Ozone therapy in dentistry: from traditional applications towards innovative ones. A review of the literature

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Ozone therapy in dentistry: from traditional applications towards innovative ones. A review of the literature

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Abstract. Ozone (0_3) is a natural gas deriving from dioxygen (0_2) and acting as a strong oxidant. Despite this characteristic, low doses of ozone can be beneficial for the organism due to the antioxidant response implemented by this latter. Accordingly, since the 19th century, several therapeutic applications have been proposed in medicine, but even dental pathologies can benefit from the use of this substance. In particular, the introduction of ozone therapy in dentistry dates to 1930 when it was proposed as a disinfectant and wound-healing agent. Nowadays, it is known as an antioxidant, anti-inflammatory, immunomodulatory, anti-hypoxic, biosynthetic and antimicrobial agent. The main forms of ozone administration are three (gaseous ozone, ozonated water and ozonated oils) but its therapeutic indications almost cover every field of dentistry. The aim of the present review is first to describe the main traditional uses of ozone in dentistry, and, subsequently, to present the innovative applications proposed both in dental and orthopaedic implantology.

1. Ozone: history, properties and mechanisms of action

Ozone (0_3) is a natural compound which represents the triatomic form of dioxygen (0_2). It is naturally present in form of gas in the stratosphere to protect living organisms from UV radiation and it derives from dioxygen by the action of UV light and electrical discharges; however, ozone is highly instable (half-life of 40 min at 20°C) and rapidly breaks down to its diatomic allotrope which justifies the very low amounts of ozone in the atmosphere compered to dioxygen [1]. Ozone is recognised as a strong oxidant and thus it has been proposed as a disinfectant, deodorizer, cleaning and bleaching agent, food additive and air/water purifier [2-9].

The first identification of ozone dates to the 18th century when Martin van Marum, a Dutch physicist, described the rise of a gas with a typical odour and strong oxidizing properties, following an electric spark through the air [1]. However, the chemical reaction leading to the formation of ozone from the dioxygen was described in 1840 by the German chemist Christian Friedrich Schönbein who attributed to this gas the name "ozone" (from the Greek word *ozein*, "odorant") and assessed its interaction with organic compounds. After that, several generators of 0_3-0_2 mixtures were built and the biological action of ozone started to be explored. During the 19th century, Nikola Tesla patented an 0_3 generator for medical use and several diseases (like allergy, anaemia, diabetes, gangrene, fever, tuberculosis, syphilis and tetanus) were treated. From the late 1970s, ozone therapy spread worldwide and specific protocols and administration techniques were developed, like gaseous 0_3 inhalation, injection or bags, auto-hemotherapy and ozonated water or oil [1]. Additionally, the production of medical ozone was regulated, with the definition of concentration, dosages and administration frequency according to the specific disease, besides the introduction of appropriate disposable materials [1].

The therapeutic use of ozone could appear paradoxical considering the strong oxidizing action exerted by this substance. However, this can be explained considering the principle of "hormesis" which consists of "the beneficial effect of a low level exposure to an agent that is harmful at high levels" [10]. Accordingly, a mild

