascularized tissue to aggression. Inflammation is a beneficial mechanism during the first days. If this process is extended in time, its positive effect is reversed and becomes harmful [44]. Fibrosis, for example, is one of the consequences of long-term inflammation.

Inflammation is induced by the release of leukocyte inflammatory cytokines. These cytokines are predominantly angiogenic [45]. Angiogenesis begins and granulation tissue, called the "soft bone callus", is formed [46]. However, two conditions are required for this first cascade of events to occur: these are that sufficient antioxidants are present, and that the cells' repair genes are activated [47].

The transcription factor known as nuclear factor erythroid-2-related factor 2 (Nrf2) activates the transcription of over 500 genes in the human genome, most of which have cytoprotective functions [48]. Vitamin D is one of the main activator of Nrf2 production, through its autocrine and paracrine pathways. The anti-inflammatory function of vitamin D is largely documented [49].

Immunity and the immune system

The immune system is based on cells and proteins. These cells are neutrophils, lymphocytes, monocytes, macrophages and their progenitors: the haematopoietic stem cells. Proteins are made of interleukins, antibodies and TNF.

Immunity is the protection against a particular disease or illness that is offered by particular substances in the blood, which organizes defense of the living body and maintain homeostasis. Immunity helps removing microorganisms or exogenous materials, abnormal endogenous materials, waste products and diseased cells. When the body's immune function weakens, it becomes more susceptible to infections or to the development of malignant tumours. Excessive levels of specific immune

response lead to allergic reactions or to the development of autoimmune diseases [50]. The main requirement for a functional immune system is the presence of sufficient levels of antioxidants.

PREVENTION OF OXIDATIVE STRESS DURING OSSEOINTEGRATION IN ORAL SURGERY

In oral surgery, implant placement and bone augmentation are routinely performed to replace missing teeth. However, the success of the surgery and the stability of the bone over time are not guaranteed, even though the average rate of success exceeds 90%. Failures in implants or in bone augmentations are usually related to the patients' levels of oxidative stress, which may be the result of biological disorders, chronic hypoxia or chronic inflammation.

Biological disorders: deficiency in vitamin D and high levels of LDL cholesterol

Vitamin D is synthesized mainly after sun exposure and normal vitamin D serum levels are 30-100ng/ml. However, due to modern lifestyles in which people spend most of their time indoors, human skin is not enough exposed to the sun to create sufficient vitamin D. Indeed, about 70-80% of patients are deficient in vitamin D [51].

In the liver and kidney, vitamin D that circulates in the blood undergoes two hydroxylations through endocrine activity to produce the active hormone 1, $25(OH)_2$ cholecalciferol. Meanwhile, in other tissues, through autocrine and paracrine pathways [52], the Vitamin D Receptor (VDR) is stimulated. Both pathways enable vitamin D to perform its role as a neuromediator; it regulates cell growth and stimulates the production of antioxidants.

Vitamin D induces expression of antimicrobial peptides that act against bacteria such as Staphylococcus aureus [53,54]. Vitamin D levels of <20ng/ml can lead to organ dysfunction, high infection rates and extended lengths of stay in hospital [55]. Vitamin D has also been shown to have anti-inflammatory properties, as it inhibits Cox-2 expression and suppresses proinflammatory mediators [56].

Vitamin D supplementation is recommended at levels of around 400-600 International Units (IU)/day. This amount is adequate for the prevention of most skeletal abnormalities but is insufficient to stimulate the autocrine and paracrine pathways. To improve cell growth and antioxidant levels, supplementation must be at a daily dose of 4000 to 10000IU/day. Vitamin D serum level blood testing should be performed systematically before any surgery and the patient advised regarding supplementation in case of deficiency [57].

Numerous studies have reported a correlation between high levels of total and LDL cholesterol with low mineral density of bone. LDL cholesterol is oxidized in osteoblasts and is frequently implicated in slow bone metabolism, during which bone synthesis from the bone marrow is slowed and stem cells are oriented to produce fat cells. The bone becomes fattier than normal and takes a yellowish color. In contrast, High-Density Lipoprotein (HDL) cholesterol acts as an antioxidant [58,59].

Chronic inflammation

Patients with inflammatory diseases such as autoimmune diseases, obesity, diabetes, or infected with HIV have high levels of inflammatory cytokines, leading to oxidative stress.

In the specific field of oral surgery, the main issue is the amount of pathogens that are present in the mouth.

The gingival epithelium functions as a barrier to bacteria. However, in many people, this epithelium is contaminated and not always bacteria proof. This is of particular concern in the junctional epithelium [60], which can form a gateway for bacteria to contaminate the underlying bone. This occurs particularly in patients who exhibit a thin biotype, which does not prevent bacterial invasion. Pathogens spread to the periodontium and induce long-term inflammation. The oxidative stress caused by this long-term inflammation induces bone resorption and fibrosis [61]. This mechanism could explain the frequent presence of fibrosis and resorption at the point of re-entry after bone augmentations.

Before oral surgery, antibiotic prophylactics are prescribed to reduce the level of bacterial contamination at the surgical site. However, sometimes, prophylaxis has a limited effect on the number of bacteria present. This has been found particularly among patients who are allergic to penicillin. This population has been shown to carry a three- to four-fold increased risk of surgical site infection in comparison with non-allergic patients [62]. Indeed, alternatives to amoxicillin do not show as effective an effect [63,64]. Also, allergics recruit insufficient neutrophils to ward off pathogens. Furthermore, allergy is an autoimmune disease in which T-lymphocytes have been

identified as the main responsible for the long-term release of inflammatory cytokines [65]. Moreover, allergic patients are often deficient in vitamin D [66]. These patients are consequently in chronic oxidative stress and deficient in the correct immune response [67,68]. Our proposed solution for these patients is to reduce their oxidative stress levels through supplementation with vitamins and immunity boosters: probiotics, omega 3 fats, magnesium, zinc, and vitamins D, B, C, K and E. This supplementation should start at least seven to 10 days before surgery.

