Evidence-based efficacy of ozone for root canal irrigation.

Lynch E.

Ask the Experts

EVIDENCE-BASED EFFICACY OF OZONE FOR ROOT CANAL IRRIGATION

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QUESTION: As a follow-up to the recently published information on ozone as a means of caries treatment, can you provide some information on the use of ozone in root canal therapy?

ANSWER: Ozone has been proposed as a dental antiseptic agent based on reports of its antimicrobial effects in both gaseous and aqueous forms. Ozone is effective when it is prescribed in sufficient concentration, used for an adequate time, and delivered correctly into root canals after the traditional cleaning, shaping, and irrigation have been completed. Ozone will not be effective if too little dose of ozone is delivered or it is not delivered appropriately. Ozone should be used after the conventional cleaning, shaping, and irrigation of root canals, and the ozonated liquid in the canal system should be agitated with ultrasound.

PROVEN ANTIMICROBIAL EFFICACY OF OZONE

Ozone is one of the most powerful antimicrobial agents available for use in medicine or dentistry. As failure of root canal therapy is mainly caused by microorganisms, it is not surprising that there are enormous advantages to killing these pathogens. Numerous peerreviewed published research papers
have proven the antimicrobial effectiveness of ozone as a gas and as ozonated water.\textsuperscript{2-20} In model dental unit water lines, ozone achieved a 57\% reduction in biofilm and a 65\% reduction in viable bacteria in spite of being used in a very low dose and with a short time of application.\textsuperscript{21} Ozone rapidly kills otherwise hard to kill microorganisms.

RECOMMENDED USE OF OZONE IN ROOT CANAL THERAPY

Ozone works best when there is less organic debris remaining. Therefore, the recommendation is to use either ozonated water or ozone gas at the end of the cleaning and shaping process. I personally still use my conventional irrigants during this earlier phase and I finally irrigate with ozonated water (TherOzone, Santa Monica, CA, USA) using ultrasonics. I also bubble ozone gas (HealOzone, KaVo, Biberach, Germany) into this ozonated water and use ozonated oil (Lime Technologies Ltd., Capetown, South Africa) as a medicament.

COMPARISON OF THE USE OF OZONE AND SODIUM HYPOCHLORITE IN ROOT CANAL THERAPY

Oxygen has a dramatically toxic effect to microaerophilic and anaerobic bacteria. Virtej and colleagues\textsuperscript{22} compared the antimicrobial performance of four systems used as root canal irrigants. Seventy instrumented and initially sterile roots with open access cavities and containing a paper point were carried by one volunteer in the oral cavity for 1 week. After removal, the samples were taken for microbiologic analysis. The root canals were then disinfected with the Endox Endodontic System (Lysis S.r.l., Nova Milanese [MI], Italy), MTAD (Dentsply Tulsa Dental, Tulsa, OK, USA), 3\% sodium hypochlorite (NaOCl), or HealOzone, and thereafter, the samples were repeated for microbiologic analysis. The roots were then sealed and incubated for a
further week, after which bacterial
growth was again determined.
After disinfection, there was a significant
decrease in the absolute
bacterial count between each disinfection
method and the positive
control group. There was no statistically
significant difference
between the 3% NaOCl, MTAD,
and HealOzone groups. The Endox
device showed the least antibacterial
effect with significant differences
to MTAD and HealOzone.
Bacterial regrowth after 1 week of
incubation was detected in all
specimens of the control group,
whereas the test groups showed
several bacteria-free specimens.
The authors concluded that ozone
has great potential in endodontic
antimicrobial use and that MTAD
and HealOzone seem to be as
effective as 3% NaOCl in reducing
mixed bacterial infection in the
root canal system.22
I would speculate that the antimicrobial
effect of the ozone would
have been even greater if it had
been used as I recommended
above. I personally feel that conventional
irrigation (including
NaOCl) should be used during
cleaning and shaping, and ozonated
water (ideally with ozone gas)
should be used as the final irrigant
with ultrasonication.
Cardoso and colleagues23 concluded
that the ozonated water, used as an
irrigant agent, significantly reduced
the number of Candida albicans
and Enterococcus faecalis in root
canals in human teeth.
A review23 identified four
studies investigating the
bactericidal effect of ozone as
compared with 2.5 to 5% sodium
hypochlorite as irrigation solutions
in endodontics.
Nagayoshi and colleagues6 found
nearly the same antimicrobial
activity (against E. faecalis and
Streptococcus mutans) and a
lower level of cytotoxicity of ozonated
water as compared with
2.5% NaOCl. They stated,
“Ozone is known to act as a
strong antimicrobial agent against
bacteria, fungi, and viruses. In the
present study, we examined the
effect of ozonated water against
Enterococcus faecalis and Streptococcus mutans infections in vitro
in bovine dentin. After irrigation
with ozonated water, the viability
of E. faecalis and S. mutans invading
dentinal tubules significantly
decreased. Notably, when the
specimen was irrigated with sonication,
ozonated water had nearly
the same antimicrobial activity
as 2.5% sodium hypochlorite
(NaOCl). We also compared the
cytotoxicity against L-929 mouse
fibroblasts between ozonated
water and NaOCl. The metabolic
activity of fibroblasts was high
when the cells were treated with
ozonated water, whereas that of
fibroblasts significantly decreased
when the cells were treated with
2.5% NaOCl. These results
suggest that ozonated water
application may be useful for
endodontic therapy."6

Muller and colleagues24 found 5%
NaOCl superior to gaseous ozone
in eliminating microorganisms
organized in a cariogenic biofilm.
This study reported less than one
log reduction of bacteria after
using ozone gas above biofilms in
culture media, which was only a
similar reduction to that achieved
by using 0.2% chlorhexidine or
photoactivated disinfection.24
However, it should be noted that
ozone is a potent oxidant and will
undergo a redox reaction with
reductants in a culture media. In
addition, the authors did not
bubble the ozone into the biofilm.
Ozone should be delivered under
pressure into a root canal irrigant
or lesion by pressing the delivery
tube onto the surface so that ozone
can penetrate the root canal irrigant
or lesion. In vivo root canal
contents and caries, unlike artificial
biofilms, contain many molecules
such as iron, which can increase
the antimicrobial effectiveness of
ozone in teeth and can help
produce the powerful hydroxyl
radicals in vivo to further increase
the antimicrobial effectiveness
of ozone.
Moreover, another study25 has
found that the irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine, or the application of gaseous ozone was not sufficient to inactivate *E. faecalis*. The methodology used was obviously spartan, as no tested agent had any antimicrobial effect. It is highly probable that the ozone (oxidant) reacted preferentially with the reductants in the brain–heart infusion used for the inoculation in a simple redox reaction rather than with the bacterial strain. Hems and colleagues concluded that "ozone had an antibacterial effect on planktonic *E. faecalis* cells and those suspended in fluid, but little effect when embedded in biofilms. Its antibacterial efficacy was not comparable with that of NaOCl under the test conditions used." Unfortunately, these authors used an extremely low dose of ozone in their experiments. The concentration of ozone mentioned in the paper was only 0.68 ppm. This concentration was immediately after production and would have reduced further by the time it was used. This was clearly a biased comparison as the dose of NaOCl used was enormous in comparison to the ozone. Surprisingly, immediately following ozone sparging, 1 mL of this broth had ozone inactivation by a transfer into 9 mL of neutralizing broth. This neutralization does not appear to have been similarly used with the NaOCl, again biasing the experiment. Given the methodology used in this paper, and the low dose and time of application of ozone used, it is surprising that ozone was as effective as it was reported.

**USE OF OZONATED OILS AS MEDICAMENTS**

An investigation evaluated histologically and histobacteriologically the response of periradicular tissues to the endodontic treatment of infected root canals performed in a single visit or in two visits using either ozonated oil or calcium hydroxide in camphorated paramonochlorophenol (CMCP) as an intracanal medication. After 6 months, the animals were sacrificed and the specimens were
processed for histologic and histobacteriologic analyses. The root canals treated in a single visit showed a success rate of 46%. When a calcium hydroxide/CMCP-based interappointment intracanal medication was used, 74% of the cases were categorized as successful. In cases where ozonated oil was used as the intracanal medication, the success rate was 77%.

Siqueira and colleagues evaluated the antibacterial activity of the ozonated oil and calcium hydroxide pastes against bacterial species commonly associated with the etiology of periradicular diseases. Of the tested medicaments, ozonated oil was the most effective against the evaluated bacterial species.

**BIOCOMPATIBILITY OF OZONE IN ROOT CANAL THERAPY**

A high level of biocompatibility of aqueous ozone on human oral epithelial (BHY) cells, gingival fibroblast (HGF-1) cells, and periodontal cells has been published. Huth and colleagues investigated whether gaseous ozone and aqueous ozone exerted any cytotoxic effects on BHY cells and HGF-1 cells compared with established antiseptics (2 and 0.2% chlorhexidine digluconate [CHX]; 5.25 and 2.25% sodium hypochlorite [NaOCl]; 3% hydrogen peroxide [H₂O₂]) over 1 minute and compared with the antibiotic metronidazole over 24 hours. Cell counts, metabolic activity, Sp-1 binding, actin levels, and apoptosis were evaluated. Ozone gas was found to have toxic effects on both cell types. Essentially, no cytotoxic signs were observed for aqueous ozone. CHX (2%, 0.2%) was highly toxic to BHY cells, and slightly toxic (2%) and nontoxic (0.2%) to HGF-1 cells. NaOCl and H₂O₂ resulted in markedly reduced cell viability (BHY, HGF-1), whereas metronidazole displayed mild toxicity only to BHY cells. Taken together, aqueous ozone had the highest level of biocompatibility of the tested antiseptics. Nonetheless,
ozone gas performed well compared with the established endodontic irrigants, which showed equal or even higher cytotoxic potentials than ozone gas. In addition, ozone gas applied into the moist root canal, as currently performed with the HealOzone device, dissolves in canal fluids, thereby resulting in aqueous ozone, which then comes into contact with tissues. Other reports also reported a high biocompatibility of aqueous ozone. Irrigation of the root surface of avulsed teeth did not reveal a negative effect on periodontal ligament cell proliferation. A clinical report regarding the healing accelerating effect of ozonated water did not document detrimental effects on cells. A clinical report regarding the healing accelerating effect of ozonated water did not document detrimental effects on cells.30

EFFECT OF AQUEOUS OZONE ON THE NF-κB SYSTEM

The transcription factor NF-κB plays a crucial role in inflammatory/immune processes and apoptosis. NF-κB is also thought to be of primary importance in the regulation of periodontal/periapical inflammatory reactions and the pathogenesis of periodontal diseases and apical periodontitis. Huth and colleagues reported that aqueous ozone exerts inhibitory effects on the NF-κB system, suggesting that it has anti-inflammatory and immune-modulatory capacities.

OZONE IS A POTENT OXIDIZER

Ozone has been proven to be one of the most powerful oxidants we can use in dentistry.33

OZONE SYSTEMS AVAILABLE FOR USE IN ROOT CANAL THERAPY

KaVo produces the HealOzone, which delivers 2,100 ppm ozone at a flow rate of 615 cc per minute and has been proven to be safe.34,35 TherOzone produces an excellent unit to produce ozonated water for root canal irrigation and numerous other applications. In addition, other systems are available (such as that supplied by Lime Technologies) that blow ozone into root canals, but manufacturer’s directions must be followed in order to prevent any potential lung inhalation. Lime Technologies also sells ozonated oils for use as root canal medicaments.
USE OF OZONE TO MANAGE ANY
CARIES REMAINING IN THE
ACCESS CAVITY

Ozone has been proven to help reduce cariogenic microorganisms and this could be beneficial to reduce potential contamination of the canal systems during instrumentation.20,36–49

ENHANCED HEALING ASSOCIATED WITH OZONE USE

Ozone also can play a key part in the healing process.6,29–32,50–59

CONCLUSION

Of course, more research on the use of ozone in root canal therapy will add to our knowledge in endodontics.

ASK THE EXPERTS

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Thousands of dentists worldwide use ozone in root canal therapy and it is claimed that millions of teeth have received root canal therapy with ozone having been used as the final irrigant. No adverse event has been recorded after use of the HealOzone or ozonated water in root canal therapy.

Ozone is an effective, easy, cheap, and fast treatment to help disinfect root canals. Ozone is much stronger than chlorine and acts 3,000 times faster without producing harmful decomposition products.60

As ozone is the most powerful antimicrobial and oxidant we can use in endodontics, and as aqueous ozone revealed the highest level of biocompatibility compared with commonly used antiseptics, then it is fairly obvious that ozone should be used to help combat the microorganisms associated with infected root canals.

Ozone has a place in the 21st century oral health care,61 and we should use its proven powerful antimicrobial efficacy and potent oxidant ability to reduce microorganisms during root canal therapy.

DISCLOSURE

Professor Edward Lynch is a consultant and principal investigator for research grants from CurOzone USA (Aurora, Ontario, Canada) administered by Queens University, Belfast, Northern Ireland, UK.

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ORIGIINAL RESEARCH

Bovine pulp tissue dissolution ability of HealOzone®, Aquatine Alpha Electrolyte® and sodium hypochlorite

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Keywords
dental pulp, ozone, sodium hypochlorite, superoxidised water, tissue dissolution.

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Abstract
The aim of this study was to evaluate the bovine pulp tissue dissolution ability of HealOzone, Aquatine Alpha Electrolyte® and 0.5% sodium hypochlorite, used alone or in combination. Thirty bovine pulp fragments were weighed, divided into six groups and placed individually in Eppendorf tubes containing the tested solution until total dissolution occurred. The groups were: G1: saline (negative control), G2: Aquatine Alpha Electrolyte®, G3: 0.5% NaOCl (positive control), G4: Saline + HealOzone, G5: 0.5% NaOCl + HealOzone, G6: Aquatine Alpha Electrolyte® + HealOzone. HealOzone was activated for 2 min with a #6 cup covering the test tube opening on a fixed platform. Two blinded observers using 2¥ loupes magnification assessed the samples continuously for the first 2 h, and then every hour for the next 8 h. Dissolution speed was calculated by dividing pulp weight by dissolution time (mg min⁻¹). G3 (NaOCl) and G5 (NaOCl + HealOzone) dissolved the pulp tissue completely. The mean dissolution speed for G3 was 0.396 mg min⁻¹ (SD 0.032) and for G5 was 0.775 mg min⁻¹ (SD 0.2). Student’s t-test showed that G5 dissolved bovine pulp tissue faster than G3 (P = 0.01). Only groups containing sodium hypochlorite dissolved pulp tissue, whilst HealOzone enhanced speed of dissolution.

Introduction
Root canal irrigants should have the ability to dissolve pulp remnants (1), as removal of pulpal tissue is inadequate with mechanical preparation alone because of the morphological complexities of root canals (2). It is suggested that post-operative pain is more prevalent in vital that in non-vital cases (3) and pulp remnants can cause post-operative pain (4).

Sodium hypochlorite (NaOCl) is recommended as the main irrigant because of its broad antimicrobial activity, its capacity to prevent formation of and dissolve the organic part of the smear layer, and its ability to dissolve tissue remnants (1). However, it has been shown to have a cytotoxic effect on vital tissues, eliciting severe inflammatory reactions if it reaches the periapex (5), with 5.25% producing greater toxic and caustic effects than 0.5% and 1% solutions (5). Low NaOCl concentrations have reduced tissue dissolution capability (6) although this can be improved through increasing its temperature (3,4). It has been postulated that such solutions have reduced systemic toxicity when compared to higher concentration solutions (7).