oxidative stress exerted by ozone at low levels has been shown to trigger a metabolic cascade (the so called "eustress") with favourable effects on the organism, encompassing the activation of antioxidant pathways [11]. In fact, considering an exposure to toxic levels of atmospheric ozone, an injury and inflammatory response take place through the activation of the redox sensitive nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which causes the transcription of pro-inflammatory cytokines [12]; conversely, an exposure to low concentrations of ozone is able to exert antioxidant and anti-inflammatory effects by means of the expression of proteins involved in the antioxidant response, among which the Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2). In fact, this component can activate more than two hundred cytoprotective antioxidant genes (Antioxidant Responsive Element, ARE) to contrast toxicity. For instance, a systemic treatment with ozone in healthy volunteers raised the levels of Nrf2 in peripheral blood mononuclear cells, thus enhancing the activity of the antioxidant enzymes superoxide dismutase and catalase [13]. This same pathway, with up-regulation of antioxidant enzymes and down-regulation of cytokines, was demonstrated in rats' kidneys with adenine-induced chronic kidney disease: the systemic administration of ozone was responsible for the reduction of the renal insufficiency and tubointerstitial injury [14]. Other evidences have shown that ozone therapy has increased Nrf2 in patients with multiple sclerosis [15] but also in murine models of streptozotocin-induced pancreatic damage, besides protecting the lung and myocardium of rats from ischemia-reperfusion injury [16, 17]. Further cell functional pathways dependent on Nrf2, with an activation exerted by ozone, are related to diabetes, gastrointestinal diseases, autoimmune diseases, infective diseases, and probably aging and cancer [1]. On the basis of this consideration, several studies have been conducted on ozone, proposed as an adjuvant or alternative therapeutic modality for different conditions, including pain management, gastrointestinal diseases, lung diseases, diabetes, ischemia, cancer and infective diseases [1].

Besides being an antioxidant and anti-inflammatory agent, at therapeutic doses ozone has demonstrated immunomodulatory, anti-hypoxic, biosynthetic and also antimicrobial effects on bacteria, virus, protozoa and fungi; for instance, in vitro exposition of bacteria to ozone causes the oxidation of phospholipids and lipoproteins of the bacterial cell envelope, disrupting the cytosolic membrane and allowing ozone itself to infiltrate the microorganisms and oxidize glycoproteins and glycolipids, thus blocking the enzymatic function [18]. In fungi, ozone is able to inhibit cell growth at certain stages, whereas in viruses it damages the viral capsid and upsets the reproductive cycle by disrupting the virus-to-cell contact with peroxidation [19]. Recently, ozone has gained attention due to its possible effects against SARS-CoV-2 virus, the respiratory virus which emerged in China in December 2019 and rapidly spread worldwide causing a pandemic; besides being used as a disinfectant agent, ozone might exert a cytoprotective role to contrast the organ damage induced by the virus [20].

In addition to that, in cell culture assays, ozone not only reported no cytotoxic effects on fibroblasts or keratinocytes but conversely induced fibroblasts migration, which suggests a positive role on the wound-healing process [21]. In fact, the different effect exerted towards microorganisms and human cells can be understood considering the lack of antioxidant defences by the former. Indeed, the transient oxidative stress caused by ozone is lethal for microorganisms but not for human cells [18].

The use of ozone has interested even dentistry since when Dr. E.A. Fisch firstly reported in 1930 the application of ozonized water as disinfectant and wound-healing agent after dental surgery. Today, the main forms of application to administer ozone are gaseous ozone (through an ozone generator converting oxygen into ozone), ozonated water (to be used as a mouthwash) and ozonated oils (deriving from a chemical reaction between ozone and pure plants extracts to form an oil or a jelly-like product) [22,23]. The aim of the present review is to briefly describe the main traditional applications of ozone in dentistry, and, subsequently, to emphasise the latest evidence concerning the innovative uses proposed in this field.

2. Applications in dentistry

Nowadays, almost every field of dentistry encompasses ozone therapy as a possible treatment modality.

2.1. Oral medicine

Ozone has been shown to stimulate the wound healing process, by increasing the production of the tissue growth factor β1 (TGF-β1) [24]. Considering planimetric, immunohistochemical and micromorphological examinations of the wounds of the oral mucosa, patients applying ozonized water on wounds have shown an acceleration of the healing process with an earlier closing with respect to control patients; therefore, several oral mucosal lesions, including herpes, aphthous ulcers, candidiasis, lichen planus and denture stomatitis, can benefit from ozone therapy [22].