Gingival contamination and inflammation can also be reduced through use of the antibiotic Azithromycin, which has an immunomodulatory effect: it has a greater potential to inhibit inflammatory mediator expression at peri-implant wound sites than Amoxicillin and shows long-term activity after just a single dose: higher and more sustained concentrations in periodontal tissues than Amoxicillin when used as pre-op prophylactic antibiotic. Azithromycin has the capacity to inhibit inflammation, oxidative stress, and apoptosis of high glucose-induced podocytes [69,70]. Then, it makes sense to propose a prophylaxis with a monodose of Azithromycin before the surgery (4 capsules of 250mg the night before): this single dose is enough to cover the whole week after surgery with a high level concentration of azithromycin in soft tissue. For bone augmentations, this single dose should be repeated each week during the first month: 1g as single dose at D7, D14 and D21. This protocol has a unique objective: to prevent the long term gingival contamination and inflammation which is the main factor of bone graft resorption during the first weeks. In addition, a single dose of amoxicillin should be added one hour before the surgery (3g).

Chronic hypoxia

In oral surgery, hypoxia occurs when gingiva is submitted to chronic tension or when bone is under chronic pressure. As the blood supply to the cortical bone is organised by the soft tissue through the periosteum, any hypoxia will lead to soft-tissue and bone oxidative stress, followed by the resorption of the underlying bone. The rule of tension-free flap closure must be applied on every occasion, in order to avoid ischemia. Flap release is one of the most important steps of the surgery, as it will guarantee a tensionless situation.

The implant placement, with high primary stability, might also create bone compression. The spongiosa is flexible, so no pressure occurs even if the implant is placed with high torque. In contrast, the cortical bone is a rigid element with a reduced blood supply. So, any pressure will be followed by marginal bone loss or even cratering if the cortical bone is thick [71]. The ideal solution is to avoid any contact between the crestal cortical bone and the implant neck: by over drilling the cortical bone of about 2mm in depth, or by placing the implant 2mm sub-crestally.

Regarding bone grafts, they are specified to become stiff after a few months. Mechanically, the regenerated bone has low flexibility and behaves as a cortical bone when submitted to pressure. Therefore, the same behaviour should be applied during implant placement in a dense bone graft: using a reduced torque and crestal over drilling in order to do not induce the bone graft oxidative stress.

Growth factors and oxidative stress

Growth factors are routinely used in oral surgery. Platelet-Rich Fibrin (PRF), prepared without anticoagulant, offers the simplest and most efficient method, as it combines platelets, leukocytes and the fibrin matrix [72]. After 20 years of clinical use of PRF, the literature contains plenty of evidence regarding its benefits and the mechanism of its action. PRF induces angiogenesis and anti-inflammation pathways [73,74], is osteogenic [75] and inhibits osteoclastogenesis [76]. Recent studies indicate that the main mechanism of action of PRF is its antioxidants promotion and consequently its improvement of local immunity at the site. Use of PRF also enables production of a sticky bone graft. Reducing the mobility of the graft (which is induced by muscle activity during smiling, eating, talking and coughing) avoids fragmentation of the bone callus, which would be inflammatory.

A solution of the osseointegration puzzle can now be proposed. The first required condition is production of antioxidants, which is enhanced by immune supplementation with various supplements that include vitamin D. Growth factors are antioxidants ➔ Immunity ➔ angiogenesis. In order to maintain the vasculature, pressure and tension should be avoided. Motionlessness and high biomaterial compatibility serve to reduce inflammation and fibrosis.

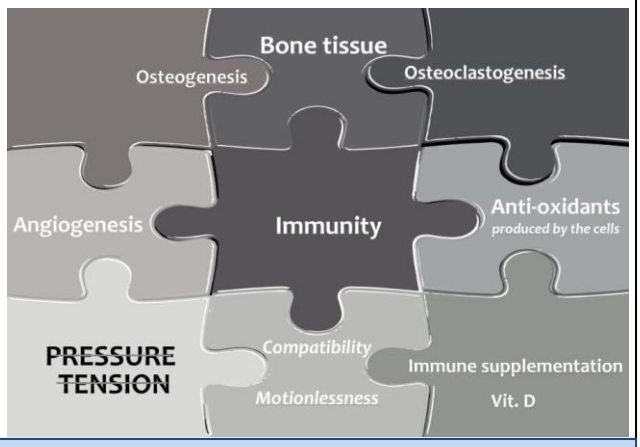

Figure 1: The Oral osseointegration puzzle.

CONCLUSION

The stability of bone in the oral cavity and the presence of several pathological conditions are tied to the levels of global oxidation stress. Bone loss that occurs after implants or bone grafts should be analysed as an immune disease that is secondary to high level of oxidants. However, the integrity of the gingival epithelium may be the key factor that affects bone maintenance; the thickness of the keratinized tissue is the most important barrier to periodontium contamination and inflammation. The application of this concept should enable retention of the osseointegrated bone or implants.

Management of oxidative stress and use of osteoimmunology principles offer the best methods to achieve predictability and reproducibility in oral surgery.

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