Aqueous and gaseous ozone have been tested as antimicrobial agents for endodontic treatment although different studies reveal varying results. An investigation using the HealOzone delivery system in extracted teeth after carriage in the oral cavity suggests that ozone has good potential as an antimicrobial in endodontics (8) and it has been shown to be dose-, strain- and

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time-dependent against biofilm cells (9). Conversely a different investigation using a positive/negative culture analysis with longer time exposure to gaseous ozone and ozonated water found that these substances were not able to inactivate Enterococcus faecalis (10).

Ozonated water had no negative effect on periodontal cells (11) and when compared against other established antimicrobials ozonated water was shown to have the highest level of biocompatibility (12).

Superoxidised water is a form of disinfectant which is generated at the point of use by passing a saline solution over titanium-coated electrodes. According to the manufacturer Aquatine Alpha Electrolyte® (Optident Dental, Ilkley, West Yorkshire, UK) is a solution which is described as ‘brine’ consisting of a mixture of oxidising substances including hypochlorous acid, with 200 p.p.m. available free chlorine (AFC) at a pH of 5.0–6.5.

Electrochemically activated solutions have been suggested as irrigants as they provide efficient cleaning of root canal walls (13); Aquatine has been shown to have an antimicrobial effect against E. faecalis biofilms in a bovine root model (14).

The aim of this study was to evaluate the bovine pulp tissue dissolution ability of HealOzone, Aquatine and 0.5% NaOCl, used solely or in associations.

Materials and methods

Thirty bovine pulp fragments were used in this study. The
animals were slaughtered for commercial purpose and therefore this study exerted no influence on the animals’ fate. The teeth were stored frozen and left to thaw overnight at room temperature (20°C circa) before being split. The pulps were removed and divided into fragments using a #12 scalpel blade (Swann-Morton, Sheffield, UK) and weighed using precision scales (Sartorius BP61S, Göttingen, Germany). The pulp fragments were then divided into six groups of five and placed individually in 1.5 mL Eppendorf tubes filled with the test substance. The groups were as follows: G1: saline (negative control) which is Optident Sterilox electrolyte solution® (Optident Dental, Ilkley, West Yorkshire, UK), not activated, G2: Aquatine (Optident Dental, Ilkley, West Yorkshire, UK), G3: 0.5% NaOCl (positive control) (Teepol Bleach, Teepol, Orpington, Kent, UK), G4: HealOzone + Saline, G5: HealOzone + 0.5% NaOCl, G6: HealOzone + Aquatine. Details of the above solutions are presented in Table 1.

Following complete immersion of pulp fragments in the solutions, HealOzone gaseous ozone delivery system (KaVo, Biberach, Germany) with a 4.2 ¥ 106 mg m-3 ozone concentration was activated for 120 s with a #6 cup covering providing an airtight seal against the Eppendorf tube opening (Fig. 1) while resting in a fixed platform. All other groups remained in a resting state in the same fixed platform. Two observers blinded to the experimental groups visually assessed the samples using 2¥ loupes for magnification.
The samples were continuously monitored for the first 2 h, and then every hour for the next 8 h, or until complete dissolution occurred. Time taken for dissolution was recorded in minutes (min) and dissolution speed was calculated by dividing pulp weight (mg) by dissolution time (mg min⁻¹).

The NaOCl concentration was tested by iodometric titration and was 0.5%. Statistical analysis involved use of Student's t-test (a = 0.05) to compare the means between the NaOCl groups.

Table 1 Test solutions’ characteristics and manufacturers

<table>
<thead>
<tr>
<th>Name of chemical Description</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquatine Alpha Electrolyte Chlorine concentration 200 p.p.m., pH 5</td>
<td>Optident Dental, Ilkley, West Yorkshire, UK</td>
</tr>
<tr>
<td>Optident Sterilox electrolyte solution 10% sodium chloride</td>
<td>Optident Dental, Ilkley, West Yorkshire, UK</td>
</tr>
<tr>
<td>Sodium hypochlorite 0.5% by dilution, confirmed via iodometric titration</td>
<td>Teepol Bleach, Teepol, Orpington, Kent, UK</td>
</tr>
<tr>
<td>HealOzone 4.2 ¥ 106 mg m⁻³ ozone concentration</td>
<td>KaVo, Biberach, Germany</td>
</tr>
</tbody>
</table>

Figure 1 Pulp fragment immersed in solution with a HealOzone delivery cup achieving an airtight seal against the Eppendorf tube opening.

Ozone, EAS and NaOCl Pulp Dissolution Ability G. Rossi-Fedele et al.

Results

Results are summarised in Table 2 and Figure 2.

Only G3 (NaOCl) and G5 (NaOCl + HealOzone) were able to dissolve pulpal tissue; no dissolution activity was evident in the remaining groups. The mean dissolution speed for G3 was 0.396 mg min⁻¹ (SD 0.032) and for G5 was 0.775 mg min⁻¹ (SD 0.2), and thus NaOCl + HealOzone dissolved bovine pulp tissue faster.
than NaOCl alone and this was statistically significant
($P = 0.01$).

Discussion

The aim of this study was to evaluate the bovine pulp
tissue dissolution ability of HealOzone, Aquatine and
0.5% NaOCl, used solely or in associations.

Bovine pulp tissues are regarded as comparable to
human pulp tissues despite some minor differences (15),
and have been previously used to test the dissolution
ability of different endodontic irrigants (16,17).

As greater NaOCl volume and contact surface area
leads to greater tissue dissolution ability (2,18), it could be
speculated that the Eppendorf tubes used in these experiments
could have reduced the dissolution time compared
with normal clinical (in vivo) conditions, as is most likely
the case in previously reported in vitro investigations
(2–4,6,16–18).

The results of this study appear to be in line with
previous investigations where only NaOCl has shown
tissue dissolution ability (16,19); one study has shown
the mean dissolution speed for 0.5% sodium hypochlorite
solutions as being 0.31 mg min$^{-1}$ (16), which is very
close to the dissolution speeds obtained in our assays. This
might be explained by the differences in the volume of
irrigant in contact with the pulp fragments (20 mL vs.
1.5 mL in our research) and perhaps a difference in the
temperature of the solutions. An investigation into the
tissue dissolution capacity of 5% dichloroisocyanurate,
which in water releases hypochlorous acid that contains
‘free chlorine’, and therefore a potential for tissue dissolution, did not have any significant action against necrotised porcine palates (19); this is similar to our findings using Aquatine.

It has been suggested that 0.5% NaOCl solutions are ineffective as necrotic tissue solvents after contact for 7 min (6). Our investigations showed that when 0.5% NaOCl was evaluated beyond 7 min, its dissolution capacity was efficient as no pulp fragments remained; they were completely dissolved.

A previous investigation suggests that a similar time to the one used in our research to activate the irrigants (2.5 min vs. 2 min in our research) was able to achieve total elimination of microorganisms in an in vitro model with a low gas concentration (4 g m⁻³) (9). HealOzone application for 40 s had similar antimicrobial ability when compared to NaOCl and MTAD (8); 2 min is a clinically relevant time period and, in our model, halved the time required to totally dissolve the pulp fragments.

The results in this study were reported as dissolution speed to compensate for fragment weight variability even though there was no statistical difference between total fragment weights amongst groups.

It has been suggested that ozone is approximately 10 times more soluble in water than oxygen and its half life in pyrogen-free water is 9–10 h (at pH 7 and 20°C) (20).

Table 2 Mean weight (mg) and dissolution time (min) amongst test groups

<table>
<thead>
<tr>
<th>Solution</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
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</table>
Saline Sterilox 0.5% NaOCl HealOzone + Saline HealOzone + NaOCl HealOzone + Sterilox

Weight (mg) 12.2 13.2 13.4 13.4 12.4 13.2

Time to dissolution no dissolution no dissolution 34 min no dissolution 16.4 min no dissolution

Tissue dissolution speed (mg min\(^{-1}\))

TD TDHO

Figure 2 Mean speed (mg min\(^{-1}\)) of pulp dissolution within the NaOCl groups. TD: Group 3 (NaOCl), TDHO: Group 5 (NaOCl + HealOzone).

Conversely a different investigation suggests that when ozonated water is maintained at 22°C for 180 min its concentration decreases significantly with time and after 10 min is almost halved (21). Therefore, it is unclear whether using the HealOzone generating device, 2 min activation is likely to produce ozone present in solution for a clinically relevant period of time.

The mechanism for ozone to activate sodium hypochlorite is not fully understood. Volume of irrigation, agitation, temperature and solution concentration, mechanisms previously suggested to activate NaOCl, were unchanged between the groups and ozone activation...
did not cause an increase in temperature during the assays.

It has been suggested that the amount of available chlorine is responsible for the tissue-dissolving properties of hypochlorite solutions (22). We can hypothesise that ozone, being a strong oxidising agent, might be able to activate NaOCl directly, leading to the formation of oxygen molecules as well as extremely reactive atomic oxygen that possibly enhances the tissue dissolution capacity of the solution.

When comparing the tissue dissolution capacity of 0.5% NaOCl and Aquatine solutions it is necessary to take into account the differences in solvent concentration, as well as the differences in behaviour of the compounds. A 0.5% NaOCl solution will have a solvent concentration (mainly dissociated as hypochlorite ion, because of its high dissolution constant) 18 times larger than a 200 p.p.m. AFC Aquatine solution (AFC consists of hypochlorite ion and a larger proportion of HOCl because of a very low dissolution constant). Subsequently tissue dissolution might depend on the amount of hypochlorite ion rather than the AFC.

The ozone gas concentration provided by the HealOzone system has been shown to be slightly less cytotoxic than 2.5% NaOCl (23). An aqueous form of ozone at a concentration of 1.25–20 mg mL\(^{-1}\) showed less cytotoxicity on human oral epithelial and gingival fibroblast cells when compared with 0.2% and 2% chlorhexidine digluconate, 2.25% and 5.25% sodium hypochlorite, 3%
hydrogen peroxide (12). Ozonised water has no negative effect on periodontal cells remaining on tooth surfaces after irrigation for 2 min at a concentration of 2.5–3.5 ng mL-1 (11).

Exposure to ozone causes acute changes in pulmonary function and development of symptoms (24) and therefore the HealOzone system has a safety mechanism that will stop the delivery of the gas if the seal between the delivery cup and the tooth is broken.

Reduced toxicity is one of the proposed advantages of superoxidised waters when compared to NaOCl (25). Another electrochemically activated water (Microcyn, Oculus Innovative Sciences, Petaluma, CA, USA) was tested and found to be significantly less cytotoxic than antiseptic hydrogen peroxide concentrations (26). Microcyn is different to Aquatine and therefore results may not be directly transferable.

It is worth noting that it is not completely clear if 0.5% NaOCl solutions activated with HealOzone would minimise known side-effects such as tissue irritation and loss of resistance to mechanical stress of dentine when compared to more concentrated solutions that have a similar tissue dissolution capability.

In conclusion, only NaOCl was able to dissolve pulp tissue and HealOzone contributed to reducing the time for this. It could be speculated that dissolution properties of NaOCl could be enhanced by HealOzone allowing clinical use of more diluted concentrations with potentially reduced side-effects.
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Abstract

OBJECTIVES: To determine the disinfecting effect of ozonized oxygen (120 seconds from the HealOzone generator, KaVo) on Enterococcus faecalis, representing bacteria that are difficult to eliminate in the root canals of human teeth, and to compare it with the conventional irrigants: sterile physiologic sodium chloride solution (negative control group), 3% hydrogen peroxide solution, 0.2% chlorhexidine solution, 1.5% sodium hypochlorite solution, and 3% sodium hypochlorite solution (positive control group).

METHOD AND MATERIALS: The roots (n = 10 in each group) were sterilized, contaminated with the test microorganisms in a quantitative preparation, rinsed with the test solutions, and dried. The residual concentration of E faecalis was determined through another irrigation stage with the sodium chloride solution.
RESULTS: The positive control group showed a significantly lower concentration of microorganisms than all the other groups, whereas the negative control group showed a significantly higher concentration compared to the other groups. The test groups showed low concentrations.

CONCLUSION: Ozonized oxygen appears to be suitable for disinfecting root canal systems in cases where sodium hypochlorite is not indicated.

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Clinical Reversal of Root Caries using Ozone, Double Blind, Randomised, Controlled 18-Month Trial

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Abstract:

Objective: To assess the effect of an ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery primary root carious lesions (PRCL’s), in an older population group. Design: A total of 89 subjects, (age range 60 – 82, mean ± SD, 70.8 ± 6 years), each with 2 leathery PRCL’s, were recruited. The 2 lesions in each subject were randomly assigned for treatment with ozone or air, in a double blind design, in a general dental practice. Subjects were recalled 3, 6, 12 and 18 months. Lesions were clinically recorded at each visit as soft, leathery or hard, scored with a validated root caries severity index. Results: There were no observed adverse events. After 3 months, in the ozone treated group, 61 PRCL’s (69%) had become hard and none had deteriorated, whilst in the control group, 4 PRCL’s (4%) had become worse (p < 0.01). At the 6 month recall, in the ozone group, 7 PRCL’s (8%) remained leathery, the remaining 82 (92%) PRCL’s had become hard, whilst in the control group, 10 PRCL’s had become worse (11%) and one had become hard (p < 0.01). At 12 and 18 months, 87 Subjects attended. In the ozone group at 12 months, 2 PRCL’s remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCL’s had progressed from leathery to soft, i.e. became worse, 65 PRCL’s (75%) were still leathery, and one remained hard (p < 0.01). At 18
months, 87 (100%) of ozone treated PRCL’s had arrested, whilst in the control group, 32 lesions (37%) of the PRCL’s had worsened from leathery to soft (p < 0.01), 54 (62%) PRCL’s remained leathery and only one of the control PRCL’s had reversed (p < 0.01). 

**Conclusions:** Leathery non-cavitated primary root caries can be arrested non-operatively with ozone and remineralising products. This treatment regime is an effective alternative to conventional "drilling and filling".

Key words: Root caries, reversal, arrest, ozone, toothpaste, mouth-rinse, spray.

**Introduction:**

**Elderly Populations and Root Caries:**

The demographic profile of developed countries has moved from a young to an older population. This ageing is associated with better nutrition, increased standards of living, and advances in medical and pharmacological management of disease.

In a 1996 study, 2,280 subjects who were 60 years old or over from three different areas of the UK were examined clinically to assess their dental health and needs. Root caries was found to be common and there was an age-related increase in risk of the disease. In a national survey of adult dental health conducted in the Republic of Ireland in 1989/1990, a total of 1,527 subjects aged 25 and older were examined for root caries. It was suggested that the prevalence of root caries was highest in older age groups, residents of non-fluoridated communities, and those earning low incomes. As tooth loss masked the potential prevalence of root caries and as more people are retaining their natural teeth into middle and old age, the incidence of primary root surface caries (PRCL’S) was thought likely to increase.