2.2. Restorative dentistry

Tooth decay represents one of the most common pathological conditions among humans. The two main human odontopathogens are represented by *Streptococcus mutans* (involved in decays of deep fissures of the tooth) and *Streptococcus sobrinus* (with a role in smooth-surfaces decays) [25]. Ozone can prevent dental decay not only through its antimicrobial properties, but also by oxidizing the pyruvic acid produced by cariogenic bacteria into acetate and dioxide [26]. Several trials have demonstrated that odontopathogens are significantly reduced when exposed to ozone from an ozone-generator. Holmes [27] stated that an application of ozone for 40 seconds, followed by the use of remineralising products, can reverse and arrest non-cavitated primary root caries, although according to Baysan and Lynch [28] this effect is decreased in case of cavitated lesions. Ozone especially appears as a valuable method for the treatment of caries in patients with dental anxiety, considering the proven reduction of this parameter with respect to the treatment with traditional rotatory instruments [29].

2.3. Endodontics

Ozone in form of gas, water or oil, has shown a significant potential as antimicrobial agent in endodontics considering that its action has been demonstrated against several bacterial strains, like *Micobacteria*, *Streptococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Peptostreptococcus*, *Enterococcus faecalis* and *Candida albicans* [30]. Ozonated water can be applied as an intracanal irrigant and in infected necrotic canals: its disinfectant action, when used as an irrigant by means of sonication, has been shown to be comparable to the one reported by 2.5% NaOCl [31]. Ozonized oils can be used as an intra-canal dressing to reduce the odour emanating from infected tooth, this because of the toxic effect on anaerobic bacteria [19]. In general, the effect of ozone has been shown to be more significant when used in canals having the least amount of organic debris [22].

2.4. Oral hygiene and periodontics

One of the most frequent events taking place in the oral cavity, especially in case of limited oral hygiene, is the formation of dental plaque, generally on the tooth surfaces and the gingival sulcus, with an eventual progression to gingivitis and periodontitis, respectively representing a reversible and irreversible inflammatory condition of the gums. Dental plaque consists of a biofilm which is to say a complex of bacteria into a polymeric matrix with channels to provide nutrients and water. Several studies in literature have proved the efficacy of ozonized water to decrease viable bacteria, inhibit the accumulation of dental plaque and contrast gingivitis/periodontitis [22]. This antimicrobial action is exerted especially against Gram-negative bacteria (including *Porphyromonas gingivalis,* one of the periodontopathogens most related to periodontitis), but also Gram-positive bacteria and *Candida albicans* [32]. Ozone has been successfully used as a pretreatment rinse to irrigate periodontal pockets before performing Scaling and Root Planing [22]. As well, other forms of periodontitis, less related to plaque accumulation, can benefit from the use of ozonized water or oil, with an improvement of periodontal clinical indexes and bacterial count [33].

2.5. Prosthodontics

The surfaces of the oral cavity are not the only ones which can be interested by plaque accumulation. In fact, this event might occur even on dentures which are frequently inhabited by different micro-organisms, especially *Candida albicans*, with an increased risk of denture stomatitis. According to its antimicrobial activity, ozone has been proposed as a denture cleaner, with an effective action against Candida albicans and other microbes adhering on denture surfaces, like methicillin-resistant Staphylococcus aureus and viruses [34,35]. Even partial denture alloys can be cleaned by means of ozone, with no compromising of the physical properties [36].

In the field of dental fixed prosthodontic, titanium implants have represented a breakthrough as substitutes of loss dental roots. However, implants-related infections still represent the major concern for dental and orthopaedic surgery [37]. Karapetian et al., [38] have compared the results obtained by conventional, surgical and ozone therapy to treat periimplantitis. Despite the main challenge still remains the decontamination of the implant surface and of the surrounding tissues, as well as the bacterial recontamination, the Authors reported the highest effectiveness for ozone therapy.

