Original Article

Antioxidant Efficacy of Ozonated Water and blue®m Mouthrinse on Dental Implants

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Abstract

Background: This study aims to assess the antioxidant capacity of Blue®m mouthrinse and ozonated water, and their impact on dental implant osseointegration. **Materials and Methods:** The current study is a split-mouth study. Ten individuals with two or more edentulous sites, aged 25–55, were divided into two groups at random. Group A: Ozonated water was used to irrigate the osteotomy site. Group B: Blue®m mouthrinse was used to irrigate the osteotomy site. The stability of the implant was evaluated using a resonance frequency analysis device. To evaluate bone formation, cone-beam computed tomography was used. The peri-implant gingival blood flow was measured using ultrasound Doppler flowmetry at baseline and 10 days. Superoxide dismutase (SOD) levels were measured both at baseline and 3 months. **Results:** The results of the current trial showed that bone formation, implant stability, vascularity, Doppler velocity rate, and levels of SOD improved in both the groups but significantly increased in Group A that are treated with ozone (O_3) therapy. **Conclusion:** All the implants osseointegrated without any uneventful healing. Within the limited sample size, O_3 therapy favors osseointegration and increased implant stability, anti-oxidant capacity, and gingival blood flow. When the two groups were compared, the O_3 therapy showed superior results.

Keywords: Antioxidant capacity, Blue®m mouthrinse, Dental implant, Osseointegration, Oxidative stress, Ozone therapy.

Introduction

The goal of modern dentistry is to repair or replace damaged teeth with prosthetics in order to return the patient to normal in terms of shape, function, comfort, appearance, speech, and health. Implant dentistry is unique in that it can accomplish this objective despite stomatognathic system atrophy, disease, or damage.[1]

Osseointegration is the direct structural and functional connection between the surface of a load-bearing implant and organized, live bone. It is necessary for endosseous dental implants to function clinically over the long term, for stability, and as a prerequisite for loading.[2]

An implant's primary stability stems from its mechanical interaction with cortical bone, which stops it from moving in the bone bed once it is placed. Since compressed bone holds the implant firmly in place, it is often referred to as "Mechanical Stability." Secondary stability, also known as "Biological Stability," is attained at the implant/bone interface by bone regeneration and remodeling. It is the outcome of new bone cells growing at the implant site.[3]

As a non-invasive diagnostic technique, resonance frequency analysis (RFA) has become a growing trend due to its accuracy. It is a commonly used technique for objectively and reliably assessing implant stability at various stages. This approach analyzes the initial resonance frequencies using transducers attached to implant mounts or abutments. The measurement unit is the implant stability quotient (ISQ).[4]

Tissue damage during dental implant surgery results in pain and inflammation at the placement site. A surgical inflammation could be defined as the inflammatory response to trauma and surgery. From an inflammatory process perspective, the surgical inflammation can be understood as consisting of multiple overlapping phases.[5]

The inflammatory phase initiates the process of wound healing through coagulation, hemostasis, and enhanced vascular permeability for inflammatory leukocytes. These cells are responsible for reactive oxygen species (ROS) generation and phagocytosis.[6]

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Oxidative stress is strongly linked with inflammatory processes. High levels of ROS released by inflammatory cells result in the formation of an oxidative microenvironment at the site of the wound. Crucially, it has been demonstrated that a hydrogen peroxide ($H_2O_{2)}$ gradient in the wound serves as a chemoattractant for inflammatory cells. Macrophages and endothelial cells produce ROS in response to metal corrosion products. The cells possess defense systems built in to fend off ROS. These include antioxidant enzymes that regulate how cells react to oxidative stress, such as glutathione peroxidase, catalase, and superoxide dismutase (SOD).[7]

Superoxide, an oxygen (O_2) radical generated in inflammatory pathways, is hindered by the antioxidant enzyme SOD.[8]

Ozone (O_3) is commonly administered in three routes (i.e., gaseous O_3, ozonated water, ozonated plant oils). Ozonated water is the most preferred form for usage in dentistry. It has been found that ozonated water improved local O_2 supply, inhibited bacterial growth, and enhanced hemostasis. Numerous effects of O_3 on the human body are well-established, including immunostimulant and analgesic, antioxidant, antibacterial, bioenergetic, and biosynthetic.[9]