Ageing individuals tend to have gum tissue recession, and the associated exposed root surfaces are more susceptible to caries. A survey conducted in the United Kingdom in 1988 by Downer found prevalence in the 55-64 year old group with a mean of 0.7 teeth having root caries, and 1.2 having had restorations placed in their roots. The prevalence of PRCL’s was reported to be over 80% in elderly institutionalised people. Hand et al. reported that 1.8 in 100 susceptible exposed root surfaces in adults over age 65 years became carious annually. Saliva contains a number of important minerals such as phosphate, calcium and fluoride that could aid PRCL remineralisation in the right environment, and acts as an important buffer for oral acids. As salivary flow decreases, for example in the pharmacological management of disease, there may be an increase in the incidence of decay. The micro flora of PRCL’s has been shown to contain large numbers of acidogenic and aciduric microorganisms, which correlate with the severity/hardness of root caries.

**The Detection of Root Caries:**

More accurate detection systems need to be developed for the detection of PRCL’s. Root carious lesions are classified as soft, leathery or hard based on differences in the degree and pattern of mineralisation. Nyvad and Fejerskov suggested that soft lesions showed extensive demineralisation with no evidence of an intact mineralised surface layer, whilst hard lesions appeared to have a generally uniform distribution of mineral throughout the lesion, and leathery lesions had a broad range of histological appearances. These authors concluded that soft and leathery lesions were active, whilst hard lesions were arrested. Soft lesions are the most severe type of root carious lesions according to a validated clinical severity index and contain more micro-organisms. Research suggests that soft and
leathery lesions can remineralise and may become hard\textsuperscript{3,12,23,24}. Remineralised lesions acquire a smooth and hard surface and remain unchanged over many years.

**The Management of Root Caries:**

The term 'root caries' was used by Hazen \textit{et al.}\textsuperscript{25} and the term primary root carious lesion (PRCL) was proposed by Lynch in 1986\textsuperscript{20,23}. A PRCL is defined as an area on the surface of the tooth, at or apical to the cemento-enamel junction, that has undergone a carious process.

Restorative management of root caries is a challenge in view of the difficulties of visibility, moisture control, access to carious lesions, proximity of the pulp, proximity to the gingival margin, and the high organic content of the dentine. Many restorative materials on roots of teeth are associated with problems such as microleakage\textsuperscript{26} and marginal adaptation\textsuperscript{27} necessitating frequent restorative replacement\textsuperscript{28}.

Previous studies have shown that root caries prevalence increases with age\textsuperscript{6,29,30} from about one in 9 root surfaces at risk for under-30s, to approximately two in three for the over 60’s\textsuperscript{4}. Attachment loss and exposure of root surfaces to the oral environment are accepted to be nearly universal prerequisites to the development of root caries\textsuperscript{31-33}.

Papas \textit{et al.}\textsuperscript{34} emphasised the high involvement of posterior teeth with root caries The microbial colonisation of PRCL’s has been extensively investigated. Attention has focused on the causative micro-organisms in the aetiology of root caries\textsuperscript{12,23,35-37}. These studies showed the importance of the acid-producing group of bacteria in PRCL’s. Further studies linked salivary and plaque conditions\textsuperscript{38,39}. It is now recognised that the most advantageous treatment for root caries is remineralisation\textsuperscript{40}. The use of pharmaceutical agents\textsuperscript{41,42} and fluoride-containing dentifrices may provide some protection for high caries risk patients. Various pharmaceutical agents have been used, such as fluoride\textsuperscript{43-46}, and chlorhexidine or chlorhexidine in combination with thymol\textsuperscript{47}.

Keltjens \textit{et al.}\textsuperscript{48} concluded that high-risk patients with dentures supported by natural teeth with high salivary mutans streptococi, root and enamel caries would benefit both from fluoride and chlorhexidine therapy. Root coverage by removable partial dentures correlates with root caries prevalence\textsuperscript{49}.

Recent publications conclude that ozone should be considered as an alternative pharmaceutical management strategy\textsuperscript{19,50-54} rather than the traditional drill and fill approach. Ozone (a pale blue-coloured gas, chemical formulae O\textsubscript{3}) plays an important role as a natural constituent in the higher layer of the Earth’s atmosphere. It has been used for many years in medicine, and within recent years in dentistry. A device that has a CE mark, know as the HealOzone (CurOzone, USA and KaVo GmbH & Co, Germany) has been available commercially in Europe for more than 2 years. Ozone is a very powerful antimicrobial agent. Recently, Baysan \textit{et al.}\textsuperscript{51,52} reported that ozone application either for 10 or 20 seconds was effective to kill the great majority of microorganisms in PRCL’s (>99% microbial killing after 10 seconds Ozone application).

O\textsubscript{3} is naturally produced by the photo-dissociation of molecular O\textsubscript{2} into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating HO\textsubscript{3}, which, in turn, decomposes to hydroxyl radical. Further reactions convert O\textsubscript{3} to an even more powerful oxidant, the hydroxyl radical (OH\textsuperscript{•}).

In view of its powerful oxidising properties, O\textsubscript{3} can attack many biomolecules such as the cysteine, methionine and the histidine residues of proteins. The effects of ozone on cell
structures, metabolism and microorganisms is well documented in published papers\textsuperscript{19,55-59} in both dentistry and medicine. Research has shown that ozone disrupts the cell walls of microorganisms within seconds, leading to immediate functional cessation. This effect within a very short time is of great clinical significance, as the potential for microbial resistance to this treatment modality is insignificant. Baysan et al.\textsuperscript{19,51} have published reductions from $\log_{10} 6.0$ to $\log_{10} 0.46$ colony forming units after just 20 seconds of ozone. Studies have shown that just 10 seconds of ozone treatment is sufficient to produce reversal of PRCL’s\textsuperscript{19,50-54}.

Aim:

The aim of this study was to assess the effect of an ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCL’s, in an older population group.

Method:

After ethics committee approval, all participants were recruited from consecutive subjects presenting with 2 leathery non cavitated PRCL’s at UKSmiles Dental Practice, Berkshire, UK. Each subject had given their informed consent for both dental examinations and ozone treatment to be undertaken. A total of 89 subjects, all over 60 (age range 60 – 82, mean \pm SD, 70.8 \pm 6 years) with 2 leathery lesions each (178 PRCL’s in total were entered into the study). All lesions entered fulfilled the criteria of the middle severity lesion group in the Perceived Treatment Need Index\textsuperscript{12,23}. Lesions were randomly assigned into two groups; Group 1; treated with ozone, or Group 2; no ozone for the control PRCL’s.

All subjects were prescribed a course of oral hygiene instruction by a member of the practice hygiene team followed by scaling and polishing non fluoride containing paste at baseline only. Professional instruction was given on brushing, the use of floss and interdental brushes. Each subject was advised not to consume fermentable carbohydrates between meals. They were informed of the relationship between caries incidence, and increased frequency of consumption of fermentable carbohydrates.

Lesions were examined using a visual/tactile method at baseline. Each PRCL was classified subjectively in terms hardness and severity\textsuperscript{12,23}. Leathery lesions were selected which were deemed to require drilling & filling. All subjects were offered a pharmacological treatment as an alternative to the traditional drilling and filling method and informed consent obtained. All subjects accepted the pharmaceutical approach to manage their PRCL’s rather than the traditional drill and fill method.

The lesions were assigned into two groups by a dentist, using a computer generated random table; Group 1 lesions were treated with 40 seconds of ozone, and Group 2 lesions were left as controls. Following initial oral hygiene instruction, subjects were given ozone or air treatment. The treatment method was explained and demonstrated. Two dentists were involved in this study; the first assessed the PRCL’s and the second dentist assigned them to Group 1 or 2 with a computer-generated random table. The first dentist then carried out the treatment for 40 seconds, applied the mineral wash, dispensed the remineralising products, and instructed the subjects. A double blind system was employed, and the ozone treatment was applied by a different operator than the one recording the clinical criteria used to define the severity of the lesions.

The ozone delivery system, HealOzone (CurOzone USA and KaVo, Germany) was employed. The HealOzone is a device that takes in air and produces ozone gas. The ozone is then delivered via a hose into a disposable sterile cup at a concentration of 2,100 ppm \pm 10%. The ozone gas is refreshed in this disposable cup at a rate of 615 cc/minute changing the
volume of gas inside the cup over 300 times every second. The cup forms a seal around the lesion being treated so that ozone cannot leak into the oral cavity.

The HealOzone unit was fitted with a modified control integrated electronic chip. The HealOzone unit’s display and sound were exactly the same when delivering ozone or air. In this way the dentist treating the subjects was unaware which tooth had been treated with ozone, and which tooth was used as the control lesion. The second dentist recorded if the PRCL was treated or untreated. If the lesion was assigned to Group 1 and ozone treated, the HealOzone was switched to produce ozone. If assigned as a control, the HealOzone was switched to produce air only. Otherwise, the HealOzone unit functioned as normal so that the first dentist was unaware if the PRCL’s were treated or untreated.

After treatment (lasting 40 seconds) a professionally applied remineralising solution containing xylitol, fluoride, calcium, phosphate and zinc (HealOzone remineralising solution) was applied to the lesion.

Instructions were given to each subject to use the re-mineralising tooth paste twice each day, and the mineral mouth wash on two separate occasions each day, and to use the remineralising spray, sprayed into the mouth 4 times a day after breakfast, lunch, dinner and supper. Standard soft toothbrushes were dispensed, and subjects were advised to use a new toothbrush every month. Subjects were recalled at 3, 6, 12, and 18 months. At each appointment, the lesions were re-treated to the original treatment protocol, again employing two dentists. The subjects were given further supplies of the remineralising products and new toothbrush supplies were dispensed.

The reproducibility of the data was tested at the 12-month recall. 1 week after assessment by the first dentist, 15 subjects (30 PRCL’s) were recalled and examined by a third dentist. There was a good agreement in the classifications of hardness and severity of PRCL’s (kappa = 0.80). Twenty Subjects with 40 PRCL’s were also re-assessed 24 hours later, at the final 18-month recall visit, by the usual dentist who had used the same criteria throughout the study period. Two lesions that were marked as soft were re-assigned to leathery on the second visit (kappa = 0.95).

Data sets were collected at each recall and the codes were only broken at the end of the 18 month period. Statistical analyses using chi square statistics was carried out on these collected data sets.

**Results:**

Results: 89 subjects started this study at baseline. At 18 months, 87 subjects had completed the study. Two subjects had moved out of the practice area, and were not available for the 12 or 18-month re-assessment visits. There were no observed or reported adverse events at any of the treatment sessions or afterwards (See Table 1).

After 3 months, in the ozone treated group, 61 PRCL’s (69%) had become hard and none had become worse, whilst in the control group, 4 PRCL’s (4%) had become worse (p < 0.01). At the 6 month recall, in the ozone group, 7 PRCL’s (8%) remained leathery, the remaining 82 (92%) PRCL’s had become hard, whilst in the control group, 10 PRCL’s had become worse (11%) and one had become hard (p < 0.01). At 12 and 18 months, 87 Subjects attended. In the ozone group at 12 months, 2 PRCL’s remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCL’s had progressed from leathery to soft, i.e. became worse, 65 PRCL’s (75%) were still leathery, and one remained hard (p < 0.01). At 18 months, 87 (100%) of ozone treated PRCL’s had reversed, whilst in the control group, 32 lesions (37%) of the PRCL’s had worsened from leathery to soft (p < 0.01), 54
(62%) PRCL’s remained leathery and only one of the control PRCL’s had reversed (p < 0.01).

Table 1

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Ozone Treated</th>
<th>2 subjects had dropped out at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leathery</td>
<td>89</td>
<td>28</td>
</tr>
<tr>
<td>Hard</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 months</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>12 months</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>18 months</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Control Group</th>
<th>2 subjects had dropped out at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leathery</td>
<td>89</td>
<td>85</td>
</tr>
<tr>
<td>Hard</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>75</td>
</tr>
<tr>
<td>18 months</td>
<td>32</td>
<td>54</td>
</tr>
</tbody>
</table>

Table 1 shows the data sets (numbers of lesions in the first column and percentages in the second column) at baseline, 3, 6, 12 and 18 months.

Discussion:

Statistics show that the proportion of elderly people is rapidly increasing in all developed countries. The conventional approach of drill & fill, or tissue amputation, to treat a carious lesion is problematic in view of the difficulties of visibility, moisture control, access to the carious lesion, proximity of the pulp and proximity to the gingival margin. The high organic content of the dentine leads to potential problems with bonding restorative materials to achieve a long-lasting seal. Many elderly subjects have medical conditions that make dental treatment a challenge, such as sudden muscle spasms and movement. This study aimed to assess the effect of a novel ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery PRCL’s, in an older population group, which if successful would be a preferable treatment option to “drilling and filling”.

The restoration of root caries poses a number of problems, in particular visibility and isolation from saliva, gingival secretion and haemorrhage. Restorative materials used to restore PRCL’s have required frequent replacement. Preventive treatment regimes for PRCL’s may be considered to have a better long-term prognosis than restorative treatment options.

Clinical observations suggest that carious lesions can be arrested at any stage of lesion development i.e., even at the cavitation stage if plaque-free conditions are maintained. In this respect, Bradshaw et al. reported that mutans streptococci become increasingly sensitive to fluoride ions as the pH falls. It is possible that the routine topical application of fluoride ions could to some extent inhibit the metabolism of such cariogenic organisms. The remineralisation observed in clinically arrested lesions and the conversion of clinically active to inactive lesions supports the non-restorative management of root carious lesions using...
dentifrices containing fluoride (Fluoride congruent to 0.1% w/w) for a period of 18 months. Papas et al. reported the efficacy of a dentifrice containing 1,150 ppm sodium fluoride with soluble calcium and phosphate salts. Keltjens et al. concluded that high-risk patients with dentures supported by natural teeth with high salivary mutans streptococci, root and enamel caries would benefit both from fluoride and chlorhexidine therapy. However no study has approached a reversal rate of 100% with any of these preventative regimes.

This study has shown that dentistry has the ability to reverse lesions with just 40 seconds of ozone treatment. At 18 months, 100% reversal and remineralisation had been achieved. The studies by Baysan, Baysan et al. and this study draws together important strands of research and publications on fluoride, oral health & hygiene, and the microbiology of caries. It is possible to eliminate the ‘protected’ niche environment of aciduric and acidogenic microorganisms, and oxidise the bacterial by-products that are responsible for the perpetuation of the acidic ecological niche. Of the 87 non-ozone treated PRCL’s, only a single lesion (1%) showed reversal, despite improved oral hygiene instruction and care, regular brushing and the use of a remineralising dentifrice, spray and mouth-rinse. These measures alone cannot produce predictable caries reversal. This study has shown that over time, leathery PRCL’s can gradually become worse, becoming soft. The requirement for intervention is a necessity, and these lesions should not just be observed and left, especially in this caries risk population, who had presented with 2 active caries lesions and who resided in an area with no water fluoridation. This new technology has the potential to have a dramatic effect on the improvement of the dental health of our ageing populations. There is not a single study to show that once a lesion had remineralised, it is ever involved in the active carious process again. This study used a validated set of clinical detection criteria for PRCL’s and hard root caries lesions are arrested.

O₃ has the unique feature of decomposing to a harmless, non-toxic and environmentally safe material (oxygen). O₃ has been used in medicine for many years. The first O₃ generator was developed by Werner von Siemens in Germany as early as 1857, and the first report of it being used therapeutically was for the purpose of purifying blood by C. Lender in 1870. In 1885, Dr. Charles J. Kenworthy first published medical applications of O₃. To date, O₃ therapy has been a recognised treatment modality in sixteen nations. Research by Baysan showed that the HealOzone system has no potential to leak into the oral cavity due to the unique ozone delivery system. No side effects have ever been documented in either the dental research centres or the numerous dental practices in the UK and Europe that use this technology, and it can be considered to be completely safe. Important features of the HealOzone for use in ozone treatment are that it is entirely self-contained, requiring only a power source; it uses air (no cost); each treatment is of low cost; is very fast (40 seconds, compared to 25 minutes for an average filling); and no injections or tissue destruction is involved.