2.6. Oral surgery

Ozone therapy has several applications in oral surgery. It is recognised as an important tool in the management of post-surgical pain, along with laser therapy [39]. Additionally, ozone promotes wound

healing, improves the properties of erythrocytes and favours the release of the oxygen in the tissues, thus representing a method to supply ischemic zones with blood and to treat patients with wound healing impairments following surgery [19].
A more and more frequent condition which oral surgeons must deal with is represented by osteonecrosis of the jaw, a side effect occurring after a surgical trauma (e.g. tooth extraction), head/neck radiotherapy or treatment with bisphosphonates; it is due to a loss of blood supply to the bone, with a break down of this latter and a possible exposition of the alveolar bone [22]. Medical ozone gas insufflation was proved to be effective in the treatment of post-surgery osteonecrosis of the jaw, especially in case of lesions greater than 2.5 cm [40].

2.7. Gnathology
The temporomandibular joint, connecting the jaw with the temporal bone of the skull, is frequently interested by processes known as temporomandibular disorders, encompassing pain and/or dysfunction of masticatory muscles and the joint. Even in this case, the use of ozone has been documented as successful. Daif [41] stated that intra-articular gas injection into the superior joint space succeeded in treating internal derangement of the temporomandibular joint. As well, the same result was obtained by Hammuda et al. [42] using ozonated water to perform temporomandibular joint arthrocentesis. Additionally, Doğan et al., [43] have stated that ozone therapy is more effective for the treatment of temporomandibular-joint related pain with respect to medications.

2.8. Orthodontics
The risk of enamel demineralization around orthodontic brackets remains one of the main side effects related to the application of fixed appliances, and several enamel pre-treatments have been proposed to contrast this event, including ozone application. Ghobashy et al. [44] assessed that the application of ozonized olive oil on enamel significantly reduced demineralization around orthodontic brackets. Anyway, according to a previous study by Kronenberg et al., [45] the protective effect was significantly higher for Cervitec/Fluor Protector. The study of Cehreli et al., [46] even concluded that prophylactic ozone pre-treatment didn't affect the shear bond strength of neither total nor self-etch adhesive systems.

3. Recent applications of ozone for dental and orthopaedic biomaterials
The beneficial effects reported by ozone could be even extended to both dental and orthopaedic implantology; in these fields, new treatments are being developed with the aim of preventing/resisting implant-associated infections, as well as to promote osseointegration [18, 47, 48]. Focussing on this latter, the potentiality of ozone is still poorly explored and few studies have been conducted.
An interesting experimentation by Sunarso et al., [49] was aimed to develop and investigate the properties of a superhydrophilic titanium implant, functionalized by ozone gas (O_3-Ti) for 24h, to modulate osteoconductivity and inhibit inflammatory responses towards titanium, as well as to compare the functionalized titanium with untreated titanium (unTi). After 3h of ozone treatment, water contact angle of titanium was much lower compared with unTi and this trend was proportional with ozone treatment time, reaching a water contact angle of zero degree after 24h. Accordingly, a superhydrophilic surface was obtained thanks to an increase of hydrophilic OH groups and a reduction of hydrophobic carbon contaminants, without alteration of the original topography and roughness which were similar to that of unTi. As regards the modulation of osteoconductivity, bone marrow cells were cultured on specimens of the two both surfaces. After 5h of incubation, cells grown on functionalized titanium appeared larger and cytomorphometric parameters (aspect ratio, size and cell perimeter attached to titanium) were significantly higher than those on unTi. Considering cells attachment and proliferation, attached cells number on O_3-Ti at 5h of incubation was comparable with that on unTi, whereas cell proliferation was significantly higher after 4 days on the former surface compared with unTi. Additionally, this study assessed the effect of ozone on cell differentiation and mineralization by considering the activity of the ALP (alkaline phosphatase) enzyme and the bone-like nodule formation on the substrates: at day 7 of differentiation, ALP activity was significantly higher on O_3-Ti with respect to unTi. At day 14, cell differentiated on O_3-Ti exhibited larger bone nodules compared with those grown on unTi. At day 21, O_3-Ti has generated a further higher bone nodule area, but without a statistical significance to unTi in this case. Finally, the Authors of the study evaluated the effect of mouse macrophages response on the two surfaces considered, in order to evaluate inflammatory responses towards titanium. For both ozone-functionalized titanium and untreated titanium, proliferation of RAW264.7 macrophages was comparable on day 1 and 3. However, production of