O_3's capacity to dissolve in plasma's aqueous component makes it capable of causing mild oxidative stress. H_2O_2, a ROS, is produced when O_3 reacts with water and polyunsaturated fatty acids. O_3 concurrently produces a variety of lipid ozonation products. The transcriptional factor mediating nuclear factor-erythroid 2-related factor 2 (Nrf_2) is activated more when moderate oxidative stress from O_3 occurs. The transcription of antioxidant response elements (ARE) is activated by the Nrf2 domain. When ARE transcription is induced, a variety of antioxidant enzymes respond to the brief oxidative stress by increasing their concentration levels.[10]

O_3 enhances the production of type I collagen and also increases the production of alkaline phosphatase (ALP), thereby promoting new bone formation.[11]

blue®m mouthrinse, which has been represented as having no adverse effects and an extended shelf life. It does not contain any antibacterial agents; instead, its effectiveness is based on the release of active O_2 and lactoferrin.[12]

Lactoferrin triggers the differentiation and multiplication of osteoblasts, which leads to the formation of mineralized new bone and also inhibits osteoclastogenesis by decreasing the potency of precursor cells to produce osteoclasts. The mouthwash's active O_2 normalizes and regulates harmful microbes while hastening the healing of wounds.[13]

The purpose of this study was to evaluate the antioxidant capacity of ozonated water and blue®m mouth rinse and their effect on osseointegration of dental implants.

Methodology

Study design

This study was conducted at the Department of Periodontics, St. Joseph Dental College. The Institutional Scientific Review Board and ethical committee approved the protocol of this clinical prospective study with IEC number CEC/21/2021-22 and the CTRI number for this study was 065676. A minimum total sample size of 10 was found to be sufficient for an alpha of 0.05, power of 80%, and 1.0 as effect size.

Volunteers who fulfilled the inclusion requirements participated in a crossover randomized controlled study. Participants in this study were those who sought therapy for bilateral partial tooth loss with implant procedures at St. Joseph Dental College. Participants had to be in good health or have a well-controlled medical condition, be between the ages of 25 and 55 years, have one missing tooth on each side, and be willing to sign a consent form. Patients with a history of head-and-neck radiation therapy, life-threatening illnesses, or a complete contraindication to implants were not included.

It is a split-mouth study with two edentulous sites in 10 patients. The assessment of antioxidant capacity was done by the evaluation of SOD levels. Implant stability was evaluated using RFA. Peri-implant gingival blood flow was assessed using Doppler flowmetry. Radiographic assessment was done by cone-beam computed tomography.

Surgical phase

The edentulous sites were divided into two groups.

Intervention

The surgical field was prepared and the edentulous site was anesthetized. A mid crestal incision was given, and then a full-thickness mucoperiosteal flap was raised. 2 mL of blood was drawn from the site. Osteotomy was done using the standard protocol.

Group A: The implants were placed with ozonated water as an irrigant for 10 min.

An O_3 generator based on ambient air was used to create ozonated water (ADC. Inc., Dentoozone India). The generator comes with a metal applicator tip, pipe, electric cord, and machine. The equipment was prepared for usage after every accessory was connected. The pipe was submerged in the water while the water was being ozonated. 0.6–1 ppm was the concentration of the ozonated water utilized in the present study. Ozonated water was used within 20 min after its preparation (Figure 1).

Group B: blue®m mouth rinse with a concentration of 1 µL/mL was used as an irrigant for about 10 min.

Primary stability of the implant was measured in ISQ values using RFA (Penguin RFA) device. Using number 3-0 silk sutures, the mucoperiosteal flap was adapted and sutured over the implants. Post-operative instructions were given to the patients.

Prosthodontic phase

In both groups, conventional loading was scheduled. 2 mL of blood was drawn from the implant site. The ISQ readings were then used to evaluate the implant's secondary stability.