Recent surveys of adults aged more than 65 years in the UK revealed that all were vulnerable to root caries. The proportions of people with restorations on root surfaces ascribable to root caries rose steadily with 35% of 55-64 year olds, and 43% of those aged 65 and above in 1998. It is important to remember that in 1968, only 21% of people aged 65-74 had any teeth compared to 66% in 1998. Such data illustrates the need to provide an increasing dentate elderly population with a simple, effective means of preventing and reversing root caries. The purpose of this study was to build on recent research, and further investigate this treatment regime for the management of root caries.

Filling materials fail at alarming rates. Costs can be measured in terms of pain, discomfort, and in financial terms such as lost productivity. In England and Wales, restorations carried
out in the NHS dentistry cost a total of £1.25 billion in 2001. This does not include private treatment, which is currently estimated to be 50% of dentists’ income. The total costs of all dental treatment in England and Wales probably exceeded £3.26 billion in 2001 (General Dental Council UK, Annual Statistics, 2001). Most of these fees are ascribable to fillings, root fillings, dentures, crowns and bridges. Published reports suggest 50% of restorative items are replacements for previous restorations, and about half of these restorations are being replaced due to secondary caries. If only 50% of all fillings could be avoided with the use of ozone, enormous sums of money could be saved. The cycle of filling preparation and subsequent replacement eventually may eventually lead to more complex restorative care requirements with increasing cost implications, such as the progression from a simple cavity, to a multi-surface one, to the fracture of the crown requiring root canal treatment, followed by restoration with a crown and core.

In the United States, dental treatment is estimated to cost $52 billion per year, and half of this cost may be associated with restorative treatment and the cost of missed workdays and lost production due to oral disease. Despite advances in clinical and laboratory research, approximately 50% of the U.S. population over the age of 65 shows evidence of root caries. In all countries, from the advanced to poor and developing countries, there is a huge potential for a cost-effective way to prevent and reverse caries. In the ageing population, and those with reduced manual dexterity, a preventative and early intervention strategy needs to be found. In this respect, the use of ozone should be also considered for medically compromised patients, domiciliary care patients and homebound elderly people. The equipment required is limited and essentially portable compared to that required for conventional drill & fill. Therefore elderly patients who have limited access to the dental services can benefit from this treatment. In many poor, developing and highly populated countries, equipment, dental supplies, and dental services are inadequate due to high costs and lack of dentally trained personnel.

The benefits of ozone treatment can represent one of the major prevention strategies for these high-risk population groups. The processes involved have been shown to be multi-factorial. Traditionally, clinicians have detected root caries by visual-tactile or visual methods, which disclose cavitation, but fail to reflect the dynamic process of carious lesions. Increased understanding of the process of carious lesions and new management strategies in reversing the clinical severity of PRCL’s can radically alter traditional drilling and filling of lesions and shift the emphasis to a pharmaceutical approach to the management of root caries using Ozone.

**Conclusion:**

The restorative management of PRCL’s has become challenging, especially for the high percentage of the elderly population and particularly for those people in special care units that are experiencing reduced financial support from health services. There is a major requirement for an improved management strategy for root caries. Emilson et al. reported that it was possible to convert active root caries to inactive lesions by an intensive prophylactic program. The pharmaceutical approach for the management of root caries in elderly people should therefore be considered.

This study showed that regular ozone application for 40 seconds and the use of remineralising products, arrested leathery non cavitated primary root caries in a general dental practice population, without the need for dental tissue removal. The use of ozone may supply the key to predictable caries arrest and reversal.
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*Am J Dent, 2004, Accepted for publication*


54. **Lynch E.** Leczenie prochnicy za pomocą ozonu. *Quintessence dla lekarzy stomatologow* 2003; 11:198-200


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Here are 63 of Professor Bocci's peer reviewed research papers which really help explain why the healozone is so useful to promote healing, kill microorganisms and why it helps keep pulps vital

**Ozone: a new therapeutic agent in vascular diseases. [Review]**
Bocci V. Zanardi I. Travagli V.
[Journal Article. Review]
UI: 21446774
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB In this article, we scientifically evaluate the bio-oxidative procedure known as oxygen-ozone therapy. Research over a decade has established a comprehensive framework for understanding and recommending this type of autohemotherapy in vascular diseases. In contrast, a non-specific immunomodulation therapy, using heavily oxidized and denatured blood, has been recently used in studies involving a total of approximately 3000 patients and has led to 'disappointing' results. Such a treatment appears to be an inappropriate example of the so-called minor autohemotherapy, and its poor outcomes may discourage any further studies. Therefore it appears necessary to clarify that the use of only a minimal ozone dose and a valid experimental protocol is likely to produce beneficial results. Millions of people suffer from
chronic limb, brain, and heart ischemia, and such patients may benefit if appropriate ozone therapy could be implemented. Accordingly, we propose the need for a well designed, multicenter, clinical trial to be conducted.

Important details to be clarified about the effect of rectal ozone on the portal vein oxygenation.
Bocci V. Zanardi I. Travagli V.

Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice.
Valacchi G. Lim Y. Belmonte G. Miracco C. Zanardi I. Bocci V. TravaglI V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 21134039
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Ozone is well recognized as a bactericidal agent and its beneficial effect on wound healing could be a consequence of this property. Because ozone itself does not penetrate the cells but immediately reacts with polyunsaturated fatty acids, its effects should be the results of oxidative reaction. For this reason, ozonated oils could be a way to deliver ozone messengers to the skin. This paper evaluated the therapeutic effects of three different grades of ozonated sesame oil in acute cutaneous wounds made in the skin of SKH1 mice. Specifically, wound closure rate, histological parameters, and the level of key proteins such as vascular endothelial growth factors and cyclin D1 have been analyzed in relation to the peroxide level present in the ozonated oil. Treatment with moderately ozonated sesame oil--expressed as peroxide value about 1,500)--has a faster wound closure rate in the first 7 days than treatment with oil containing either lower or higher peroxide value, and even with controls. Moreover, under the same treatment, an earlier and higher response of cells involved in wound repair, a higher angiogenesis, as well as an enhanced vascular endothelial
growth factors and cyclin D1 expression were observed. The present study shows the validity of ozonated sesame oil in cutaneous wound healing and emphasizes the importance of the ozonation grade. Copyright 2010 by the Wound Healing Society.

Ozonation of human HIV-infected plasmas for producing a global vaccine: How HIV-patients may help fight the HIV pandemic.
Bocci V. Zanardi I. Travagli V.
[Letter]
UI: 21178445
Authors Full Name
[View Annotation(s)]
AB A vaccine against HIV able to generate properly neutralizing antibodies and an efficacious cytotoxic T lymphocyte response is of paramount importance. We are proposing a novel approach based on the collection of thousand small HIV-infected human plasma samples for preparing a global vaccine, able to counteract HIV diversity and mutagenicity. The pooled plasmas will undergo several steps for sterilizing and inactivating HIV, possibly other contaminant viruses and other pathogens. The critical step is the prolonged and controlled exposure of plasmas to ozone so that finally each ml of plasma has interacted with a precise dose of ozone. To inactivated plasma, both therapeutic human albumin and ozonated ethyl oleate are added for enhancing a proficient absorption and reaction with the immune system of the vaccine. The need of a partner collaboration for developing the production and the preliminary testing of the vaccine is essential.

Potentiality of oxygen-ozonetherapy to improve the health of aging people. [Review]
Bocci V. Zanardi I. Travagli V.
[Journal Article. Review]
UI: 20735348
5. [ ] Authors Full Name
[View Annotation(s)]
AB During the last century the lifespan of human beings has increased from about 49 to almost 80 years owing to the great advances of biomedical sciences. This fact has strongly
stimulated the idea that it may be possible to prolong productive life for another twenty years but paradoxically the problem of both hyper- and hyponutrition severely jeopardizes this objective all over the world. Many causes of premature aging have been discussed in order to prevent it or find suitable medical treatment. So far only a moderate restriction of caloric intake that does not alter essential nutrients has proved capable of keeping animals and humans healthier for a prolonged time. This modality appears to activate critical longevity genes that can prolong survival: although this is a valuable line of research it will take considerable time to produce valid drugs to selectively activate these relevant genes. Meantime we propose to evaluate an easy and well accepted treatment based on a weekly quasi-total body exposure to oxygen-ozone inside a thermostatically controlled cabinet. The rational exposure to a minimal amount of ozone acting as a mild stressor induces a striking improvement of crucial metabolic activities capable of preserving a good health for several years.

The failure of the ACCLAIM trial is due to an irrational technology.
Bocci V.
[Letter]
UI: 18996606
Authors Full Name
Bocci, Velio.
[View Annotation(s)]
[View Abstract]
AB The excessive blood oxidation devised with the Celacade System does not procure any advantage in chronic heart failure's patients. The irrationality of the procedure delays a therapeutic advantage and ought to be fully revised. Copyright Copyright 2008 Elsevier Ireland Ltd. All rights reserved.

Ozone and ozonated oils in skin diseases: a review. [Review]
Travagli V. Zanardi I. Valacchi G. Bocci V.
[Journal Article. Review]
UI: 20671923
Authors Full Name
Travagli, V. Zanardi, I. Valacchi, G. Bocci, V.
[View Annotation(s)]
[View Abstract]
AB Although orthodox medicine has provided a variety of topical anti-infective agents, some of them have become scarcely effective owing to antibiotic- and chemotherapeutic-resistant
pathogens. For more than a century, ozone has been known to be an excellent disinfectant that nevertheless had to be used with caution for its oxidizing properties. Only during the last decade it has been learned how to tame its great reactivity by precisely dosing its concentration and permanently incorporating the gas into triglycerides where gaseous ozone chemically reacts with unsaturated substrates leading to therapeutically active ozonated derivatives. Today the stability and efficacy of the ozonated oils have been already demonstrated, but owing to a plethora of commercial products, the present paper aims to analyze these derivatives suggesting the strategy to obtain products with the best characteristics.

8. [ ] The irrationality of a non-specific immunomodulation therapy used in cardiovascular diseases deserves a critical comment.
   Bocci V. Zanardi I. Travagli V.

8. [ ] Are dialysis devices usable as ozone gas exchangers?
   Travagli V. Zanardi I. Gabbrielli A. Paccagnini E. Bocci V.

9. [ ] A study aimed to compare the efficiency of the ozone transfer of four hydrophilic dialysis filters, and one hydrophobic gas-exchange device (GED) has been performed. Obviously, the former should be specifically used only for dialysis. Unfortunately, some clinicians incautiously use them as GEDs. It has been shown that: (i) dialysis filters present a wide range of gas-exchange yield (from 0 up to 70%), often related to variability according to the treatment time; (ii) by scanning microscopy, it has been noticed that hollow fibers are somewhat altered by ozone; and (iii) because their constitutive materials may not be ozone-resistant, they may release toxic compounds harmful for the patients. On the contrary, the appropriate GED is ozone-transfer efficient, is ozone-resistant, and is suitable for
blood autotransfusion and ozonation.

10. [ ]

**Effects of ozone blood treatment on the metabolite profile of human blood.**
Travaglì V. Zanardi I. Bernini P. Nepi S. Tenori L. Bocci V.
[Journal Article]
UI: 20335512
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Metabonomic characterization of the effects caused by ozone and other stressors on normal human blood was performed. Samples of blood obtained from healthy subjects were treated ex vivo with increasing concentrations of ozone and/or with UV radiation and heat. (1)H-NMR analysis of plasma samples after treatments showed the quantitative variation of some metabolites and the formation of new metabolites normally absent. Both the increment of some metabolites like formate, acetoacetate, and acetate and the decrement of pyruvate were of particular interest. Moreover, the oxidation of ascorbic acid and the transformation of uric acid into allantoin after ozonation within the therapeutic concentration range were observed. In the ozonated spectra, 2 unidentified peaks appeared at 2.82 ppm and 8.08 ppm. They are related to the direct antioxidant activity of albumin in the presence of ozone and they could be considered as specific markers of the blood ozonation.

11. [ ]

**Properties of sesame oil by detailed 1H and 13C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements.**
Sega A. Zanardi I. Chiasserini L. Gabbrielli A. Bocci V. Travaglì V.
[Journal Article]
UI: 19900426
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Gaseous ozone chemically reacts with unsaturated triglyceride substrates leading to ozonated derivatives with a wide potential applications, ranging from the petrochemical to the pharmaceutical industry. To date, an ultimate understanding of the ozone reactivity during sesame oil ozonation process as well as detailed (1)H and (13)C NMR assignments are lacking. A practical advantage of NMR is that a single NMR sample measurement can explain many issues, while similar analysis by traditional methods may require several independent and time-consuming measurements. Moreover, significant
relationships among NMR spectra and both conventional chemical analysis and viscosity measurements have been found. Eventually, NMR could play an important role for quality attributes of ozonated oil derivatives.

Oxygen-ozone therapy in medicine: an update.
Bocci V. Di Paolo N.
[Journal Article]
UI: 19752547
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Oxygen-ozone therapy, initially started as an empirical approach, has now reached a stage where most of the biological mechanisms of action of ozone have been clarified, showing that they are in the realm of orthodox biochemistry, physiology and pharmacology. Here we have reviewed a few relevant clinical applications and have shown that ozone therapy is particularly useful in cardiovascular disorders and tissue ischemia. In chronic viral infections, it is unable to eliminate the viremia but it may display supportive help by stimulating the immune system. Recently, its use has been successfully extended to the herniated disk pathology and therapy of primary caries in children. Copyright 2009 S. Karger AG, Basel.

Randomised, double-blinded, placebo-controlled, clinical trial of ozone therapy as treatment of sudden sensorineural hearing loss.
Bocci V. Travagli V. Zanardi I.
[Comment. Letter]
UI: 19379546
Authors Full Name
Bocci, V. Travagli, V. Zanardi, I.
[View Annotation(s)]
My Projects Annotate

Topical applications of ozone and ozonated oils as anti-infective agents: an insight into the patent claims. [Review]
[113 refs]
Travagli V. Zanardi I. Bocci V.
[Journal Article. Review]

• Abstract Reference
• Complete Reference
• [Find Similar]
• [Find Citing Articles]

My Projects Annotate
Orthodox medicine has been very active in the field of topical anti-infective agents and current chemotherapy has procured valid antibiotics, antivirals, vaccines, antibodies, and antiparasitic drugs to be parenterally and/or topically used. However, these drugs may cause side effects and sometimes provide unsatisfactory results because pathogens become drug-resistant. Another drawback is represented by their cost, which compromise their use or their availability in poor Countries. Therefore, there is a critical need for new strategies to treat dermatological affections. The old intuition for using ozone in the treatment of necrotic wounds, especially if due to anaerobic bacteria, is now justified by the studies about reactive oxygen species generation by granulocytes and macrophages as the first line of defense during an infection. As a consequence, the disinfectant value of ozone has been increasingly appreciated during the last fifteen years. This review summarizes the patents filed and issued, with particular emphasis to the more recent patents, about the anti-infective topical use of ozone: i) in the gaseous form; ii) after gaseous ozone saturation of aqueous, not-oily pharmaceutical vehicles and solvents; iii) where gaseous ozone chemically reacts with unsaturated substrates leading to therapeutically active ozonated derivatives. We hope that recent advances and a better understanding of the ozone chemistry and biology will be able to create the mental attitude to prove the validity of large-scale therapeutic use of both ozone and ozone derivatives as topical anti-infective agents by performing multicenter, randomized clinical studies, as aptly requested by orthodox medicine.

