

Ozone Therapy in Dentistry: A Systematic Review

Ozonoterapia en Odontología: Una Revisión Sistemática

Iris Jasmin Santos German^{*}; Antonio de Castro Rodrigues^{*}; Jesus Carlos Andreo^{*}; Karina Torres Pomini^{*}; Farooque Jamaluddin Ahmed^{*}; Daniela Vieira Buchaim^{*}; Geraldo Marco Rosa Júnior^{**}; Jéssica Barbosa de Oliveira Gonçalves^{*} & Rogério Leone Buchaim^{*}

GERMAN, I. J. S.; RODRIGUES, A. C.; ANDREO, J. C.; POMINI, K. T.; AHMED, F. J.; BUCHAIM, D. V.; ROSA JÚNIOR, G. M.; GONÇALVES, J. B. O. & BUCHAIM, R. L. Ozone therapy in dentistry: A systematic review. *Int. J. Odontostomat.*, 7(2):267-278, 2013.

ABSTRACT: The purpose of this systematic review was to verify the literature available regarding the effectiveness and the biological effects of ozone therapy in periodontics, orthodontics and dental implants. Studies were searched in September 2012. Analyzed sources included the databases PubMed, Lilacs and SciELO, through a combination of key words, dental implants, periodontics, orthodontics, therapeutics. Studies between 2002 and 2012 were included. In vitro and in vivo studies English and Spanish language publications, excluding posters, letters to the editors and conferences. In Vitro and in Vivo studies showed the inactivation of the major periodontal pathogens by ozone. There were divergent results and lack of evidence for the activity of ozone on adhesion of orthodontic brackets, in implantology and orthodontics. Ozone could be considered as a promissory alternative therapy in dentistry. However, well-designed studies are needed to assess the application of ozone in order to evaluate its clinical effectiveness in the field of dentistry.

KEY WORDS: dental implants, periodontics, orthodontics, therapeutics.

INTRODUCTION

Ozone is a gas with the chemical formula O₃, and is the third most powerful oxidant. Medical ozone is produced by oxygen and its passage through a voltage gradient, using ozone generators that react to dioxygen molecules, forming ozone (O₂ + O → O₃) (Ripamonti *et al.*, 2011; Bocci, 2006; Swilling, 2004).

Several biological actions (Fig. 1) of medical ozone include the increase in the synthesis of biologically active substances such as interleukins, leukotrienes and prostaglandins that are beneficial in the reduction of inflammation and in cicatrization, the activation of aerobic processes (glycolysis, Krebs cycle, beta-oxidation of fatty acids), secretion of vasodilators (e.g. nitric oxide-NO), activation of the mechanism of protein synthesis and increase in the quantity of ribosome and mitochondria in the cells, thus raising the functional and potential activity of tissue regeneration (Seidler *et al.*, 2008).

The ozone oxidant potential induces the destruction of cellular walls and cytoplasmatic

membranes of bacteria (Thanomsub *et al.*, 2002). The gas was named ozone (a Greek word that means "smell") by the German chemist Christian Frederick Schonbein of the University of Basel in Switzerland 1840 (Seidler *et al.*; Veranes *et al.*, 1999).

During the First World War, ozone gas was used for the treatment of post-traumatic gangrene, infected wounds, burns and fistulas and other anaerobic infections, putrefied wounds, suppurations of bone fractures and various inflammations in German soldiers (Azarpazhooh & Limeback, 2008). By the middle of 1932, German dentist Edward A. Fisch, perceived the therapeutic possibilities of using ozone as a medical therapy (Altman, 2007; Bocci, 2005).

Currently, there are divergences in the developed studies regarding ozone therapy action. Therefore, the studies available in the literature must be reviewed in order to evaluate the obtained results and estimate the effectiveness of ozone therapy and the methods used. The purpose of this study was to

^{*} Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil.

^{**} University of Sacred Heart, Bauru, São Paulo, Brazil.