proinflammatory cytokines inhibiting osteoblasts and facilitating osteoclasts (TNFα and IL-6) was lower on O_3-Ti for both naive RAW264.7 cells and lipopolysaccharide-stimulated RAW264.7 cells.
Accordingly, this study showed that titanium treated by ozone gas resulted in superhydrophilic properties, promoted favorauble cellular characteristics, facilitated mesenchymal stem cells differentiation and the subsequent bone-like nodule formation, and finally reduced the production of proinflammatory cytokines by macrophages. These results are in accordance with those obtained by Harmankaya et al., [50].
The abovementioned study of Sunarso et al., [49] was aimed to develop an ozone gas-treated titanium surface which resulted to be superhydrophilic and reported high osteoconductivity and anti-inflammatory properties. Besides the functionalization of new titanium surfaces by means of ozone, other forms of ozone application have been tested. For instance, El Hadary et al., [51] evaluated the effect of ozonated plant oils on the osseointegration of dental implants placed in rabbit tibiae under the influence of the immunosuppressive agent Cyclosporin A (with daily subcutaneous injection of 10 mg CsA/kg body weight for 14 days before the surgery). Animals were randomly assigned to two different groups, with Group A receiving an application of ozonated sunflower oil applied directly into each osteotomy site (excessive oil was allowed to flood over surrounding bone and soft tissues), whereas in Group B implants were inserted without previously performing the abovementioned experimental protocol. The authors performed radiographic evaluations at the day of the surgical intervention (T_0) and after 8 weeks (T_1, when animals where sacrificed). At this latter time, the researchers also performed examinations with scanning electron microscope (SEM) and light microscope. As to the radiographic bone density, no significant intergroup and intragroup differences for bone density values were assessed, as well as no significant percentual changes between T_0 and T_1. The SEM examination revealed that close bone implant contact was achieved in both studies groups at T_1, but in case of ozone application implants had almost engaged the bone, whereas in the control group a wide space was still observed. Finally, at light microscopic examination, new bone complementary to the threads was assessed next to the implant in both groups with a tendency to migraine to the space formed between the implants and the bone surfaces. However, in control groups, the formation of the primary osteons, a characteristic of the immature tissue, was observed; on the contrary, in those cases where ozonized oil had been applied before implant insertion, the newly formed bone showed numerous Haversian systems which represent a feature of mature bone formation.
The improved osseointegration described in the previous studies has been recently confirmed by Yücesoy et al., [52] who aimed to evaluate how continuous heavy orthopaedic forces could affect the stability of different miniscrew implants (sandblasted, large-frit and acid-etched) and surrounding bone tissue healing, at three different loading periods under treatment with photomodulation and ozone therapy. Miniscrew implants were inserted on tibias of rabbits, randomly divided into three groups (control, photobiomodulation, and ozone therapy). In the three groups, implants were loaded with 500 gf at three different times (0, 4 and 8 weeks, respectively) and implant stability quotient level, bone volume and bone-to-implant contact were then measured. By means of the Infinite Focus Microscopy, the ozone therapy group revealed significantly higher bone volume measurements (in mm^3) at the 4-week loading time, whereas at the 8-week loading time both the photobiomodulation and the ozone therapy group revealed significantly higher values than controls. Considering histologic analysis, the two both experimental groups revealed significantly higher scores at the 4-week loading time with respect to controls, whereas after 8 weeks it was only the photomodulation group to reveal the highest scores. Accordingly, both photomodulation and ozone therapy appeared to be effective in promoting osseointegration in miniscrew implants.