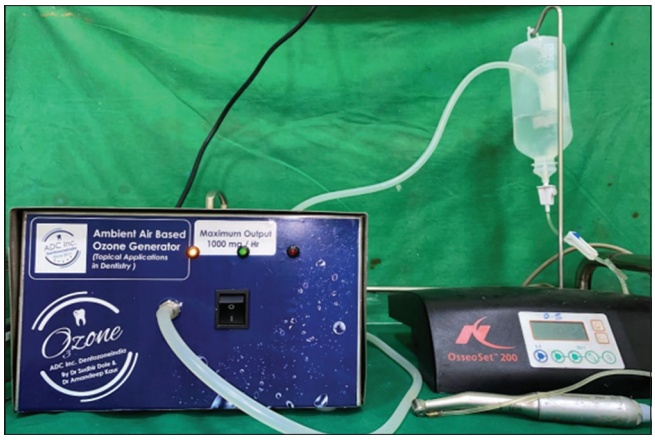

Figure 1: Ozonator Device

Healing abutments were then placed after 3 months for the mandibular implants and 6 months for the maxillary implants. After the abutments had been in place for 15 days, abutment level closed tray putty impressions were taken and sent for crown fabrication. Following that, each implant received a functional metal ceramic crown.

SOD evaluation

(Copper-Zinc) SOD activity was determined by use a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol. At pH 8.2, 50% of pyrogallol autoxidize in the presence of ethylenediaminetetraacetic acid. The principle of this method is based on the competition between the pyrogallol autoxidation by O_2 and the dismutation of this radical by SOD.

Results

Data were entered into MS-Excel and analyzed in Statistical Package for the Social Sciences v26 (IBM Corp, Armonk, N.Y., USA). Descriptive statistics were represented with means and standard deviations, independent and paired *t*-tests were used to find significance, $P < 0.05$ is considered statistically significant.

Intra-group comparison of bone mineral density (BMD) at 6 6-month interval shows a statistically significant increase in both the groups. A statistically significant rise was seen in both groups, according to an intragroup study of the RFA and SOD at 3-month intervals. Doppler velocity rates were compared within groups at baseline and after 10 days, showing a statistically significant rise in both groups.

Upon comparing the two groups at 6-month intervals (Table 1), the BMD increased statistically significantly in the current study, with the ozonated water group experiencing a greater increase than the blue®m mouthrinse group.

As RFA and SOD were compared among the groups at baseline and 3 months (Table 2), the results showed a statistically significant rise in the group that administered ozonated water treatment as opposed to the group that administered blue®m mouthrinse.

Table 1: Intergroup comparison of the mean bone mineral density scores at baseline and 6 months using independent sample *t*-test

Time	Group	*n*	Mean	SD	SEM	*t*	*P*
Baseline	A	10	524.4100	134.89440	42.65735	−0.924	0.372
	B	10	480.3400	67.51988	21.35166		
6 months	A	10	651.4000	75.61547	23.91171	−3.835	0.001
	B	10	533.7800	60.75466	19.21231		

SD: Standard deviation, SEM: Standard error of mean

Table 2: Intergroup comparison of the mean resonance frequency and superoxide dismutase scores at baseline and 3 months using independent sample *t*-test

Group	*n*	Mean	SD	SEM	*t*	*P*
Resonance frequency analysis (ISQ)						
Baseline						
A	10	64.90	3.75	1.18743	−1.271	0.220
B	10	62.00	6.16	1.94936		
3 months						
A	10	87.80	6.14	1.94251	−2.966	0.008
B	10	79.10	6.95	2.19823		
Superoxide dismutase						
Baseline						
A	10	6.61	0.940	0.297	0.098	0.923
B	10	6.65	0.874	0.276		
3 months						
A	10	15.38	4.288	1.35	4.56	0.000
B	10	10.8	0.949	0.300		

SD: Standard deviation, SEM: Standard error of mean

Table 3: Intergroup comparison of the mean Doppler velocity rate scores at baseline and 10 days using independent sample *t*-test

Time	Group	*n*	Mean	SD	SEM	*t*	*P*
Baseline	A	10	14.09	1.26	0.398	0.267	0.793
	B	10	14.25	1.41	0.44752		
10 days	A	10	52.9	3.93	1.24	−11.607	0.000
	B	10	24.5	6.666	2.10		

SD: Standard deviation, SEM: Standard error of mean

Intergroup comparison of Doppler velocity rates at baseline and 10 days (Table 3), statistically significant increase in the group treated with ozonated water compared to the group treated with blue®m mouthrinse.