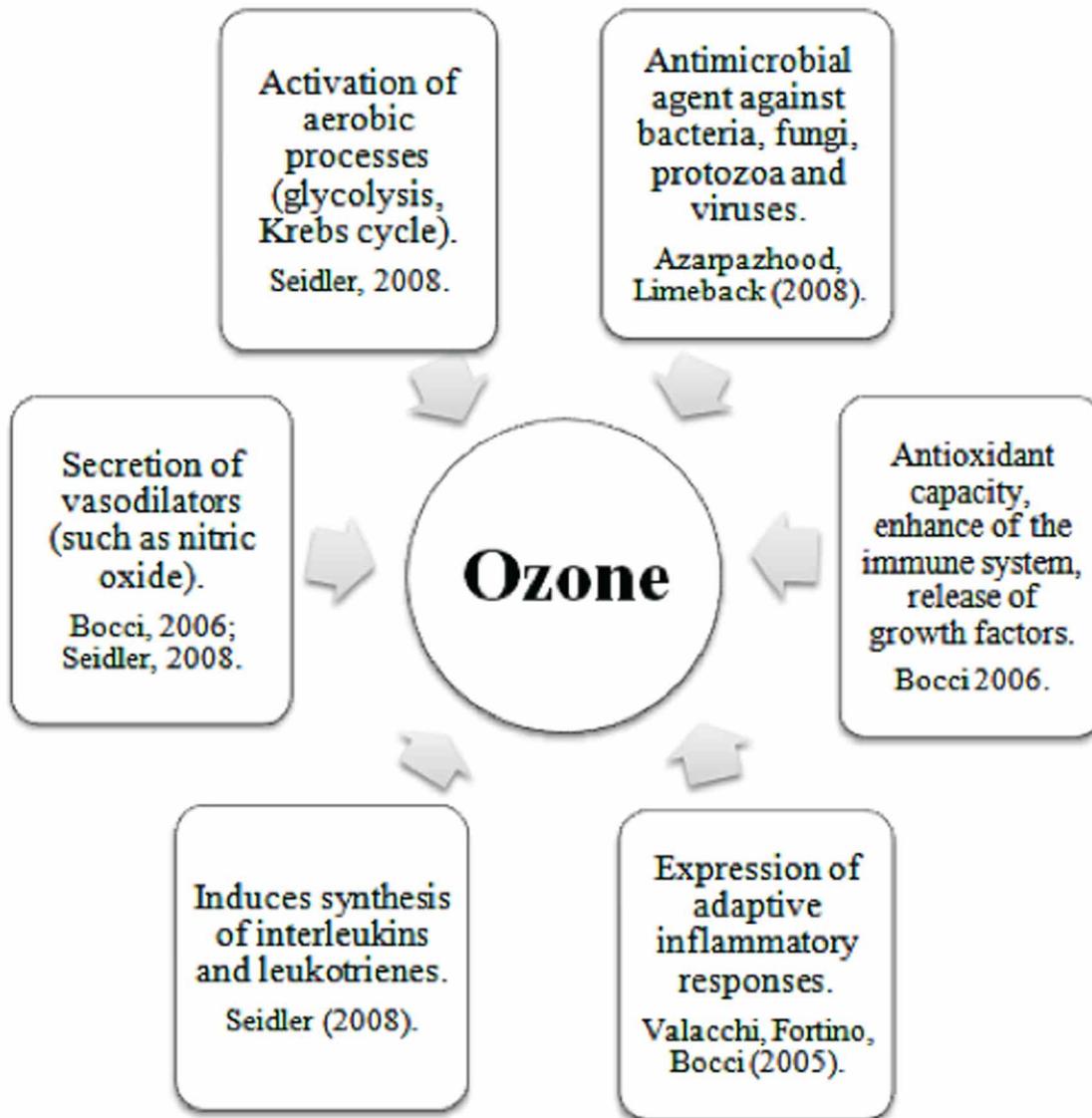


Fig. 1. Major biological effects of ozone therapy.

perform a review regarding the effectiveness of ozone therapy in the area of dentistry, with emphasis on the current uses, focusing on the areas of periodontics, orthodontics and dental implants. The authors aimed at answering the following questions: "What is the action of ozone on periodontal pathogens?" "What are the effects of ozone on the adhesion of brackets to dental enamel?" "How does ozone act in the bone integration of dental implants?"

These questions are important for future studies in order to understand the properties of ozone and its contribution in clinical uses.

MATERIAL AND METHOD

Data sources. The literature review included studies available in the databases, PubMed, LILACS and SciELO, utilizing the following descriptors: ozone, dentistry, periodontics, orthodontics and dental implants. Studies published between the years 2002 and 2012 were included. A combination of the key words was used, which helped to identify studies related to ozone therapy in dentistry. Books regarding ozone therapy were also referred to in order to obtain more information on the theme

(Altman; Bocci, 2002, 2005). The search was limited to studies written in English and Spanish. Both in

vitro and in vivo studies regarding ozone therapy in dentistry were selected (Fig. 2).

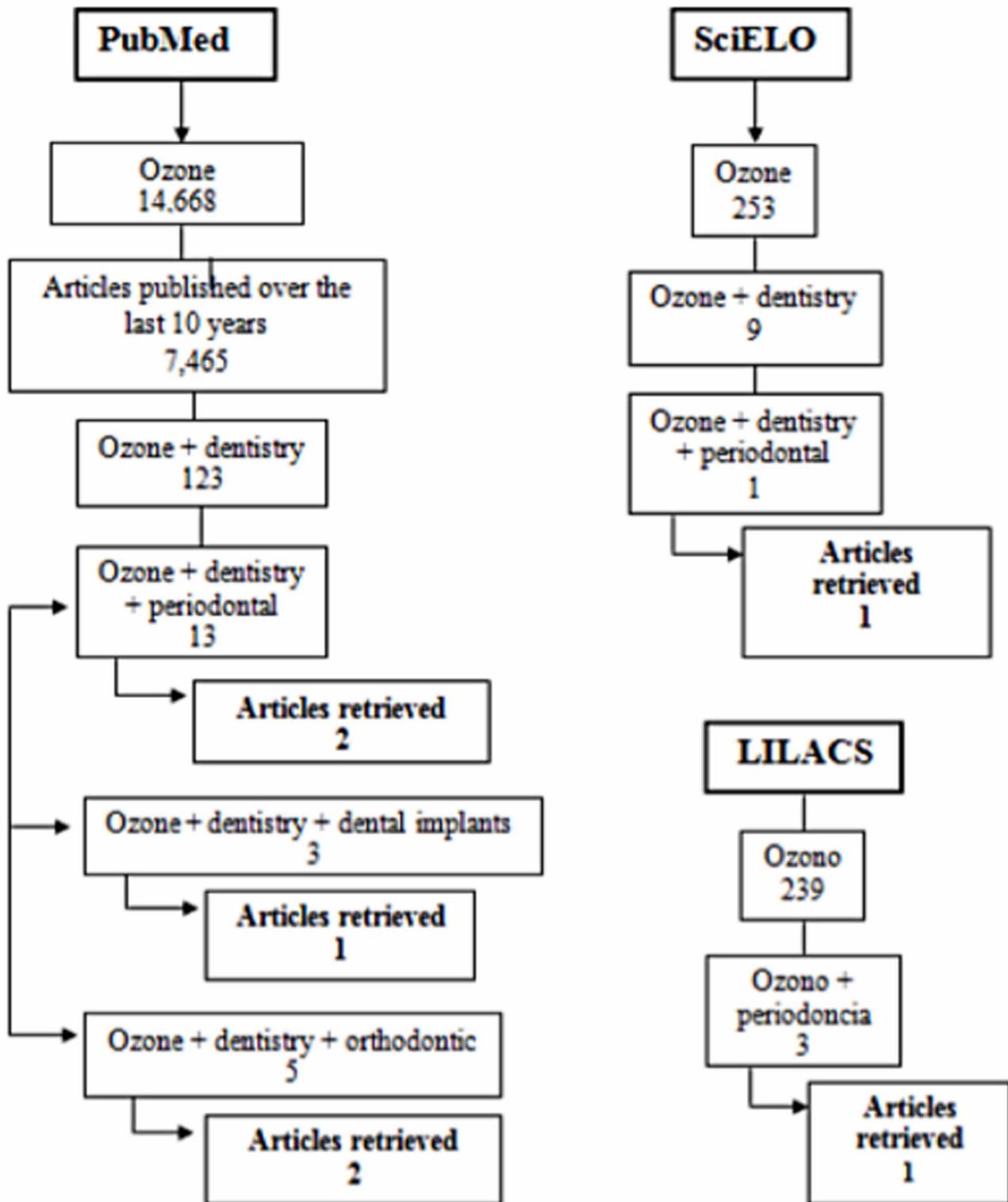


Fig. 2. Search strategy for literature review on the use of ozone therapy in dentistry.