References: 113

May oxygen-ozone therapy improves cardiovascular disorders?. [Review] [112 refs]
Bocci V. Travagli V. Zanardi I.
[Journal Article. Review]
UI: 19519366

AB In an aging population vascular disorders well exemplified by the chronic limb ischemia, chronic heart failure, cerebral ischemia and age-related macular degeneration represent a
serious medical and socio-economical problem. While there is always a not easily identifiable first pathogenic noxa, all of these diseases are characterized by ischemia, chronic inflammation and tissue degeneration. Orthodox medicine has provided several optimal drugs targeting various pathological situations but, even with their concomitant applications, it is not possible to reduce the chronic oxidative stress. Here it is proposed to associate the approach of ozonated autohemotherapy as a modifier of the biological response capable to block the pathological progress. [References: 112]

The ozone paradox: ozone is a strong oxidant as well as a medical drug. [Review] [236 refs]
Authors Full Name
AB After five decades characterized by empiricism and several pitfalls, some of the basic mechanisms of action of ozone in pulmonary toxicology and in medicine have been clarified. The present knowledge allows to understand the prolonged inhalation of ozone can be very deleterious first for the lungs and successively for the whole organism. On the other hand, a small ozone dose well calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms and reactivate the antioxidant system. In detail, firstly ex vivo and second during the infusion of ozonated blood into the donor, the ozone therapy approach involves blood cells and the endothelium, which by transferring the ozone messengers to billions of cells will generate a therapeutic effect. Thus, in spite of a common prejudice, single ozone doses can be therapeutically used in selected human diseases without any toxicity or side effects. Moreover, the versatility and amplitude of beneficial effect of ozone applications have become evident in orthopedics, cutaneous, and mucosal infections as well as in dentistry. [References: 236]

How much ozone bactericidal activity is compromised by plasma components?.

16. [ ]
17. [ ]
AB AIMS: Evaluation of bactericidal effect of different concentrations of ozone when used (a) as a gas, or (b) dissolved in saline. The addition of hydrogen peroxide or 4-hydroxynonenal dissolved in saline was also tested, as well as the effect of human plasma.

METHODS AND RESULTS:

Staphylococcus aureus, methicillin-resistant Staph. aureus (MRSA), and Pseudomonas aeruginosa, suspended in their culture media were tested. While all bacteria suspended in protein-free saline were killed at high ozone concentrations, they survived when as little as 5% human plasma was present. Hydrogen peroxide was 100-fold less active than ozone and needed to remain in contact with bacteria for at least 60 min. 4-hydroxynonenal (2 micromol l(-1)) was inhibitory for proliferation of both Staph. aureus and MRSA, but not for Ps. aeruginosa. CONCLUSIONS: Ozone and the cascade of its derivative products are potent bactericidal agents, but even small amounts of human plasma, hence of hydro- and liposoluble antioxidants, in bacterial suspensions inhibit oxidation and protect bacteria.

SIGNIFICANCE AND IMPACT OF THE STUDY: Any substantial in vivo cytocidal effect of ozone and its derivatives can be excluded. On the other hand, topical and continuous action of various ozone preparations remains valuable in a variety of skin and mucosal infections.

Physico-chemical characterization of sesame oil derivatives.
Zanardi I. Travagli V. Gabbrielli A. Chiasserini L. Bocci V.
Lipids. 43(9):877-86, 2008 Sep.
[Evaluation Studies. Journal Article]
UI: 18679737

AB Ozone treatment of commercially available vegetable oils gives rise to the formation of chemical species that are responsible for the therapeutic properties of ozonated oil derivatives in dermatological diseases. In the last years, these products have been successfully used as a topical disinfectant in a number of serious skin affections. The medical application of empirically prepared ozonated oil has yielded striking improvements with unexpected and rapid healing, compelling us to begin a long-range study aiming first to define the main characteristics of the most common ozonated vegetable oils,
about which there is usually no medical consensus because of the lack of standardization of their technological parameters. Sesame oil was selected because of its great amount of polyunsaturated acyl groups, as well as natural antioxidants. Moreover, we have determined the kinetics and optimal conditions of ozonation (e.g., ozone concentrations, time of exposure, temperature) for obtaining an ozonated oil characterized by well-established technological and physico-chemical properties, namely an accurate peroxide value determination. On the basis of the results, we have gained an understanding of the modifications of the vegetable oils during the ozonation process.

**My Projects** Annotate

**Does ozone really "cure" cancer?**
Bocci V.
[Comment. Letter]
UI: 18537157
Authors Full Name
Bocci, Velio.
[View Annotation(s)]
**My Projects** Annotate

**Non-specific immunomodulation in chronic heart failure.**
Bocci V.
[Comment. Letter]
UI: 18572074
Authors Full Name
Bocci, Velio.

**Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice.**
Valacchi G. Lim Y. Belmonte G. Miracco C. Zanardi I. Bocci V. Travagli V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 21134039
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Ozone is well recognized as a bactericidal agent and its beneficial effect on wound healing could be a
consequence of this property. Because ozone itself does not penetrate the cells but immediately reacts with polyunsaturated fatty acids, its effects should be the results of oxidative reaction. For this reason, ozonated oils could be a way to deliver ozone messengers to the skin. This paper evaluated the therapeutic effects of three different grades of ozonated sesame oil in acute cutaneous wounds made in the skin of SKH1 mice. Specifically, wound closure rate, histological parameters, and the level of key proteins such as vascular endothelial growth factors and cyclin D1 have been analyzed in relation to the peroxide level present in the ozonated oil. Treatment with moderately ozonated sesame oil—expressed as peroxide value about 1,500)—has a faster wound closure rate in the first 7 days than treatment with oil containing either lower or higher peroxide value, and even with controls. Moreover, under the same treatment, an earlier and higher response of cells involved in wound repair, a higher angiogenesis, as well as an enhanced vascular endothelial growth factors and cyclin D1 expression were observed. The present study shows the validity of ozonated sesame oil in cutaneous wound healing and emphasizes the importance of the ozonation grade.

May oxygen-ozone therapy improves cardiovascular disorders?. [Review] [112 refs]
Bocci V. Travagli V. Zanardi I.
[Journal Article. Review]
UI: 19519366
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB In an aging population vascular disorders well exemplified by the chronic limb ischemia, chronic heart failure, cerebral ischemia and age-related macular degeneration represent a serious medical and socio-economical problem. While there is always a not easily identifiable first pathogenic noxa, all of these diseases are characterized by ischemia, chronic inflammation and tissue degeneration. Orthodox medicine has provided several optimal drugs targeting various pathological situations but, even with their concomitant applications, it is not possible to reduce the chronic...
oxidative stress. Here it is proposed to associate the approach of ozonated autohemotherapy as a modifier of the biological response capable to block the pathological progress. [References: 112]

My Projects Annotate

Ozonation of human blood induces a remarkable upregulation of heme oxygenase-1 and heat stress protein-70.
[Journal Article]
UI: 18274635
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB Heme oxygenase-I (HO-1) has emerged as one of the most protective enzymes and its pleiotropic activities have been demonstrated in a variety of human pathologies. Unpublished observations have shown that HO-1 is induced after the infusion of ozonated blood into the respective donors, and many other experimental observations have demonstrated the efficacy of oxidizing agents. It appeared worthwhile to evaluate whether we could better define the activity of potential inducers such as hydrogen peroxide and ozonated human plasma. Human vascular endothelial cells at confluence were challenged with different concentrations of these inducers and the simultaneous production of nitric oxide (NO); and HO-1 was measured by either measuring nitrite, or bilirubin formation, or/and the immune reactivity of the protein by Western blot using a rabbit antihuman HO-1 and Hsp-70. The results show that production of both NO and HO-1 is fairly dose dependent but is particularly elevated using human plasma after transient exposure to a medium ozone concentration. At this concentration, there is also induction of Hsp-70. The results clarify another positive effect achievable by the use of ozone therapy.
My Projects Annotate

A physicochemical investigation on the effects of ozone on blood.
AB Ozonation of either human whole blood or saline-washed erythrocytes causes considerable damage to the latter and this result has opened a controversy. With the benefit of hindsight, it appears logical that once erythrocytes are deprived of the potent antioxidants of plasma, they become very sensitive to the oxidant effects of ozone. The aim of the present work was to perform a physical-chemical evaluation of some critical parameters able to clarify this issue. We have ascertained that when whole blood is exposed to the appropriate ozone doses used in human therapy, no damage ensues while saline-washed erythrocytes undergo conspicuous haemolysis. The dogma that ozone is always toxic is incorrect because its reactivity below the concentration of 80 μg/mL can be controlled by the plasmatic antioxidant system.

My Projects  Annotate

Bocci V. Zanardi I. Travagli V. Di Paolo N.
Artificial Organs. 31(9):743-8, 2007 Sep.
[Journal Article]
UI: 17725702
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB We have investigated the performance of a new gas exchange device (GED), named L001, specifically devised for the ozonation of human blood during extracorporeal circulation. This procedure, defined with the acronym "EBOO," means "extracorporeal blood oxygenation-ozonation." The innovative GED is made of microporous, ozone-resistant, polipropylene hollow fibers with an external diameter of 200 microm, a thickness of 50 microm, and a membrane surface area of 0.22 m². The material is coated with phosphorylcholine on the external side in contact with the circulating blood, while a gas mixture, necessarily
composed of medical oxygen and ozone (about 99 and 1%, respectively), flows inside the fibers in opposite direction. The new GED has been tested by using a buffered saline solution containing KI and by varying several parameters, and it has shown to be very versatile and efficient. Its main characteristics are minimal foreign surface contact, high gas transfer, and negligible priming volume. This device appears to be a practical, nontoxic, and rather inexpensive tool for performing ozonation of blood for already defined human diseases.

My Projects Annotate

The case for oxygen-ozonetherapy. [Review] [76 refs]
Bocci V.
[Journal Article. Review]
UI: 17444419
Authors Full Name
Bocci, V.
[View Annotation(s)]
[View Abstract]
AB Ozone is a very reactive gas that is toxic to the respiratory system. However, under controlled conditions, it can be therapeutically useful in several human diseases. An unfavourable combination of factors (ozone is one of the worst troposphere pollutants) and past misuse have led to misgivings about ozonetherapy. However, basic and clinical work developed over the past 10 years has clarified the fundamental mechanisms of action of ozone in biology and medicine. Interestingly, judicious doses of ozone dissolved in blood trigger a cascade of well-defined chemical compounds acting on multiple cellular targets according to well-known molecular, biochemical and pharmacological pathways. Ozonetherapy is proving to be very useful in age-related macular degeneration, ischaemic and infectious diseases, and in wound healing disorders, where conventional medicine has failed. Critical evaluation of the potential therapeutic utility of this simple, inexpensive medical application by national and international health authorities is warranted and may lead to clinical benefit for a large proportion of the world's population. [References: 76]
My Projects Annotate

A realistic evaluation of the action of ozone on whole human blood.
27. [ ] Travagli V. Zanardi I. Bocci V.

• Abstract Reference
• Complete Reference
• [Find Similar]
• [Find Citing Articles]
We have clarified the role of the ozone concentration in relation to the resistance of human erythrocytes in whole human blood or in blood diluted either in saline or in distilled water. Spectrophotometric data related to haemoglobin were evaluated by exposing samples of fresh human blood directly to ozone doses (ratio 1:1 volume), within the therapeutic range (0.21-1.68 mM) and to one toxic dose (3.36 mM). Furthermore, the same determinations have been carried out after previous dilution of the same blood with either pure water or physiological saline (1 ml blood + 29 ml diluent) followed by ozonation with the above reported ozone doses. Addition of either saline or water implies a dilution of plasma antioxidants and also total haemolysis after water dilution. Particularly the latter case represents a most unphysiological situation because the osmotic shock causes the solubilization of the erythrocytic content. While it is possible to demonstrate that after haemolysis there is an ozone-concentration dependent transformation of some oxyhaemoglobin to methaemoglobin, no such process occurs after ozonation of whole blood. The results of this study fully confirm our previous data that judicious ozone doses neither damage erythrocytes, nor induce oxidation of intracellular haemoglobin. We hope that our conclusions will definitively clarify the absence of toxicity of ozonotherapy.
showing that exposure by inhalation to prolonged tropospheric ozone damages the respiratory system and extrapulmonary organs. The skin, if extensively exposed, may also contribute to the damage. The undoubtful strong reactivity of ozone has contributed to establish the dogma that ozone is always toxic and its medical application must be proscribed. Although it is less known, judiciously practiced ozonetherapy is becoming very useful either on its own or applied in combination with orthodox medicine in a broad range of pathologies. The opponents of ozonetherapy base their judgment on the ozone chemistry, and physicians, without any knowledge of the problem, are often skeptical. During the last 15 years, a clear understanding of the action of ozone in biology and medicine has been gained, allowing today to argue if it is true that ozone is always toxic. The fundamental points that are discussed in this paper are: the topography, anatomical and biochemical characteristics of the organs daily exposed to ozone versus the potent antioxidant capacity of blood exposed to a small and precisely calculated dose of ozone only for a few minutes. It is becoming clear how the respiratory system undergoing a chronic oxidative stress can release slowly, but steadily, a huge amount of toxic compounds able to enter the circulation and cause serious damage. The aim of this paper is to objectively evaluate this controversial issue. [References: 175]

Biochemical modifications induced in human blood by oxygenation-ozonation.
Bocci V. Aldinucci C.
Journal of Biochemical & Molecular Toxicology. 20(3):133-8, 2006.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 16788956
Authors Full Name
Bocci, Velio. Aldinucci, Carlo.

AB Some biochemical effects determined on human blood after addition of a gas mixture composed of oxygen (approximately 96%) and ozone (approximately 4%) have been evaluated. Ozone was used in a mild concentration ranging between 0.21 and 1.68 mM. Within few minutes after rapid mixing of the equal gas-liquid volumes, the ozone was consumed because by instantaneously reacting with biomolecules, generating
reactive oxygen species (particularly hydrogen peroxide) having very short lifetime and lipid oxidation products. The following results are oxygen-ozone dose dependent: (1) The pO(2) values have risen from about 40 up to 400 mmHg. (2) By testing the highest ozone concentration, the total antioxidant capacity of blood decreased within 1 min from 1.35 to 0.91 mM but regained its normal values within 20 min owing to the rapid reduction of oxidized antioxidants operated by erythrocytes. (3) Similarly, intraerythrocytic reduced glutathione after ozonation decreased from the initial value of 5.71 to 4.56 micromol/g Hb. (4) Both hemolysis and methemoglobin showed a negligible increase. (c) 2006 Wiley Periodicals, Inc.

**How an ill-conceived methodological approach can condemn the medical use of ozone therapy.**
Bocci V. Travagli V.
International Journal of Biological Macromolecules. 37(5):287-8; author reply
[Comment. Letter]
UI: 16413051
Authors Full Name
Bocci, V. Travagli, V.