4. Conclusions

Ozone therapy is showing more and more its potentiality in every field of dentistry. Ascertained the biocompatibility, its main effectiveness can be reconducted to the antimicrobial action against several microorganisms. The potentiality of ozone has also recently expanded to both dental and orthopaedic implantology thanks to the potentiality of ozone to promote osseointegration. Future studies are required to determine precise indications and guidelines in order to fully take advantage of ozone therapy.

5. References

[1] Galiè M, Covi V, Tabaracci G and Malatesta M 2019 *Int J Mol Sci* **20**
[2] Leduc C, Daneault C, Pelletier É, Cloutier JN, Epiney M and Lanouette R 2018 *Appita J* **71** 242–51
[3] Alresheedi MT, Basu OD and Barbeau B 2019 *Chemosphere* **226** 668–77

[4] Englezos V, Rantsiou K, Cravero F, Torchio F, Giacosa S, Río Segade S, Gai G, Dogliani E, Gerbi V, Cocolin L et al. **2019** *J Clean Prod* **233** 582–9
[5] Jin X, Wang W, Wang S, Jin P, Wang XC, Zhang W, An W and Wang Y 2019 *J Environ Sci-China* **81** 17–27
[6] Zanacic E, Stavrinides J and McMartin DW 2016 *Water Res* **104** 397–407
[7] Martinelli M, Giovannangeli F, Rotunno S, Trombetta CM and Montomoli E 2017 *J Prev Med Hyg* **58** E48–52
[8] Brodowska AJ, Nowak A and ´Smigielski K 2018 *Crit Rev Food Sci Nutr* **58** 2176–2201
[9] Cisterna B, Boschi F, Croce AC, Podda R, Zanzoni S, Degl'Innocenti D, Bernardi P, Costanzo M, Marzola P, Covi V et al. 2018 *Microsc Microanal* **24** 564–73
[10] Goldman M. 1996 *Science* **271** 1821–2
[11] Bocci VA, Zanardi I and Travagli V 2011 *J Transl Med* **9** 66
[12] Rubio V, Valverde M, Rojas E. 2010 *Sci Pollut Res Int* **17** 369–82
[13] Re L, Martínez-Sánchez G, Bordicchia M, Malcangi G, Pocognoli A, Morales Segura MA, Rothchild J and Rojas A 2014 *Eur J Pharm* **742** 158–62
[14] Yu G, Liu X, Chen Z, Chen H, Wang L, Wang Z, Qiu T and Weng X 2016 *Iran J Basic Med Sci* **19** 1136–43
[15] Siniscalco D, Trotta MC, Brigida AL, Maisto R, Luongo M, Ferraraccio F, D'Amico M and Di Filippo C 2018 *Biology* **7** 10
[16] Meng W, Xu Y, Li D, Zhu E, Deng L, Liu Z, Zhang G and Liu H 2017 *Biomed Pharm* **88** 1090–7
[17] Wang Z, Zhang A, Meng W, Wang T, Li D, Liu Z and Liu H. 2018 *Eur J Pharm* **835** 82–93
[18] Monzillo V, Lallitto F, Russo A, Poggio C, Scribante A, Arciola CR, Bertuccio FR and Colombo M 2020 *Materials* **13** 1731
[19] Tiwari S, Avinash A, Katiyar S, Iyer AA and Jain S 2017 *Saudi J Dent Res* **8** 105–11
[20] Martínez-Sánchez G, Schwartz A and Donna VD 2020 *Antioxidants* **9** E389
[21] Borges GÁ, Elias ST, da Silva S, de Oliveira Magalhães P, Macedo SB, Ribeiro APD and Guerra ENS 2017 *J Cranio-Maxillo Surg* **45** 364–70