Study selection. Letters to the editor, abstracts, conferences, comments, books and panels were not included; however all of them were reviewed in order to identify possible important data that could contribute to the development of this review.

Data extraction. After reviewing the titles of the researched studies, seven pertinent studies were found related to the study objectives (accessed on September 26, 2012). Several studies stemming from the search were read with the purpose of identifying relevant information on the theme in question.

RESULTS

Results are presented in three sections according to the purposes of the study.

Action of ozone on periodontal pathogens. Three *in vivo* (Kshitish & Laxman, 2010; Martínez Abreu & Abreu Sardinias, 2005; de Ramon *et al.*, 2004) and one *in vitro* study (Huth *et al.*, 2011) evaluating the action of ozone on periodontal microbiota were found.

In 2004, de Ramon *et al.*, (Table IA) analyzed the periodontal responses in terms of the clinical, microbiological and immunologic aspects in patients with moderate and severe periodontal pockets, comparing the technique of scaling and root planning (control group) with the application of ozone (experimental group).

After 30 days, the average clinical evaluation showed the following data: in the experimental group, initial periodontal index (Russell's Index) was 5.72 and the final index was 5.59. Initial supragingival plaque index was 31.30 and the final index was 49.99. Initial gingival bleeding index was 62.43 and the final 26.29.

In the experimental group the microbiological analysis the reduction of periodontal pathogens (*Aggregatibacter actinomycetemcomitans* (Aa), *Bacteroides forsythus* (Bf), and *Porphyromonas gingivalis* (Pg) was about 50%). In the immunologic analysis, the initial TNF- α was 101.23 and at the end it decreased to 36.01. Initial IL-1 β also reported a decrease from an initial period of 96.12 to 34.28 after 30 days of treatment.

Martínez Abreu & Abreu Sardiñas (Table IA) developed a randomized controlled clinical study. The

study was divided into two groups: the control (treated with 0.2% chlorhexidine) and the experimental group (treated with the ozonated oil Oleozon). A microbiological analysis was performed on the gingiva of the teeth with periodontitis after 21, 90 and 180 days respectively, during a nine-month postoperative period.

The microbiological and clinical evaluation was determined to be:

Satisfactory: Absence or lack of gram-negative microorganisms.

Unsatisfactory: Abundant presence or prevalence of gram-negative microorganisms.

After the postoperative period of 180 days, microbiological results were satisfactory in 98% of the patients in the experimental group and 78% in the control group. Regarding the clinical evaluation, satisfactory results after 180 days were present in 98% of the patients in the experimental group and 95% in the control group.

In 2010, Kshitish & Laxman (Table IB) developed a double-blind randomized study regarding the use of ozonated water in the treatment of periodontitis. The control group was irrigated with 0.2% chlorhexidine. Clinical parameters such as gingival index and gingival bleeding index were analyzed, in addition to the ozone activity on oral microorganisms: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythensis* (Tf), Herpes Simplex virus (HSV-1 and HSV-2), Epstein Barr virus (EBV) human cytomegalovirus (HCMV) and *Candida albicans*.

Results of the ozone irrigations showed 12% of reduction of the plaque index. The gingival index decreased 29% and bleeding index 26%. After seven days, ozone showed presence of Aa in 25%, Pg 50%, Tf 25%, HSV-1 62.5%, HSV-2 0%, HCMV 25%, EBV 37.5% and *Candida albicans* 12.5% of the cases.

In an *in vitro* study, Huth *et al.* (2011) (Table IB) determined the efficacy of the use of gaseous/aqueous ozone in the reduction of several periodontal pathogens (Aa, Pg, *Tannerella forsythia* (Tf) and *Parvimonas micra* (Pm) in both planktonic state and biofilm. Aqueous ozone in a concentration of 20 mg ml⁻¹ resulted in the reduction of approximately 99% of the total *A. actinomycetemcomitans* in planktonic state, and 70% in biofilm. Gaseous ozone (concentration of 1, 2, 4, 8, 16 g m⁻³) was capable of reducing this pathogen by

Table IA. Ozone therapy in periodontics.