**The dual action of ozone on the skin.** [Review] [47 refs]
Valacchi G. Fortino V. Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't.
Review]
UI: 16307642
Authors Full Name
Valacchi, G. Fortino, V. Bocci, V.
[View Annotation(s)]
[View Abstract]
31. [ ] AB The aim of this brief review is to summarize the recent literature on the effect of ozone (O3) on cutaneous tissues. Recently it has been reported that a chronic contact with O3 can be deleterious for the skin. Our group and others have shown a progressive depletion of antioxidant content in the stratum corneum and this can then lead to a cascade of effects resulting in an active cellular response in the deeper layers of the skin. Using an in vivo model we have shown an increase of proliferative, adaptive and proinflammatory cutaneous tissue responses. On the other hand the well known activity of O3 as a potent disinfectant and
oxygen (O2) donor has been also studied for therapeutic use. Two approaches have been described. The first consists of a quasi-total body exposure in a thermostatically controlled cabin. This treatment has proved to be useful in patients with chronic limb ischaemia. The second approach is based on the topical application of ozonated olive oil in several kinds of skin infection (from soreness to diabetic ulcers, burns, traumatic and surgical wounds, abscesses and skin reactions after radiotherapy). We and other authors have observed a striking cleansing effect with improved oxygenation and enhanced healing of these conditions. It is now clear that, on the skin, O3, like other drugs, poisons and radiation, can display either a damaging effect from a long exposure or a beneficial effect after a brief exposure to O2 and O3 or to the application of ozonated oil to chronic wounds. [References: 47]

Extracorporeal blood oxygenation and ozonation (EBOO): a controlled trial in patients with peripheral artery disease.

Di Paolo N. Bocci V. Salvo DP. Palasciano G. Biagioli M. Meini S. Galli F. Ciari I. Maccari F. Cappelletti F. Di Paolo M. Gaggiotti E.

[Journal Article. Randomized Controlled Trial. Research Support, Non-U.S. Gov't]
UI: 16288443

Authors Full Name
Di Paolo, N. Bocci, V. Salvo, D P. Palasciano, G. Biagioli, M. Meini, S. Galli, F. Ciari, I. Maccari, F. Cappelletti, F. Di Paolo, M. Gaggiotti, E.

AB BACKGROUND: Since 1990 our group has been using extracorporeal circulation to ozonate blood by an original method, known as extracorporeal blood oxygenation and ozonation (EBOO), with the aim of amplifying the results observed with ozone autohemotherapy. OBJECTIVE: To verify the hypothesis that EBOO improves the skin lesions typical of peripheral artery disease (PAD) patients.

METHODS: Twenty-eight patients with PAD were randomized to receive EBOO or intravenous prostacyclin in a controlled clinical trial. The primary efficacy parameters were regression of skin lesions and pain, and improvement in quality of life and
vascularisation. RESULTS: Patients treated with EBOO showed highly significant regression of skin lesions with respect to patients treated with prostacyclin. Other parameters that were significantly different in the two groups of patients were pain, pruritus, heavy legs and well-being. No significant differences in vascularisation of the lower limbs before and after treatment were found in either group. No side effects or complications were recorded during the 210 EBOO treatments.

CONCLUSION: EBOO was much more effective than prostacyclin for treating skin lesions in PAD patients and also had a positive effect on patient general condition without any apparent change in arterial circulation. This suggests other mechanisms of action of EBOO.

Can the combination of localized "proliferative therapy" with "minor ozonated autohemotherapy" restore the natural healing process?

AB Regenerative injection therapy (RIT), also known as proliferative therapy, has been used for over 30 years in the USA in patients with spinal and peripheral joint and ligamentous pathologies. It involves the injection of mildly irritating medications onto ligaments and tendons, most commonly at origins and insertions. These injections cause a mild inflammatory response which "turns on" the normal healing process and results in the regeneration of these structures. At the same time they strengthen and become less sensitive to pain through a combination of neurolysis of small nerve fibers (C-fibers) and increased stability of the underlying structures. Oxygen/ozone therapy is a well established complementary therapy practiced in many European countries. The ozone dissolves in body fluids and immediately reacts with biomolecules generating messengers responsible for biological and therapeutic activities. This results in an anti inflammatory response, which also results in a similar trophic reaction to that of RIT. It is logical to expect that combining these two modalities would result in enhanced healing and
therefore improved clinical outcomes. Oxygen/ozone therapy, accomplished by autohemotherapy (AHT), is performed by either administering ozonated blood intravenously (Major AHT) or via intramuscular route (Minor AHT). These procedures result in stimulation of the immune and healing systems. Our concept is that the local injection of this activated blood injected directly to the ligamentous areas that are also being treated with RIT will act as a direct stimulation to the healing process. In addition, combining this with intravenous major AHT should stimulate the immune system to augment and support this process. RIT and oxygen/ozone therapy have been extensively studied separately. We propose a study of lumbosacral ligamentous pain to explore this therapeutic combination. We hope that this paper will stimulate general interest in this area of medicine and result in investigation of the "interface" between these treatment modalities.

My Projects Annotate

Major ozonated autohemotherapy in chronic limb ischemia with ulcerations.
de Monte A. van der Zee H. Bocci V.
[Case Reports. Journal Article]
UI: 15865505
Authors Full Name
de Monte, Amato. van der Zee, Hoyte. Bocci, Velio.
View Annotation(s)]
View Abstract
AB This paper reports the beneficial effects of ozone autohemotherapy (OHT) in 2 patients afflicted with painful, intractable leg ulcers. One patient had diabetes mellitus type II (DM), the other had vasculitis. Both patients had seen multiple specialists, including a dermatologist, an internist, and a vascular surgeon, but their clinical course continued to worsen. When the pain became intolerable, the patients came to our pain clinic. Chemical lumbar sympathectomy as well as epidural blockade with bupivacaine and morphine were moderately effective in reducing their pain but had no effect on the ulcers. Only after OHT treatments were performed for several months was satisfactory healing observed.
My Projects Annotate

35. [ ] Restoration of normoxia by ozone therapy may control
neoplastic growth: a review and a working hypothesis. [Review] [116 refs]
Bocci V. Larini A. Micheli V.
[Journal Article. Research Support, Non-U.S. Gov't. Review]
UI: 15865491
Authors Full Name
Bocci, Velio. Larini, Alessandra. Micheli, Vanna.
[View Annotation(s)]
[View Abstract]
AB In contrast to normal tissues, tumors thrive in hypoxic environments. This appears to be because they can metastasize and secrete angiopoietins for enhancing neoangiogenesis and further tumor spread. Thus, during chronic ischemia, normal tissues tend to die, while neoplasms tend to grow. During the past two decades, it has been shown in arteriopathic patients that ozonated autohemotherapy is therapeutically useful because it increases oxygen delivery in hypoxic tissues, leading to normoxia. Although several oxygenation approaches have been tested, none is able to restore normoxia permanently in patients with cancer. We postulate that a prolonged cycle of ozonated autohemotherapy may correct tumor hypoxia, lead to less aggressive tumor behavior, and represent a valid adjuvant during or after chemo- or radiotherapy. Moreover, it may re-equilibrate the chronic oxidative stress and reduce fatigue.
[References: 116]

Effects of ozone on isolated peripheral blood mononuclear cells.
Larini A. Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 15582356
Authors Full Name
Larini, A. Bocci, V.
36. [ ] [View Annotation(s)]
[View Abstract]
AB We have investigated the release of cytokines from isolated peripheral human blood mononuclear cells (PBMC) exposed to various ozone concentrations for 10 min and the release of both proinflammatory and immunosuppressive cytokine after 24, 48 and 76 h incubation. Ozonation was performed by exposing for 10 min equal cell numbers and volumes of cell
suspension to equal volumes of a gas mixture (1:1 ratio) composed of oxygen-ozone with precise ozone concentrations ranging from 1.0 up to 80 microg/ml (0.02 up to 1.68 mM). Markers of oxidative stress showed a significant relationship between ozone doses and both lipid peroxidation and protein thiol groups content. With the exception of the lowest ozone concentration, the cytokine production of PBMC was depressed particularly at concentrations from 40 mug/ml upwards. There was no significant effect on IL-6 production between exposed or unexposed cells, up to 72 h of incubation. IL-4 production was markedly affected by ozone exposure, showing a marked decrease even at the lowest ozone concentration (2.5 microg/ml) already after 24 h incubation. On the other hand, production of IFN-gamma and TNF-alpha was slightly stimulated by the lowest ozone dose either at all times or only after 72 h incubation, respectively. Analysis of the proliferation index (PI) is consistent with these results showing that, while the lowest concentration stimulates it, progressively increasing O(3) concentrations inhibit the PI. These data show that there is a significant relationship between cytokine production and ozone concentrations and that PBMC are very sensitive to oxidation particularly in presence of serum with low antioxidant capacity.

My Projects Annotate

Rational bases for using oxygen-ozonetherapy as a biological response modifier in sickle cell anemia and beta-thalassemia: a therapeutic perspective.

Bocci V. Aldinucci C.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 15323359
Authors Full Name
Bocci, V. Aldinucci, C.
37. [ ] [View Annotation(s)]
[View Abstract]
AB We are proposing to evaluate whether a complementary approach based on cycles of oxygen-ozone autohemotherapy (O3-AHT) already performed in millions of patients, can abate the chronic oxidative stress and improve the quality of life of serious hemoglobinopathic patients. Although a preliminary study has yielded encouraging results, it appears appropriate to perform a controlled, randomized and possibly multicentre clinical trial. The long use of this
approach in other pathologies has proved to be very useful and it is hoped that scepticism will not prevail over scientific rationale. Ozone, as any other drug, has an intrinsic toxicity that, in the proposed application, is fully tamed by the blood antioxidant system.

**Ozone as Janus: this controversial gas can be either toxic or medically useful. [Review] [76 refs]**
Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't.
Review]
UI: 15203558
Authors Full Name
Bocci, Velio.
[View Annotation(s)]
[View Abstract]
AB Ozone is an intrinsically toxic gas and its hazardous employment has led to a poor consideration of ozone therapy. The aim of this review is to indicate that a wrong dogma and several misconceptions thwart progress: in reality, properly performed ozone therapy, carried out by expert physicians, can be very useful when orthodox medicine appears inadequate. The unbelievable versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of a pathological state. During the past decade, contrary to all expectations, it has been demonstrated that the judicious application of ozone in chronic infectious diseases, vasculopathies, orthopedics and even dentistry has yielded such striking results that it is deplorable that the medical establishment continues to ignore ozone therapy. [References: 76]

**Ozone therapy. [Review] [59 refs]**
Di Paolo N. Bocci V. Gaggiotti E.
[Editorial. Review]
UI: 15112882
Authors Full Name
Di Paolo, N. Bocci, V. Gaggiotti, E.
[View Annotation(s)]
My Projects Annotate
The ozone tolerance: Enhancement of antioxidant enzymes is ozone dose-dependent in Jurkat cells.
Larini A. Bianchi L. Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 14703728
Authors Full Name
Larini, A. Bianchi, L. Bocci, V.
[View Annotation(s)]
[View Abstract]
AB We have begun to examine the biological and toxic effects of ozone on Jurkat T cells incubated thereafter for 24, 48 and 72 h. Tissue culture medium was strengthened by adding 20% fetal calf serum with an albumin content of about 6 mg/ml. Ozonization was performed by exposing for 10 min a volume of cell suspension (4 x 10^5/ml) to an equal volume of a gas mixture composed of oxygen-ozone with precise ozone concentrations ranging from 1.5 up to 72 microg/ml (31.5-1512 microM). The proliferation index declined progressively and was ozone dose-dependent. The response of enzymatic activities varied depending upon the enzyme and ozone concentrations: glucose-6-phosphate dehydrogenase begins to increase at an ozone dose of 6 microg/ml (126 microM), reached a peak at 12 microg/ml (252 microM) and rapidly declined thereafter. On the other hand activities of superoxide dismutase, glutathione peroxidase and glutathione reductase increased progressively from the ozone concentration of 12 microg/ml. Thus, as we have observed in blood, the biological response is linked to the ozone dose that must reach a threshold to be effective.

Necrotizing fasciitis successfully treated with extracorporeal blood oxygenation and ozonization (EBOO).
Di Paolo N. Bocci V. Cappelletti F. Petrini G. Gaggiotti E.
[Case Reports. Journal Article. Research Support, Non-U.S. Gov't]
UI: 12518965
Authors Full Name
Di Paolo, N. Bocci, V. Cappelletti, F. Petrini, G. Gaggiotti, E.
[View Annotation(s)]
[View Abstract]
AB A case of necrotizing fasciitis in a dialysis patient is described. Since traditional therapies were unsuccessful, extracorporeal blood oxygenation and ozonation (EBOO) was tried. This technique is no longer in the experimental stage and is used routinely in our hospital. Patient condition improved radically after EBOO.
Ozonation of blood during extracorporeal circulation. II. Comparative analysis of several oxygenator-ozonators and selection of one type.

Bocci V. Di Paolo N. Borrelli E. Larini A. Cappelletti F.
[Comparative Study. In Vitro. Journal Article. Research Support, Non-U.S. Gov't]
UI: 11831595

AB Exposure of human blood ex vivo to oxygen-ozone (O2-O3) using either dialysis exchangers or normal oxygenators gives rise to a number of problems, one of which linked to platelet activation, leads to rapid occlusion and no gas exchange. Semipermeable membranes were found unsuitable because, except for one, they were gas-transfer inefficient, allowed ultrafiltration and were more or less vulnerable to O3. Over the last three years we have examined several types of hydrophobic O3-resistant hollow fiber capillaries but, if the polypropylene surface is not properly coated, there is platelet aggregation and blood coagulation. These problems while far less relevant with O2 alone, become prohibitive in the presence of ozone. Recently new oxygenators have been prepared with special materials to make them more biocompatible and it has become possible to oxygenate and ozonate up to 5L of blood in about an hour, thus making the treatment of critical patients feasible.


Valacchi G. Bocci V.
Mediators of Inflammation. 9(6):271-6, 2000.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 11213910

AB BACKGROUND: Empirical observations have shown that ozonated autohemotherapy markedly
improves the symptoms of chronic limb ischemia (muscular pain at rest, intermittent claudication, etc) in atherosclerotic patients, but mechanisms of action remain unclear. AIMS: Human endothelial cells (HUVECs) are known to release nitrogen monoxide (NO) and we investigated the biological effects of human ozonated serum on HUVECs in culture.

METHODS: We assessed the relevance of peroxidation, the release of NO as nitrite and of three classical cytokines. RESULTS: The treatment of HUVECs with ozonated serum yields a dose dependent increase of thiobarbituric acid reactive substances (TBARS) and of hydrogen peroxide (H2O2) and a decrease of protein thiol groups (PTG). Concomitantly, in comparison to either the control or the oxygenated sample, there is a significant and steady increase of nitric oxide (NO) production; this is markedly enhanced by the addition of L-arginine (20 microM) and inhibited in the presence of the NO inhibitor, L-NAME (20 mM). The main mediator of ozone action is H2O2 as it has been shown either after its direct measurement or by the addition of 20, 40 and 100 microM. Moreover, during 24 hours incubation we have investigated the production of endothelin 1 (ET-1), E-selectin and Interleukin 8 (IL-8) and it appears that ozonation enhances IL-8, inhibits E-selectin and hardly modifies ET-1 production.

CONCLUSIONS: It appears that reinfusion of ozonated blood, by enhancing release of NO, may induce vasodilation in ischemic areas and reduce hypoxia.