Discussion

A high O_2 demand is a result of a number of cells increasing their metabolic activity during wound healing, including neovascularization, collagen synthesis, re-epithelialization, and the breakdown of necrotic tissue. Escalation of O_2 levels near the wound has been shown to accelerate wound healing. It also provides an environment that is hostile for anaerobic bacteria.[14]

O_3 is a blue gas present in the stratosphere with a concentration of 16–20 mg/mL. It gives up a nascent O_2 molecule to form O_2 gas; it has the highest oxidation potential, around 150% greater than that of chlorine when used as an antimicrobial agent. O_3 is used in medical and dental fields because it has strong oxidant properties.[15]

Dr. Peter Blijdorp's research group created the topical O_2 treatment formulation known as "blue®m," which has shown a clinical ability to limit the growth of bacterial biofilms. The formulation consists of cellulose, glycerol, lactoferrin and sodium perborate, which yields topical O_2 in a regulated way.[16]

A vital component of the wound's nutrition, molecular O_2 is essential to the healing process. Molecular O_2 is necessary for the reparative process since it is necessary for the production of collagen, matrix deposition, angiogenesis, epithelialization, and bacterial death. It also stimulates the proliferation and differentiation of osteoblasts by increasing the production of ALP and transforming growth factor-beta (TGF-β).[17]

In group B, there is increase in BMD from baseline to 6 months and implant stability (ISQ values) from baseline to 3 months. blue®m contains lactoferrin, which accelerates the bone growth by triggering the differentiation of osteoprogenitor cells and also indirectly decreases the potency of progenitor cells to convert into osteoclasts, thereby increasing new bone formation. This is in line with the studies conducted by Shaheen *et al*.,[18] Tanka *et al*.[19]

In group A, there is increase in BMD from baseline to 6 months and implant stability (ISQ values) from baseline to 3 months. On intergroup comparison, the mean BMD (gray scale) at baseline to 6 months and implant stability (ISQ values) from baseline to 3 months were significantly increased in group A than group B due to the impact of O_3 therapy on the bone surrounding implants. O_3 therapy helps in bone formation, maturation, and remodeling by activating osteoblasts, osteosynthesis and decreases osteoclastic activity. It also improves the local environment in the peri-implant interface zone by promoting hemostasis, improving local O_2 supply, and encouraging osteoblast proliferation. Higher levels of cytokines, particularly TGF-1, are expressed in response to O_3, and these cytokines can regulate and coordinate the early stages of wound healing. TGF-1 also significantly affects the production of collagen, angiogenesis, extracellular matrix, and monocyte, and fibroblast cell proliferation. This is in line with the studies conducted by Mnasour *et al*.,[20] Buyuk *et al*.,[21] Matar *et al*.,[22] Karaca *et al*.,[23]

SOD is an antioxidant enzyme that shows a transient increase during oxidative stress and protects the cells. In Group B, there is an increase in SOD levels from baseline to 3 months. In Group A, there is an increase in SOD levels from baseline to 3 months. SOD levels of Group A from baseline to 3 months were considerably higher than Group B, according to an intergroup comparison.

Therapeutic doses of O_3 generate controlled oxidative aggression that exacerbates a defense reaction, resulting in increase in antioxidant enzymes. This is in line with the studies conducted by Singh *et al*.,[24] Guadalupe *et al*.[25]

Vascular channels show desirable outcomes and are also important clinical parameters to evaluate the efficiency of the agents. In Group B, there is increased vascularity from baseline to 10 days. Blue®m contains sodium perborate, which liberates nascent O_2 on reaction with oral fluids. Surge in O_2 levels recruits endothelial progenitor cells to the wound, increases vascular endothelial growth factor (VEGF), and promotes angiogenesis. To transform procollagen into collagen, hydroxyproline needs O_2.[26]

In Group A, there is increased vascularity from baseline to 10 days. This study showed that VEGF was expressed significantly in group A than in Group B as a result of ozonation, which dramatically enhanced vascularity in the former. VEGF is thought to be the primary vascularization growth factor during the last stages of wound healing. As a result, the increased vascularity caused by O_3 promoted osteoblast differentiation and proliferation leading to peri-implant bone formation. This is in accordance with the studies conducted by Buyuk *et al*.[21]

Conclusion

The findings of this study suggest that both ozonated water and blue M mouthrinse exhibit promising antioxidant effects when used around dental implants. Ozonated water, with its potent oxidative properties, may help to reduce microbial load and enhance tissue healing. Utilizing ozonated water as an irrigating solution could offer a valuable adjunct to implant maintenance and peri-implant health. However, further clinical studies are needed to establish long-term efficacy, optimal application protocols. Integrating these biocompatible agents into implant care routines may contribute to improved peri-implant tissue health and implant stability.

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