| Author (date) | Purpose | Methodology | Results | Conclusion | Study limitations |
|--|---|---|---|---|--|
| Ebensberger <i>et al.</i> (2002) | To investigate the effects of irrigating ozonated water on the proliferation of cells in the periodontal ligament attached to roots of extracted human teeth. | 23 third cuspid teeth were extracted from patients aged between 20-35 years. Ozonated water was irrigated for 2 minutes (Ozone generator prototype Sirona MI, Sirona, Bensheim, Germany, in concentration of 2.5-3-5 µg ml ⁻¹) and the expression of the marker of Proliferating Cell Nuclear Antigen (PCNA) was evaluated. | The number of positive cells compared to the total number of cells was 7.8% after irrigation with ozone and 6.6% after irrigation with saline solution. Aqueous ozone did not have negative effects on the cells of the periodontium. Positive marker cells were found alone or in groups in the cement or in the remnant periodontal ligament. | Irrigation with ozonated water showed high rates of cells in comparison to saline solution (control group), but with no significant differences. There were no differences in the number and distribution of positive PCNA cells in the coronal and apical part of the roots of the cuspid teeth. | <i>In situ</i> , the teeth would be submitted to functional stress, which could alter the expression of fibroblasts and the DNA synthesis. |
| de Ramon <i>et al.</i> (2004) | To analyze the clinical, microbiological and immunological responses in patients with moderate/severe periodontal pockets using ozone therapy. | 72 maxillary hemiarches were analyzed with at least 4 teeth and 6 mm of probing depth. One hemiarch was treated with ozone and the other with scaling and root planing (Control group). | Initial periodontal index (Russell) was 5.72 and the final was 5.59. Initial supragingival plaque index was 31.30 and the final index was 49.99. Initial gingival bleeding index was 62.43 and the final was 26.29. Initial TNF-α was 101.23 and at the end it decreased to 36.01. | Ozone had no significant effect on the removal of supragingival plaque, but reduced the gingival bleeding index, including TNF-alfa and IL-1. However, it did not produce changes in the level of periodontal insertion and pocket depth. | The dosage used was not reported. |
| Martínez Abreu & Abreu Sardiñas (2005) | To evaluate the effects of ozonated oil (Oleozon) in the treatment of moderate periodontitis. | Application of Oleozon in periodontal pockets after 21, 90 and 180 days over a total period of 9 months. n=84 patients. | Microbiological evaluation showed, over the period of 180 days, scarcity or lack of gram-negative microorganisms: 98% for the experimental group and 78% for the control group (0.2% aqueous chlorhexidine). | Improvement of the signs and symptoms was faster in the group treated with ozonated oil than in the control group. Microbial levels were more compatible with healthy periodontium in the ozonated group. | The dose of ozone used and the time of each application were not reported. |
| Huth <i>et al.</i> (2007) | To investigate the effects of aqueous ozone on the NF-kB system. | Human oral epithelial cells and gingival fibroblasts were cultivated. Aqueous ozone was applied to the cells. Aqueous ozone was generated with gaseous ozone, 75µg/ml for 15 minutes (Ozonosan-photonic) resulting in the final concentration of 20µg/ml). | The activity of the NF-kB on the oral cells in the radicular surfaces periodontally compromised was inhibited when incubated with ozone, in addition IκBα proteolysis, cytokine expression and kB-dependent transcription were prevented. | Aqueous ozone had inhibitory effects on the NF- κ B system, suggesting it has anti-inflammatory properties. | Only one concentration of ozone and time of exposure were used. Higher or lower concentration and times may generate different results. |

Table IB. (Continuation) Ozone therapy in periodontics.

| Author (date) | Purpose | Methodology | Results | Conclusion | Study limitations |
|------------------------------|--|---|--|--|---|
| Kshitish & Laxman (2010) | To evaluate and to compare the effects of irrigating ozonated water (0.082 mg/h for 5-10 minutes) and 0.2% chlorhexidine (Control group) in clinical parameters such as gingival index and gingival bleeding index, in addition to the action of ozone on oral microorganisms. | 16 patients with chronic generalized periodontitis. Study period was for 18 days. Irrigations of aqueous ozone and chlorhexidine were randomized. An evaluation of the clinical parameters and oral microorganisms was performed. | Ozonated water reduced plaque index in 12%, gingival index in 29% and bleeding index in 26% of cases. After 7 days the ozone group showed presence of Aa in 25%, Pg 50%, Tf 25%, HSV-1 62.5%, HSV-2 0%, HCMV 25%, EBV 37.5% and <i>Candida albicans</i> 12.5% of cases. | Reduction of the gingival index and bleeding index in the ozone group was significant in comparison to chlorhexidine. Ozone is a powerful agent in the inactivation of microorganisms associated to periodontitis. | Study period was too short. |
| Skurska <i>et al.</i> (2010) | A comparison of the clinical condition and salivary levels of MMP (Matrix metalloproteinase) after scaling and root planing or ozone therapy in patients with aggressive or chronic periodontitis. | 52 patients with chronic or aggressive periodontitis, aged between 25-68 years were divided into 3 groups: a) CP -S: patients with chronic periodontitis submitted to root scaling. b) CP-O: patients with chronic periodontitis treated with scaling and root planing and ozone therapy. c) AP: patients with aggressive periodontitis treated with root scaling and ozone therapy. Ozone was applied 3 times every 2 days (1 minute, 42.2 µg ozone/ml of oxygen). | After 2 months the highest scores in dental plaque reduction were found in group AP (API-31.04, PI-0.47). The lowest were in group CP-S (57.77, PI-0.78). Levels of MMP-1 increased in CP-O and AP group. The highest level of MMP-1 was found in group CP-O (0.185), whereas CP-S decreased from 0.175 to 0.107. The levels of MMP-8 decreased in all groups, but groups CP-O and AP were higher statistically than in the control group. | The scaling and root planing (SRP) combined with ozonotherapy does not contribute to further improvement in clinical periodontal parameters in patients with aggressive and chronic periodontitis. Ozonotherapy combined with SRP leads to a decrease of saliva MMP concentration in patients with aggressive periodontitis. | A vacuum mould should have been used not only to avoid toxicity but also to provide safety when the concentration used was applied in the mouth. |
| Huth <i>et al.</i> (2011) | To investigate the antimicrobial effectiveness of aqueous and gaseous ozone in comparison to the antiseptic chlorhexidine digluconate on periodontal microorganisms in planktonic state and in biofilm. | The microorganisms were cultivated and submerged in 10 ml of Brain Heart Infusion (BHI) at 37°C for 48 hours. The ozone was used in concentration of 1-53 gm ⁻³ for one minute. The combination of gaseous Aqueous ozone was prepared with bidistilled water and gaseous ozone (75 µg ml ⁻¹ for 15 minutes), resulting in a final ozone concentration of 20 µg ml ⁻¹ . | Aqueous ozone reduced 99% of the total Actinomyces committans in planktonic state and 70% in biofilm. Gaseous ozone (1, -16 g m ⁻³) reduced Aggregatibacter actinomycetemcomitans (Aa) 99.7% in isolated state and 70% in biofilm (53 g m ⁻³). | Gaseous ozone presented greater antimicrobial activity against Aggregatibacter actinomycetemcomitans. Chlorhexidine, aqueous and gaseous ozone showed dose-dependent effectiveness; however, there was greater efficacy in planktonic bacteria than in biofilm. | Antimicrobial agents were tested on biofilms with only one type of bacteria. In clinical conditions, biofilms compose multispecies that could produce changes in the host and lead to changes in the environment. |

99.7% in isolated state, and by approximately 70% in biofilm at a concentration of 53 g m⁻³.