**My Projects** Annotate

**Extracorporeal blood oxygenation and ozonation (EBOO) in man. preliminary report.**
Di Paolo N. Bocci V. Garosi G. Borrelli E. Bravi A. Bruci A. Aldinucci C. Capotondo L.
[Case Reports. Journal Article]
UI: 10741810
Authors Full Name
Di Paolo, N. Bocci, V. Garosi, G. Borrelli, E. Bravi, A. Bruci, A. Aldinucci, C. Capotondo, L.
[View Annotation(s)]
[View Abstract]
AB Autohemotherapy with ozone has been used for four decades with encouraging results but, owing to the lack of clinical studies, it has never been adopted by orthodox medicine. Confident of the valid principles of ozone therapy, we have endeavoured to increase its
therapeutic efficacy. Over a ten-year period we have developed an apparatus that makes it possible to treat large quantities of blood with ozone in extracorporeal circulation (extracorporeal blood oxygenation and ozonation EBOO). One of us volunteered to test the system and after six treatments noted the disappearance of two lipomas. This prompted us to treat a patient with Madelung disease and several patients with atherosclerotic vasculopathy. Besides showing therapeutic effects, the preliminary results indicate that EBOO is clinically valid, without side-effects and worthy of testing in various diseases.

Biological and clinical effects of ozone. Has ozone therapy a future in medicine?. [Review] [101 refs]
Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't. Review]
UI: 10795372
Authors Full Name
Bocci, V.
[View Annotation(s)]
[View Abstract]
AB Although ozone therapy has been used as an alternative medical approach for four decades, it has encountered scepticism, if not outright objection, by orthodox medicine. This prejudice is not unjustified because ozone therapy often has been used without rational basis or appropriate controls. With the advent of precise medical ozone generators, it is now possible to evaluate some mechanisms of action and possible toxicity. In contrast with the respiratory tract, human blood exposed to appropriate ozone concentrations is able to tame its strong oxidant properties and neither acute nor chronic side effects have ensued in millions of patients treated with ozonated autohaemotherapy. This paper summarises studies aimed at clarifying biological effects, defining any possible damage, the therapeutic window, and suitable doses able to express therapeutic activity. Although an unfashionable and unpopular approach, it is hoped that orthodox medicine will help to critically assess the validity of ozone therapy.

Prevention of renal injury after induction of ozone
AB On the basis that ozone (O3) can upregulate cellular antioxidant enzymes, a morphological, biochemical and functional renal study was performed in rats undergoing a prolonged treatment with O3 before renal ischaemia. Rats were divided into four groups: (1) control, a medial abdominal incision was performed to expose the kidneys; (2) ischaemia, in animals undergoing a bilateral renal ischaemia (30 min), with subsequent reperfusion (3 h); (3) O3 + ischaemia, as group 2, but with previous treatment with O3 (0.5 mg/kg per day given in 2.5 ml O2) via rectal administration for 15 treatments; (4) O2 + ischaemia, as group 3, but using oxygen (O2) alone. Biochemical parameters as fructosamine level, phospholipase A, and superoxide dismutases (SOD) activities, as well as renal plasma flow (RPF) and glomerular filtration rate (GFR), were measured by means of plasma clearance of p-amino-hippurate and inulin, respectively. In comparison with groups 1 and 3, the RPF and GFR were significantly decreased in groups 2 and 4. Interestingly, renal homogenates of the latter groups yielded significantly higher values of phospholipase A activity and fructosamine level in comparison with either the control (1) and the O3 (3) treated groups. Moreover renal SOD activity showed a significant increase in group 3 without significant differences among groups 1, 2 and 4. Morphological alterations of the kidney were present in 100%, 88% and 30% of the animals in groups 2, 4 and 3, respectively. It is proposed that the O3 protective effect can be ascribed to the substantial possibility of upregulating the antioxidant defence system capable of counteracting the damaging effect of ischaemia. These findings suggest that, whenever possible, ozone preconditioning may represent a prophylactic approach for minimizing renal damage before transplantation.

My Projects Annotate
of factors from ozonated human platelets.
Valacchi G. Bocci V.
Mediators of Inflammation. 8(4-5):205-9, 1999.
[In Vitro. Journal Article. Research Support, Non-U.S. Gov't]
UI: 10704074
Authors Full Name
Valacchi, G. Bocci, V.
[View Annotation(s)]
[View Abstract]
AB In a previous work we have shown that heparin, in
the presence of ozone (O3), promotes a dose-dependent
platelet aggregation, while after Ca2+ chelation with
citrate, platelet aggregation is almost negligible. These
results led us to think that aggregation may enhance the
release of platelet components. We have here shown
that indeed significantly higher amount of platelet-
derived growth factor (PDGF), transforming growth
factor beta1 (TGF-beta1) and interleukin-8 (IL-8) are
released in a dose-dependent manner after ozonation of
heparinised platelet-rich plasma samples. These findings
may explain the enhanced healing of torpid ulcers in
patients with chronic limb ischemia treated with O3
autohaemoteraphy (O3-AHT).

Quasi-total-body exposure to an oxygen-ozone mixture
in a sauna cabin.
Bocci V. Borrelli E. Valacchi G. Luzzi E.
European Journal of Applied Physiology &
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 10541921
Authors Full Name
Bocci, V. Borrelli, E. Valacchi, G. Luzzi, E.
[View Annotation(s)]
[View Abstract]
48. [ ] AB We have investigated the effects of quasi-total-body
exposure of healthy volunteers to either an oxygen-
ozone mixture (O(2)-O(3)) or to oxygen (O(2)) alone
during a short period in a sauna cabin. The subjects
underwent both an experimental and a control
examination, separated by a 3.5-month interval. Body
mass, blood pressure, body temperature changes,
electrocardiograms, venous blood gas and
haemocytometric analyses, total antioxidant status and
plasma levels of protein thiol groups, thiobarbituric acid
reactive substances (TBARS), plasma cytokine, hepatic
enzymes and creatine were determined before,
immediately after the 20-min period in the cabin and then 0.5, 1.0 and 24 h afterwards. We observed statistically significant variations of body temperature, venous partial pressure of O(2) values, TBARS and plasma levels of interleukin 8, particularly after O(2)-O(3) exposure. The increase in TBARS plasma levels concomitant with protein oxidation has been tentatively interpreted as being attributable to the transcutaneous passage of some reactive O(2) species, which should be considered if this approach is to be used as a biological response modifier. However, in the present study no adverse effects were noted after one session.

Ozonation of blood during extracorporeal circulation. I. Rationale, methodology and preliminary studies.
[Comparative Study. In Vitro. Journal Article. Research Support, Non-U.S. Gov't]
UI: 10532435
Authors Full Name
[View Annotation(s)]
[View Abstract]
49. [ ] AB We investigated whether exposure of blood ex-vivo to oxygen-ozone (O2-O3) through a gas exchanger is feasible and practical. We first evaluated the classical dialysis-type technique but we soon realized that semipermeable membranes are unsuitable because they are hydrophilic and vulnerable to O3. We therefore adopted a system with hydrophobic O3-resistant hollow fibers enclosed in a polycarbonate housing with a membrane area of about 0.5 m2. First we tested the system with normal saline, determining the production of hydrogen peroxide (H2O2) at O3 concentrations from 5 to 40 microg/ml. We then evaluated critical parameters by circulating swine blood in vitro; this revealed that heparin is not an ideal anticoagulant for this system. Finally, we performed several experiments in sheep and defined optimal anticoagulant dose (sodium citrate, ACD), priming solution, volume of blood flow per min, volume and concentration of O2-
O3 mixture flowing countercurrent with respect to blood and the time necessary for perfusion in vivo. The biochemical parameters showed that an O3 concentration as low as 10 microg/ml is effective; this means that gas exchange and O3 reactivity are rapid and capable of inducing biological effects. The sheep showed no adverse effects even after 50 min of extracorporeal circulation at higher O3 concentrations (20 to 40 microg/ml) but the exchanger became less effective (low pO2 values) due to progressive clogging with cells.

Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production.
Bocci V. Valacchi G. Corradeschi F. Fanetti G.
[In Vitro. Journal Article. Research Support, Non-U.S. Gov't]
UI: 9883965
Authors Full Name
Bocci, V. Valacchi, G. Corradeschi, F. Fanetti, G.
[View Annotation(s)]
[View Abstract]
AB Ozone (O3) is a controversial gas because, owing to its potent oxidant properties, it exerts damaging effects on the respiratory tract and yet it has been used for four decades as a therapy. While the disinfectant activity of O3 is understandable, it is less clear how other biological effects can be elicited in human blood with practically no toxicity. On the other hand plasma and cells are endowed with a powerful antioxidant system so that a fairly wide range of O3 concentrations between 40 and 80 microg/ml per gram of blood (approximately 0.83-1.66 mM) are effective but not deleterious. After blood ozonation total antioxidant status (TAS) and plasma protein thiol groups (PTG) decrease by 20% and 25%, respectively, while thiobarbituric acid reactive substances (TBARS) increases up to five-fold. The increase of haemolysis is negligible suggesting that the erythrocyte membrane is spared at the expense of other sacrificial substrates. While there is a clear relationship between the ozone dose and IL-8 levels, we have noticed that high TAS and PTG values inhibit the cytokine production. This is in line with the current idea that hydrogen peroxide, as a byproduct of O3 decomposition, acts as a messenger for the cytokine induction.
AB The acceptance of any complementary medical approach is conditioned by the results obtained after the same scientific scrutiny applied in orthodox medicine. Otherwise any claim of efficacy remains in the realm of fiction. In the case of ozone therapy, the mechanisms of action have remained nebulous and in a series of publications we are trying to present the biochemical, immunological and morphological evidence in favour or against ozone therapy. We have now shown that ozone (O3) dissolved in the water of either plasma or serum or physiological saline generates reactive oxygen species (ROS), of which hydrogen peroxide (H2O2) can be unequivocally demonstrated by using specific methods for its detection. Lipids present in plasma preferentially those present in lipoproteins, undergo peroxidation that is somewhat O3-dose dependent and can be observed by the measurement of thiobarbituric acid reactive substances (TBARS). While the generation of H2O2 is crucial in activating both biochemical (hexose monophosphate shunt) and immunological (via the transcription factor NF-kB) mechanisms, the role of lipid oxidation products (LOP) remains to be investigated. We have shown here that there is a small but consistent induction of some cytokines (TNF-alpha, IFN-gamma and IL-2) when human blood is directly exposed to O3 concentrations up to 100 micrograms/ml per g of blood. On the other hand, isolated blood mononuclear cells (PBMC) in tissue culture medium are far more sensitive to the oxidant action of O3 as shown by a progressive reduction of the proliferation index with comparatively far lower O3, concentrations. On the whole, these results support the concept that much of the O3 toxicity is neutralized by the powerful antioxidant system of blood. The minimal hemolysis supports this idea but as far as platelets are concerned, we must mention that they tend to aggregate in heparinized blood, even when it is exposed to an O3 concentration of 40 micrograms/ml. In spite of the lack of side-effects after autohemotherapy, this drawback must be kept in mind and avoided in clinical practice.
cellular damage by free radicals.
Leon OS. Menendez S. Merino N. Castillo R. Sam S. Perez L. Cruz E. Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 9792340
Authors Full Name
Leon, O S. Menendez, S. Merino, N. Castillo, R. Sam, S. Perez, L. Cruz, E. Bocci, V.
[View Annotation(s)]
[View Abstract]
AB There is some anecdotal evidence that oxygen-ozone therapy may be beneficial in some human diseases. However so far only a few biochemical and pharmacodynamic mechanisms have been elucidated. On the basis of preliminary data we postulated that controlled ozone administration would promote an oxidative preconditioning preventing the hepatocellular damage mediated by free radicals. Six groups of rats were classified as follows: (1) negative control, using intraperitoneal sunflower oil; (2) positive control using carbon tetrachloride (CCl4) as an oxidative challenge; (3) oxygen-ozone, pretreatment via rectal insufflation (15 sessions) and after it, CCl4; (4) oxygen, as group 3 but using oxygen only; (5) control oxygen-ozone, as group 3, but without CCl4; group (6) control oxygen, as group 5, but using oxygen only. We have evaluated critical biochemical parameters such as levels of transaminase, cholinesterase, superoxide dismutase, catalase, phospholipase A, calcium dependent ATPase, reduced glutathione, glucose 6 phosphate dehydrogenase and lipid peroxidation. Interestingly, in spite of CCl4 administration, group 3 did not differ from group 1, while groups 2 and 4 showed significant differences from groups 1 and 3 and displayed hepatic damage. To our knowledge these are the first experimental results showing that repeated administration of ozone in atoxic doses is able to induce an adaptation to oxidative stress thus enabling the animals to maintain hepatocellular integrity after CCl4 poisoning.
My Projects Annotate

Is ozone therapy therapeutic?.
Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
Ozone as a bioregulator. Pharmacology and toxicology of ozonetherapy today. [Review] [198 refs]
Bocci V.
Journal of Biological Regulators & Homeostatic Agents.
[Journal Article. Research Support, Non-U.S. Gov't. Review]
UI: 9250885
Authors Full Name
Bocci, V.
[View Annotation(s)]
[View Abstract]
AB The disinfectant activity of ozone is well recognized and ozone is used worldwide for sterilization of water. The use of ozone as a complementary medical approach is less known, because it has mostly been used in an empirical fashion without a rational basis and appropriate controls. In spite of this drawback, the use of judicious and standardized ozone dosages can elicit the formation of ROS acting as natural physiological activators of several biological functions. There is now a reasonable understanding of a few mechanisms of action and, using classical pharmacological concepts, it appears possible to formulate a rationale for optimizing clinical applications. A further exciting development is that ozone, being an oxidizer, can upregulate the intracellular anti-oxidant enzymes eventually inhibiting the constant, life-long oxidative stress responsible for degenerative diseases and aging. Among various routes for the administration of ozone, the autohemotransfusion procedure, consisting in exposing blood to ozone, i.e. to a calculated and brief oxidative stress, appears safe, simple, inexpensive and amenable to be adjusted to different pathological states It is hoped that this review will help to dispel prejudices, to clarify that ozone toxicity can be tamed, to show that ozone can act as a bioregulator and to encourage controlled clinical investigations to evaluate definitely the validity of ozonotherapy. [References: 198]
Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 8692040
Authors Full Name
Bocci, V.
[View Annotation(s)]
[View Abstract]
AB The role of ozone on earth is controversial, as in the stratosphere it is protective against excessive ultra violet irradiation, and in the troposphere it is toxic for animals and plants. The effectiveness of ozone against pathogens is well recognized and ozone appears to be the best agent for sterilization of water. In spite of this, the use of ozone in medicine has been overlooked or despised, mostly because it has been either misused or used without appropriate controls. Studies carried out in our laboratory have revealed that ozone can display relevant biological effects and that, having defined its therapeutic index, can become an important and reliable drug for the treatment of several diseases. An exciting new aspect is that ozone, being a strong oxidizer, can stimulate the increase of cellular anti-oxidant enzymes, eventually inhibiting the oxidative stress.