[22] Suh Y, Shrey P, Re K, Gandhi J, Joshi G 2019 *Med Gas Res* **9** 163–7
[23] Pattanaik B, Jetwa D, Pattanaik S, Manglekar S, Naitam DN and Dani A 2011 *Dentistry* **1** 87-92
[24] Bocci V, Luzzi E, Corradeschi F and Silvestri S 1994 *J Biol Regul Homeost Agents* **8** 108-12
[25] Loesche WJ 1986 *Microbiol Rev* **50** 353-80
[26] Kumar A, Bhagwati S, Tyagi P and Kumar P 2014 *Eur J Gen Dent* **3** 175–80
[27] Holmes J 2003 *Gerodontology* **20** 106-14
[28] Baysan A and Lynch E 2007 *Am J Dent* **20** 203-8
[29] Dahnhardt JE, Jäggi T and Lussi A 2006 *Am J Dent* **19** 267
[30] Pattanaik B, Jetwa D, Pattanaik S, Manglekar S, Naitam DN and Dani A 2011 *J Interdiscip Dent* **1** 87-92
[31] Reddy SA, Reddy N, Dinapadu S, Reddy M and Pasari S 2013 *J Int Oral Health* **5** 102–8
[32] Nagayoshi M, Fukuizumi T, Kitamura C 2004 *Oral Microbiol Immunol* **19** 240
[33] Ramzy MI, Gomaa HE and Mostafa MI 2005 *Egypt Med JNRC* **6** 229–45
[34] Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, Kakinoki Y, Nishihara T. 2005 *Oral Microbiol Immunol* **20** 206
[35] Murakami H, Mizuguchi M, Hattori M, Ito Y, Kawai T and Hasegawa J 2002 *Dent Mater* **21** 53–60
[36] Suzuki T, Oizumi M, Furuya J, Okamoto Y and Rosenstiel SF 1999 *Int J Prosthodont* **12** 179–83
[37] Arciola CR, Montanaro L, Moroni A, Giordano M, Pizzoferrato A and Donati ME 1999 *Biomaterials* **20** 323–7
[38] Karapetian VE, Lowden E and Zoller JE 2007 *Das Deutsche Zahna rzteblat*t **116** 214–8
[39] Kazancioglu HO, Ezirganli S and Demirtas N 2014 *Lasers Med Sci* **29** 1313-9
[40] Ripamonti CI, Maniezzo M, Pessi MA and Boldini S 2012 *A case report Tumori* **98** 72e-75e
[41] Daif ET 2012 *Oral Surg Oral Med Oral Pathol Oral Radiol* **113** e10-4
[42] Hammuda A, Hamid MS, Elsharrawy EA, Elsholkamy MA and Iskandar A 2013 *J Am Sci* **9**
[43] Doğan M, Ozdemir Doğan D, Düger C, Ozdemir Kol I, Akpınar A, Mutaf B and Akar T 2014 *Med Princ Pract* **23** 507-10
[44] Ghobashy Safaa Ali and El-Tokhey Heba Mohamed 2012 *J Am Sci* **8** 657–66
[45] Kronenberg O, Lussi A and Ruf S 2009 *Angle Orthod* **79** 64-9
[46] Cehreli SB, Guzey A, Arhun N, Cetinsahin B and Unver B 2010 *Eur J Dent* **4** 367–73

[47] Campoccia D, Montanaro L and Arciola CR 2013 *Biomaterials* **34** 8533-54
[48] Arciola CR, Campoccia D and Montanaro L 2018 *Nat Rev Microbiol* **16** 397-409
[49] Sunarso, Toita R, Tsuru K and Ishikawa K 2016 *J Mater Sci Mater Med* **27** 127
[50] Harmankaya N, Igawa K, Stenlund P, Palmquist A and Tengvall P 2012 *Acta Biomater* **8** 3532–40
[51] El Hadary AA, Yassin HH, Mekhemer ST, Holmes JC and Grootveld M 2011 *Oral Implantol* **37** 247–57
[52] Yücesoy T, Seker ED, Cenkcı E, Yay A and Alkan A 2019 *Int J Oral Maxillofac Implants* **34** 1337–45