The complete elimination of *P. gingivalis* and *T. forsythia* in planktonic state was obtained through the concentrations of gaseous ozone of 8 and 16 g m⁻³ and 2% chlorhexidine; in biofilm gaseous ozone of 53 g m⁻³ and 2% chlorhexidine showed effectiveness of 100%. Even in low concentrations (1, 2 and 4 g m⁻³), gaseous ozone reduced the levels of these pathogens by 99.7% in planktonic state.

Parvimonas micron, in isolated state, was totally eliminated by all agents, with the exception of low concentrations of aqueous ozone at 1.25 mg ml⁻¹ (reduction of 78% in planktonic state). However, in biofilm, *P. micra* presented greater susceptibility to ozone gas (53 g m⁻³) and 2% chlorhexidine.

Ozone effects on the adhesion of brackets to dental enamel. Two *in vitro* studies evaluating the effects of ozone application on the adhesion of orthodontic brackets were found.

In a randomized *in vitro* study, Cehreli et al., (2010) (Table II) evaluated prophylactic pretreatment with ozone, applying shear tension to the bonding of orthodontic brackets. Moreover, it analyzed the bonding failures in the interface using the modified adhesive remnant index (ARI).

Vertical force was applied to the bracket base (1N/mm²=1MPa) at a speed of 1mm/min. Each tooth was washed, and polished with pumice and rubber cups for 10 seconds. The results of the shear bond strength were: Group 1: 10.48 MPa; Group 2: 8.89 MPa; Group 3: 9.41MPa; Group 4: 9.82 MPa.

The results of modified adhesive remnant index ARI (0-3) were: Group 1: 2.38; Group 2: 1.31; Group 3: 3.00; Group 4: 1.92.

Groups that were pretreated with ozone showed greater shear strength over the bracket. The lowest shear strength was obtained in Group 2. Pretreatment with ozone in the enamel did not affect the adhesive system used to attach the brackets.

Pithon & dos Santos (2010) (Table II) determined whether aqueous ozone reduces the bond strength of orthodontic adhesives. In total, 120 bovine mandibular incisor teeth were selected randomly and divided into 4 groups. Groups 1 and 3 were cleaned

with pumice and washed with water; groups 2 and 4 were washed with ozonated water before adhesion to stainless orthodontic brackets with resin Transbond XT, 3M Unitek, Monrovia, CA, USA (Group 1 and 2); and a modified resin of glass ionometer cement (Groups 3 and 4; Fuji Ortho LC, Japan).

Results showed, on average, shear bond strength (SBS) of 22.07 Mpa for Group 1; 20.21 Mpa in Group 2; approximately 20.06 Mpa in Group 3 and over 18.73 Mpa in Group 4. In terms of the adhesive remnant index ARI (0-3), results showed: Absence of adhesive remnant in the tooth (0): Group 1=3, Group 2=9, Group 3=0, Group 4=0; less than half of the adhesive remnant in the tooth (1): Group 1=3, Group 2=6, Group 3=3, Group 4=12; more than half of the adhesive remnant in the tooth (2): Group 1=15, Group 2=12, Group 3=15, Group 4=18; total presence of adhesive in the tooth (3): Group 1=9, Group 2=3, Group 3=12, Group 4=0. Results showed that the SBS of the groups washed with ozone (Group 2 and 4) were slightly less than the brackets washed with tap water (Group 1 and 3) but with no significant differences related to the shear bond strength in both groups. Ozone did alter the sites of the resin fracture when Fuji Ortho LC was used. The adhesive remnant index (ARI) in Groups 2 and 3 was significantly different from Groups 3 and 4. Ozone did not reduce the shear strength in orthodontic bracket debonding.

Ozone action on the bone integration of dental implants. The search method used allowed us to find one *in vivo* study regarding the effects of ozonated oil on the bone integration of implants:

El Hadary *et al.* (2011) (Table III) evaluated ozonated oil (sunflower oil, 0.55 ml) under the influence of cyclosporine A in the bone integration of dental implants. In total, 20 adult New Zealand rabbits aged 9-12 months and weighing between 3 and 3.5 kg, were administered a subcutaneous daily dose of 10 mg of cyclosporine per kg for a period of fourteen days.

Two groups were formed: Group A: ozonated and Group B: non-ozonated (Control group). After eight weeks, in the ozonated group (A) the microscopic exam showed organization of the mature bone with intimate contact between the implant surface and new bone with numerous haversian systems. In group A, bone density values (\pm SD) was initially 126.7 \pm 3.3; and after eight weeks bone density was 134.1 \pm 5.0; for Group B (non-ozonated) bone density was initially 117.7 \pm 8.4; after eight weeks bone density was 124.0

Table II. Ozone therapy in orthodontics.

| Author (date) | Purpose | Methodology | Results | Conclusion | Study limitations |
|---------------------------------|---|--|---|---|---|
| Kronenberg <i>et al.</i> (2009) | Evaluating whether there is no difference in the anticaries protection of ozone and cervitec/fluorine around the brackets, in addition to assess whether Diagnodent and the quantity of I induced fluorescent light are not superior to the visual evaluation of initial carious lesions. | 20 right-handed patients with permanent dentition and deficient oral hygiene (plaque index >40%) who needed an orthodontic appliance were analyzed for 26 months. All quadrants were treated with ozone (2'100 ± 10% for 30 seconds ppm) and the other group with Cervitec/fluorine (250 ppm). | Visible plaque index (VPI) in all quadrants treated with ozone, 3.2% developed new areas of white points while in the group treated with Cervitec/fluorine protector was 0.7% of the areas. | The anticaries protective effect of Cervitec/Fluorine during the placement of the orthodontic appliance was superior to ozone. | Lack of comparison with other concentrations and times of application |
| Cebrelli <i>et al.</i> (2010) | To determine the effect of pretreatment prophylactic ozone applying shear tension to the bonding of orthodontic brackets, as well as evaluating linkage flaws in the interface using a modified adhesive remnant index (ARI). | 52 bicuspid randomized teeth were used and divided into 4 groups (13 each). Each tooth was washed, and then polished with pumice and rubber cups for 10 seconds. For light curing, a halogen lamp was used for 20 seconds on the mesial and distal area of each tooth. | Shear strength results were: Group 1: 10.48 MPa; Group 2: 8.89 MPa; Group 3: 9.41MPa; Group 4: 9.82 MPa. P=0.05 The results of the modified adhesive remnant index IAR (0-3) were: (0) Group 1 = 3, group 2 = 9, group 3 = 0; Group 4 = 0 (1) Group 1 = 3, group 2 = 6, group 3 = 3, group 4 = 12 (2) Group 1 = 15, group 2 = 12, group 3 = 15, group 4 = 18 (3) Group 1 = 9, group 2 = 3, group 3 = 12, group 4 = 0. | Groups that were pretreated with ozone showed greater shear strength. The lowest strength was obtained in group 2. Pretreatment with ozone on the enamel did not affect the shear strength. | The ozone concentration used was not mentioned. |
| Pithon & dos Santos (2010) | To determine if aqueous ozone reduces resistance to the fixation of orthodontic adhesives. | 120 bovine mandibular incisor teeth were randomly divided into 4 groups. Groups 1 and 3 were cleaned with pumice and washed with water; groups 2 and 4 were washed with ozonated water (0.6mg/L). | Results showed mean shear strength (Mpa) for group 1 of 22.07 Mpa; group 2: 20.21 Mpa; group 3: approximately 20.06 Mpa and group 4 over 18.73 Mpa. | There were no significant differences in the shear strength of brackets debonding from the enamel and washed with ozone or tap water. Adhesive remnant index (ARI) in groups 2 and 3 were significant different from groups 3 and 4. | A single ozone concentration was used. Higher or lower concentrations may generate different results. |
| Dhingra & Vandana (2011) | To evaluate the clinical effects of a single subgingival irrigation with ozonated water in patients with gingivitis and fixed orthodontic appliances and also to correlate the clinical effects with the lactate dehydrogenase (LDH) activity in gingival crevicular fluid. | Single-blind clinical study with 15 subjects aging 17 years and using orthodontic appliance and presenting 50% of sites with gingival bleeding and 50% dichotomous plaque score. | After 28 days plaque index decreased from 1.93 ± 0.22 to 0.89 ± 0.24. Gingival bleeding index reduced from 87.1 ± 6.2% to 30.9 ± 4.5%. Pocket depth showed reduction from 3.10 ± 0.12 to 2.92 ± 0.10. The activity of lactate dehydrogenase decreased from 102.7 ± 59.2 to 18.9 ± 5.9 after 28 days. | As single irrigation with ozonated water in a concentration of 0.01 mg l ⁻¹ significantly reduced all clinical parameters after from baseline to 28 days and significantly decreased the activity of lactate dehydrogenase in the gingival crevicular fluid. | Absence of control group. |

Table III. Ozone therapy in dental implants.

| Author (date) | Purpose | Methodology | Results | Conclusion | Study limitations |
|--------------------------------------|--|---|---|---|---|
| El Hadary <i>et al.</i> (2011) | To evaluate the effects of ozonated oil under the influence of Cyclosporine A in bone integration of dental implants. | Twenty white adult male New Zealand rabbits aging 9-12 months and weighing between 3-3.5 kg were tested. Daily subcutaneous dose of 10 mg of Cyclosporine per kg for 14 days was administered. Group A: treated with ozonated oil (sunflower oil) 0.550 ml in gel applied to fill each osteotomy site for the implant. Group B: non-ozonated (Control group). | In groups A and B, the initial bone density was 126.7 ± 3.3 and 117.7 ± 8.4 respectively; it was 134.1 ± 5.0 and 124.0 ± 5.6 after 2 months. The light microscopic examination demonstrated evidence of newly formed mature bone was observed in group B, whereas in control group the specimens demonstrated the formation of primary osteons. | The administration of short-term cyclosporine together with the topical application of ozonated oil may influence bone density. | Ozone concentration was not reported. |
| Hauser-Gerspach <i>et al.</i> (2012) | To investigate the antimicrobial efficacy of gaseous ozone on bacteria adhered to several surfaces of titanium and zirconium, and also to evaluate the adhesion of osteoblast-like MG-63 cells to surfaces that were treated with ozone. | Titanium and zirconium disks (5.0 mm diameter) with 2 types of surfaces were sterilized by gamma irradiation and incubated in a solution of sterile saliva serum for 15 minutes at 35°C, and used in the microbiological experiment. Ozone treatment was carried out with an Ozotop unit, 140 ppm; 2L/minute for 6-24 seconds. | P. Gingivalis was eliminated in 24 seconds with ozone (>99.94% of reduction). <i>S. sanguinis</i> was more resistant and showed greater reduction in zirconium substrates (>90% reduction). | Ozone did not affect the surface structures of the test specimens and did not influence osteoblastic cells proliferation negatively. Polished titanium and zirconium (acid etched and polished) presented lower bacterial colonization. | The study did not include aspects of the differentiation of extracellular matrix maturation, release of growth factors and expression of specific osteoblastic markers. |

± 5.6. The study demonstrated that the application of cyclosporine for short term in combination with the topical application of ozonated oil may significantly influence bone density and bone integration quality around dental implants. No statistically significant differences were found related to bone density in both groups.

DISCUSSION

The present literature review analyzed the effects of ozone on periodontal pathogens, bone integration of dental implants and adhesion of orthodontic brackets.

The literature available related to ozone therapy in dentistry showed relevance in terms of the properties of ozone therapy, namely: therapeutic action through the distribution of oxygen, expression of growth factors and cytokines and reduction of oxidative stress and acting as modifier of the biological response (Seidler *et al.*; Bocci, 2006; Estrela *et al.*, 2006; Valacchi *et al.*, 2002).

Only one in vivo study (Martínez Abreu & Abreu Sardiñas) regarding the action of ozone against periodontal pathogens was performed for a larger period (six months), which represents a fragility of the studies already developed. Additionally, the results of this article also showed reduction of 98% of gram-negative microorganisms in the group treated with ozone.

There was remarkable inactivation of the major periodontal pathogens, in both the analyzed in vivo and in vitro studies. The results obtained by de Ramon et al., confirm the effective action of ozone on gingival bleeding, with a decrease of over 50% of the main periodontal pathogens: *Aggregatibacter actinomycetencomitans*, *Bacteroides forsythus* and *Porphyromonas gingivalis*. It showed a significant reduction of TNF- α and IL-1 β . Furthermore, there was no variation in the pocket depth, or the level of periodontal insertion, which reinforces the need for mechanical removal of plaque for gingival control and periodontal maintenance (Müller *et al.*, 2007; Lindhe & Nyman, 1975).

Nevertheless, ozone had significantly effectiveness in eliminating gram-negative microorganisms, reducing periodontal pathogens, bleeding on probing (Kshitish & Laxman).

In one study (de Ramon et al.), ozone reduced plaque index over 25%. However in another article (Kshitish & Laxman) results demonstrated that dental plaque was reduced by 12%.

Although therapeutic ozone is applied in several forms, including aqueous, gaseous and oil, there is evidence of better biocompatibility in the cell with the use of aqueous ozone (Huth *et al.*, 2006). Ozone gas had greater antimicrobial activity in terms of the total elimination of pathogens (Aa, Pg, Tf and Pm) in planktonic state than in biofilm.

Regarding the effects of ozone therapy on the adhesion of orthodontic brackets, the results of the study of Cehreli et al., demonstrated that groups pretreated with ozone presented greater shear

strength. Although Pithon and dos Santos found that the shear bond strength of the brackets washed with ozone were slightly less than the brackets washed with tap water after debonding but with no significant differences.

According to the search strategy used, the only study found about ozone activity on bone integration of dental implants (El Hadary *et al.*) demonstrated an increase in bone density around dental implants in the ozonated group, but no significant differences regarding the non-ozonated group were noticed. Nevertheless, the study was carried out only for two months, under the influence of an immunosuppressive drug and it also did not report the concentration and time of application of ozone, which renders the research irreproducible.

Ozone's biological and stimulatory effects could be useful in periodontal therapeutics, in orthodontics and dental implants.

In conclusion, the studies analyzed showed several biological properties of ozone and could be considered as a promising therapy. Further studies are needed for the analysis of the application of ozone in dentistry, in order to assess its clinical effectiveness in the field of dentistry.

ACKNOWLEDGEMENTS

The authors express their thanks to the Anatomy Department of the University of São Paulo (USP), Bauru, São Paulo, Brazil, for the additional information offered for the development of this review. The authors received no financial support and declare no conflicts of interest related to the authorship of this study.

GERMAN, I. J. S.; RODRIGUES, A. C.; ANDREO, J. C.; POMINI, K. T.; AHMED, F. J.; BUCHAIM, D. V.; ROSA JÚNIOR, G. M.; GONÇALVES, J. B. O. & BUCHAIM, R. L. J. Ozonoterapia en Odontología: una revisión sistemática. *Int. J. Odontostomat.*, 7(2):267-278, 2013.

RESUMEN: El objetivo de esta revisión sistemática fue verificar la literatura disponible relacionada a la efectividad y efectos biológicos de la ozonoterapia en periodoncia, ortodoncia e implantes dentales. Se realizó una revisión sistemática en septiembre del 2012 en las bases de datos PubMed, LILACS and SciELO por medio de la combinación de las palabras clave dental implants, periodontics, orthodontics, therapeutics. Se evaluaron los artículos publicados entre los años 2002 y 2012. Fueron incluidos estudios In vitro e in vivo y publicaciones en Inglés y Español, excluyendo pósters, carta al editor y conferencias. Estudios in vitro e in vivo mostraron inactivación de los principales patógenos periodontais con el uso del ozono. Hubo resultados contradictorios y falta de evidencia sobre la acción del ozono en la adhesión de los brackets ortodónticos, así como también en la osteointegración de los implantes dentales. El ozono puede ser considerado como una terapia alternativa promisoría en la Odontología. Sin embargo, es necesaria la realización de estudios bien diseñados para, de ese modo, evaluar la aplicación del ozono y su efectividad clínica en el campo de la Odontología.

PALABRAS CLAVE: implantes dentales, periodoncia, ortodoncia, terapéutica.

REFERENCES

- Altman, N. *The Oxygen Prescription: the miracle of oxidative therapies*. 3rd ed. Vermont, Healing Arts Press, 2007.
- Azarpazhooh, A. & Limeback, H. The application of Ozone in Dentistry: a systematic review of literature. *J. Dent.*, 36(2):104-16, 2008.
- Bocci, V. A. *Oxygen-Ozone Therapy: a critical review*. Netherlands, Kluwer Academic Publishers, 2002.
- Bocci, V. A. *Ozone: a new medical drug*. New York, Springer, 2005.
- Bocci, V. A. Scientific and medical aspects of ozone therapy: State of art. *Arch. Med. Res.*, 37(4):425-35, 2006.
- Cehreli, S. B.; Guzey, A.; Arhun, N.; Cetinsahin, A. & Unver, B. The effects of prophylactic ozone pretreatment of enamel on shear Bond strength of orthodontic brackets bonded with total or self-etch adhesive systems. *Eur. J. Dent.*, 4(4):367-73, 2010.
- de Ramon, R. J.; Colmenero Ruiz, C.; Gallut Ruiz, J.; Zaera Le Gal, R. & Bascones Martinez, A. Evaluación clínica, microbiológica e inmunológica de la ozonoterapia en pacientes con bolsas periodontales moderadas-severas. *Av. Periodon. Implantol.*, 16(1):63-72, 2004.
- Dhingra, K. & Vandana, K. L. Management of gingival inflammation in orthodontic patients with ozonated water irrigation: a pilot study. *Int. J. Dent. Hyg.*, 9(4):296-302, 2011.
- Ebensberger, U.; Pohl, Y. & Filippi, A. PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rising for decontamination. *Dent. Traumatol.*, 18(5):262-6, 2002.
- El Hadary, A. A.; Yassin, H. H.; Mekhemer, S. T.; Holmes, J. C. & Grootveld, M. Evaluation of the effect of ozonated plant oils on the quality of osseointegration of dental implants under the influence of Cyclosporin A. An *in vivo* study. *J. Oral Implantol.*, 37(2):247-57, 2011.
- Estrela, C.; Estrela, C. R.; Decurcio Dde, A.; Silva, J. A. & Bammann, L. L. Antimicrobial potential of ozone in an ultrasonic cleaning system against *Staphylococcus aureus*. *Braz. Dent. J.*, 17(2):134-8, 2006.
- Hauser-Gerspach, I.; Vadaszan, J.; Deronjic, I.; Gass, C.; Meyer, J.; Dard, M.; *et al.* Influence of gaseous ozone in peri-implantitis: bactericidal efficacy and cellular response. An *in vitro* study using titanium and zirconia. *Clin. Oral Investig.*, 16(4):1049-59, 2012.
- Huth, K. C.; Jakob, F. M.; Saugel, B.; Cappello, C.; Paschos, E.; Hollweck, R.; *et al.* Effect of ozone on oral cells compared with established antimicrobials. *Eur. J. Oral Sci.*, 114(5):435-40, 2006.
- Huth, K. C.; Saugel, B.; Jakob, F. M.; Cappello, C.; Quirling, M.; Paschos, E.; *et al.* Effect of aqueous ozone on the NF-kB system. *J. Dent. Res.*, 86(5):451-6, 2007.
- Huth, K. C.; Quirling, M.; Lenzke, S.; Paschos, E.; Kamereck, K.; Brand, K.; *et al.* Effectiveness of ozone against periodontal pathogenic microorganisms. *J. Oral Sci.*, 119(3):204-10, 2011.
- Kshitish, D. & Laxman, V. K. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. *Indian J. Dent. Res.*, 21(3):341-8, 2010.
- Kronenberg, O.; Lussi, A. & Ruf, S. Preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. *Angle Orthod.*, 79(1):64-9, 2009.
- Lindhe, J. & Nyman, S. The effect of plaque control and surgical pocket elimination on the establishment and maintenance of periodontal health. A longitudinal study of periodontal therapy in cases of advanced disease. *J. Clin. Periodontol.*, 2(2):67-79, 1975.
- Martínez Abreu, J. & Abreu Sardiñas, M. Oleozón en el tratamiento de la periodontitis simple moderada. *Rev. Med. Electrón.*, 27(3), 2005. Available in: <http://www.revmatanzas.sld.cu/revista%20medica/ano%202005/vol3%202005/tema03.htm>
- Müller, P.; Guggenheim, B. & Schmidlin, P. R. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm *in vitro*. *Eur. J. Oral Sci.*, 115(1):77-80, 2007.
- Pithon, M. M. & dos Santos, R. L. Does ozone water affect the bond strengths of orthodontic brackets. *Aust. Orthod. J.*, 26(1):73-7, 2010.
- Ripamonti, C. I.; Cislighi, E.; Mariani, L. & Maniezzo, M. Efficacy and safety of medical ozone (O₃) delivered in oil suspension applications for the treatment of osteonecrosis of the jaw in patients with bone metastases treated with biphosphonates: Preliminary results of a phase I-II study. *Oral Oncol.*, 47(3):185-90, 2011.
- Seidler, V.; Linetskiy, I.; Hubáľková, H.; Stanková, H.; Smucler, R. & Mazánek, J. Ozone and its usage in general medicine and dentistry. *Prague Med. Rep.*, 109(1):5-13, 2008.
- Skurska, A.; Pietruska, M. D.; Paniczko-Drezek, A.; Dolinska, E.; Zelazowska-Rutkowska, B.; Zak, J.; *et al.* Evaluation of the influence of ozonotherapy on the clinical

parameters and MMP levels in patients with chronic and aggressive periodontitis. *Adv. Med. Sci.*, 55(2):297-307, 2010.

Swilling, J. *Beyond bypass and chelation for heart problems and cardiovascular disease*. California, Lightning Source Inc., 2004.

Thanomsub, B.; Anupunpisit, V.; Chanphetch, S.; Watcharachaipong, T.; Poonkhum, R. & Srisukonth, C. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. *J. Gen. Appl. Microbiol.*, 48(4):193-9, 2002.

Valacchi, G.; Van Der Vliet, A.; Schock, B. C.; Okamoto, T.; Obermuller-Jevic, U.; Cross, C. E.; et al. Ozone exposure activates oxidative stress responses in murine skin. *Toxicology*, 179(1-2):163-70, 2002.

Veranes, X. G.; Nápoles, Y. L.; Hechavarría, I. C.; Luque, R. F. & Pellicer, A. M. R. Resultados de los costos en ozonoterapia. *Rev. Cubana Enferm.*, 15(2):104-8, 1999.

Correspondence to:

Iris Jasmin Santos German
Department of Biological Sciences (Anatomy)
Bauru School of Dentistry
Al. Dr. Octávio Pinheiro Brisola, 9-75
Postal code: 17012-901
University of São Paulo
FOB-USP
Bauru - BRAZIL

Phone: +55 14 3235-8226

Fax: +55 14 3235-8223

Email: irisgerman@usp.br

Received: 27-04-2013

Accepted: 15-06-2013