Bocci V. Luzzi E. Corradeschi F. Silvestri S.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 7660851
Authors Full Name
Bocci, V. Luzzi, E. Corradeschi, F. Silvestri, S.
56. [ ] [View Annotation(s)]
[View Abstract]
AB After exposing human whole blood from normal volunteers to ozone concentrations ranging from 22 to 156 micrograms/ml, we have shown that, upon incubation of up to 8 hours, there is a significant release of transforming growth factor beta (TGF-beta 1). In comparison to TGF-beta 1, TGF-beta 2 production is not influenced by ozone concentrations. In line with our previous findings it appears that blood, in the presence of heparin and 5mM Ca,2+ allows a consistent
production of tumor necrosis factor a (TNF alpha) and the release of low and non-hazardous levels of free hemoglobin. These data support the contention that autohemotherapy performed after treating blood with ozone followed by reinfusion into the donor, may represent a valuable therapeutic approach for achieving immunoregulatory effects.  

Autohaemotherapy after treatment of blood with ozone. A reappraisal. [Review] [59 refs]  
Bocci V.  
[Comparative Study. Journal Article. Research Support, Non-U.S. Gov't. Review]  
UI: 8088420  
Authors Full Name  
Bocci, V.  
[View Annotation(s)]  
[View Abstract]  
AB Autohaemotherapy, involving bland treatment ex vivo of blood with ozone and prompt reinfusion into the donor, is a procedure mainly performed in central Europe, which is claimed to have therapeutic value in circulatory disorders, viral diseases and cancer. This practice is mostly performed in private clinics, and good clinical trials have not been published, which has understandably given rise to prejudice and scepticism. By analysing possible mechanisms of action and current hypotheses, this report attempts to explain how this procedure can be useful in such disparate diseases. The current state of the art is presented objectively, the lack of toxicity is documented, and the rationale and therapeutic advantages are discussed, with the aim of eliciting interest in carrying out controlled clinical trials. [References: 59]  
My Projects Annotate  

Studies on the biological effects of ozone: 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy.  
Bocci V. Luzzi E. Corradeschi F. Paulesu L.  
[Journal Article. Research Support, Non-U.S. Gov't]  
UI: 7803194  
Authors Full Name  
Bocci, V. Luzzi, E. Corradeschi, F. Paulesu, L.  
[View Annotation(s)]
AB Autohaemotherapy, after a bland treatment ex vivo of blood with ozone, is a fairly unknown medical procedure claimed to have therapeutic value in viral diseases and neoplasms. Having already shown that ozone acts as a mild inducer of cytokines, we have undertaken an investigation in normal rabbits and in normal volunteers aiming to evaluate eventual changes of some cytokine levels in plasma as well as of immunological parameters such as the Mx protein, neopterin, beta 2-microglobulin and of some acute-phase proteins after single or repeated autohaemotherapy. We have also evaluated the potential development of of side-effects. This study is the first one to show that autohaemotherapy can activate an immunological marker in normal subjects without procuring any toxic effects.

Bocci V. Luzzi E. Corradeschi F. Paulesu L. Rossi R. Cardaioli E. Di Simplicio P.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 8023701
Authors Full Name
Bocci, V. Luzzi, E. Corradeschi, F. Paulesu, L. Rossi, R. Cardaioli, E. Di Simplicio, P.
AB We have investigated the effect of various concentrations of ozone on human blood aiming to correlate the production of cytokines with depletion of reduced glutathione and hemolysis. As erythrocytes constitute the bulk of blood cells and represent the main target of ozone they have been taken as a useful marker of its oxidative activity. It appears that a transient exposure (30 sec) of blood of up to 78 micrograms ozone per ml of blood does not depress the production of cytokines even though there is a slight increase of hemolysis and a small decrease of intracellular reduced glutathione. In contrast either a constant (up to 30 sec) exposure to an ozone flux or a high ozone concentration (108 micrograms/ml) markedly decreases reduced glutathione levels and depresses cytokine production.
Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines.
Bocci V. Luzzi E. Corradeschi F. Paulesu L. Di Stefano A.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 8324077
Authors Full Name
Bocci, V. Luzzi, E. Corradeschi, F. Paulesu, L. Di Stefano, A.
[View Annotation(s)]

60. [ ] [View Abstract]
AB Ozonization of blood, normally carried out with citrated blood, may be fine for the autohemotherapy of ischemic diseases but it may be at a loss when employed in viral diseases or in immunodeficiencies. We have shown that heparin, used as an anticoagulant, with the addition of 5 mM CaCl2 favors production of cytokines by leukocytes with only a modest increase in hemolysis. High plasmatic levels of glucose, glutathione, and ascorbic acid decrease cytokine's yield because these compounds act as antioxidants and quench the inducing activity of ozone. Autohemotherapy with heparinized and Ca(2+)-supplemented blood has not revealed any side effects in volunteers.

Ozonization of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis.
Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 1435389
Authors Full Name
Bocci, V.
[View Annotation(s)]
[View Abstract]
AB In the last 3 decades major autohemotherapy after exposure to ozone has been used in Europe in uncontrolled trials carried out in patients with many illnesses, particularly chronic viral diseases and neoplasms. It appears that the treatment may activate the host's immune system by inducing the production of immunoactive cytokines and it may now be possible to rationalize the procedure, improve the regimen and assess the outcome. It is apparent, however, that such a therapeutic approach, in order to be acceptable, requires an investigative effort of biologists and clinicians. Once this is done, owing to the large range of medical applications and the simplicity of the procedure, autohemotherapy could become very valuable particularly in underdeveloped countries.

61. [ ] [View Abstract]

62. [ ] Studies on the biological effects of ozone: 2. Induction
of tumor necrosis factor (TNF-alpha) on human leucocytes.
Paulesu L. Luzzi E. Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 1768744
Authors Full Name
Paulesu, L. Luzzi, E. Bocci, V.
[View Annotation(s)]
[View Abstract]
AB The effect of ozone as a probable inducer of tumor necrosis factor (TNF-alpha) has been investigated on human blood and on Ficoll-purified blood mononuclear cells (PBMC). Samples were exposed at different ozone concentrations ranging from 2.2 to 108 micrograms/ml and incubated at 37 degrees C in an 95% air-5% CO2 atmosphere. At predetermined times, all cell supernatants were tested for TNF activity and some PBMC cultures were examined for DNA synthesis. We have shown that ozone concentration is critical in terms of TNF production and of cell mitogenesis and that, owing to the presence of erythrocytes, higher ozone concentrations are required to be effective in blood than in PBMC. Because ozonization of blood is a procedure followed in several European countries for the treatment of viral diseases and tumors, the release of factors with antiviral and immunomodulatory activities by leukocytes may explain the mechanism of action of ozone and of autohemotherapy.

Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes.
Bocci V. Paulesu L.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 2129118
Authors Full Name
Bocci, V. Paulesu, L.
63. [ ] [View Annotation(s)]
[View Abstract]
AB In this study we have investigated the effects of ozone on human blood, as well as on resuspended buffy coats and Ficoll-purified mononuclear cells. Samples were exposed at different ozone concentrations (from 2.2 micrograms to 108 micrograms/ml) for 30 sec and then incubated for different times at 37 degrees C in a 95% air-5% CO2 humidified atmosphere. Supernatants were collected and frozen at-20 degrees C until tested for interferon (IFN) activity. We have determined that the ozone concentration is critical for lymphokine
induction. In fact, while low concentrations (2.2 micrograms/ml) are effective in lymphocytes, they do not induce IFN in either whole or diluted (1:1) human blood, or resuspended buffy coats. In such cases levels as high as 42 micrograms/ml are required. On the other hand, a very high ozone concentration (108 micrograms/ml) is not effective and probably toxic. Maximal IFN production occurs 72-96 h after ozone exposure, and the kinetics of IFN release is similar to that after Staphylococcal Enterotoxin B addition. Because ozonization of blood is a medical procedure followed in several countries for treatment of viral diseases, this study can open a new field of investigation that may yield useful results both in biological and practical terms.

I have put a large number of 100 Healozone publications in peer reviewed research journals below
These will be useful
There are many more!!!!!!!!!!!!!!
If you want more then please let me know

Molar incisor hypomineralization. Morphological and chemical aspects, onset and possible etiological factors.
Fagrell T.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 22338967
Authors Full Name
Fagrell, Tobias.
[View Annotation(s)]
[View Abstract]
AB OVERALL AIM: The general objective of this thesis was to enhance

1. [ ] the understanding of Molar Incisor Hypomineralization (MIH) in areas of the histological, chemical and mechanical properties of the hypomineralized enamel, objective and subjective clinical symptoms in relation to bacteria findings. Further, to estimate a time for onset of the disturbance and investigate possible etiological factors. MATERIAL & METHODS: 22 teeth diagnosed with MIH were used in the histological and chemical studies. A number of analytical methods were used; Light microscopy, Polarized light microscopy, Scanning electron microscopy, X-ray microanalysis, Vickers hardness test and X-ray Micro Computed Tomography. Decalcified sections were stained with bacterial staining. An ozone device was tested for the ability to kill strains of oral bacteria. In collaboration with the prospective ABIS study, 17,000 individuals
were examined and possible etiological causes of severe demarcated opacities were tested. RESULTS & CONCLUSIONS: The hypomineralized enamel was mainly located in the buccal enamel of the teeth and had a high degree of porosity extending from enamel-dentin-junction with a distinct border to the normal cervical enamel. Teeth diagnosed MIH had lower hardness values in hypomineralized enamel and differences in the chemical composition. Bacteria were observed in the enamel and deep into the dentin. Ozone treatment for 20 seconds or more was effective to kill oral microorganisms. Significant relations were found between MIH in first molars and breast feeding more than 6 months, late introduction to gruel and infant formula (later than 6 months). The onset for the hypomineralized enamel was estimated to around 200 days from start of the enamel mineralization.

2. [Using ozone for dental caries treatment]. [Review] [Russian]
UI: 22332386
Authors Full Name
Grudianov, A I. Makeeva, M K.

4. [The role of remineralizing agents in dentistry: a review. [Review]
UI: 21894873
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[View Annotation(s)]
[View Abstract]
AB Minimal intervention is a key phrase in today's dental practice. Minimal intervention dentistry (MID) focuses on the least invasive treatment options possible in order to minimize tissue loss and patient discomfort. Concentrating mainly on prevention and early intervention of caries, MID's first basic principle is the remineralization of early carious lesions, advocating a biological or therapeutic approach rather than the traditional surgical approach for early surface lesions. One of the key elements of a biological approach is the usage and application of remineralizing agents to tooth structure (enamel and dentin lesions). These agents are part of a new era of dentistry aimed at controlling the demineralization/ remineralization cycle, depending upon the microenvironment around the tooth. This article details the various agents that enhance and/or promote remineralization and discusses their clinical implications.

5. [The effect of dentin hypersensitivity treatments on the shear bond strength to dentin of a composite material.
AB This study sought to evaluate the influence of a dentin desensitizer and ozone application on the bond strength to dentin of a composite resin material. The dentin desensitizing agent and ozone treatment were applied on the cervical dentin surfaces of extracted, caries-free, erupted third molars. Dentin surfaces that received no treatment were used as control samples. A dentin bonding agent was applied according to the manufacturer's instructions and an adhesion test was performed according to ISO/TS 11405. Statistical analysis showed no significant influence of the different hypersensitivity treatments on shear bond strength to dentin (ANOVA and Tukey's tests, p > 0.05). Within the limitations of this in vitro study, it appears that the short-term use of dentin hypersensitivity treatments like ozone and dentin desensitizers containing gluteraldehyde do not further affect the shear bond strength to dentin of subsequent composite resin restorations.
dental implant osseointegration. Therefore, topically applied ozonated oil may influence bone density and the quality of osseointegration around dental implants.

**Influence of ozone on the composite-to-composite bond.**

AB The study evaluated the effect of ozone application on the composite-to-composite bond. Three hundred and twenty cylindrical composite specimens were divided into two groups: group 1 was subjected to a 60 s ozone application, whereas group 2 remained untreated. Four subgroups were obtained from each group according to the intermediate repair agent: an adhesive, a silane, silane/adhesive combination, or flowable composite. Repair composite cylinders were built-up. The composite repair strength was tested after 24 h and after thermocycling with a shear test. Additionally, 4 mm x 4 mm x 2 mm composite specimens were prepared and stored 24 h in deionized water. Half of the specimens were subjected to ozone application and the other served as control. The elastic modulus (E) and the Vicker's hardness (VH) of the composite surfaces were tested immediately and after thermocycling. Significant differences among the experimental groups were detected (p < 0.001). The composite repair strength was affected by the pretreatment and by the intermediate agent, whereas, the thermocycling was not significant. The partial eta-squared statistics showed that the intermediate agent was the main factor affecting the composite repair strength, whereas the pretreatment played a minor role. No differences were observed between ozone and control groups when the same intermediate agent and the same aging conditions were applied. Repairing with flowable composite tended to achieve higher bond strengths (20.7 and 26.5 MPa in ozone and control groups, respectively, after 24 h). The use of silane coupling agent showed the lowest composite repair strengths. Ozone did not affect E and VH (p > 0.05) and the thermocycling affected only E (p < 0.05). In conclusion, the application of ozone does not impair the composite-to-composite bond.

**Effect of ozone pretreatment on the microleakage of pit and fissure sealants.**

AB The study evaluated the effect of ozone application on the microleakage of pit and fissure sealants. Four hundred and twenty specimens of composite sealants were divided into two groups: group 1 was subjected to a 60 s ozone application, whereas group 2 remained untreated. Four subgroups were obtained from each group according to the intermediate repair agent: an adhesive, a silane, silane/adhesive combination, or flowable composite. Repair composite cylinders were built-up. The composite repair strength was tested after 24 h and after thermocycling with a shear test. Additionally, 4 mm x 4 mm x 2 mm composite specimens were prepared and stored 24 h in deionized water. Half of the specimens were subjected to ozone application and the other served as control. The elastic modulus (E) and the Vicker's hardness (VH) of the composite surfaces were tested immediately and after thermocycling. Significant differences among the experimental groups were detected (p < 0.001). The composite repair strength was affected by the pretreatment and by the intermediate agent, whereas, the thermocycling was not significant. The partial eta-squared statistics showed that the intermediate agent was the main factor affecting the composite repair strength, whereas the pretreatment played a minor role. No differences were observed between ozone and control groups when the same intermediate agent and the same aging conditions were applied. Repairing with flowable composite tended to achieve higher bond strengths (20.7 and 26.5 MPa in ozone and control groups, respectively, after 24 h). The use of silane coupling agent showed the lowest composite repair strengths. Ozone did not affect E and VH (p > 0.05) and the thermocycling affected only E (p < 0.05). In conclusion, the application of ozone does not impair the composite-to-composite bond.
OBJECTIVE: This study investigated the effect of ozone pretreatment on the microleakage and marginal integrity of pit and fissure sealants placed with or without a self-etch 6th generation adhesive. STUDY DESIGN: Freshly-extracted, human third molars were randomly assigned into two main groups (n = 48): Group A: Fissures were pretreated with ozone; Group B: Fissures were left untreated. The teeth were further randomly divided into two subgroups (n = 24/each) so that half of teeth were sealed with a conventional fissure sealant (Fissurit F, Voco, Germany), while the remaining half received the same sealant bonded with a self-etch adhesive (Clearfil Protect Bond, Kuraray, Japan). Following thermal cycling (1000X), the specimens were subjected to dye penetration within 0.5% basic fuchsin for 24h. The extent of dye penetration was measured by image analysis. Kruskal Wallis and Mann-Whitney U tests were used for statistical analysis of the data (p = 0.05). Two randomly-selected sections from each group were observed under SEM RESULTS: In all groups, ozone pretreatment significantly reduced the extent of microleakage (p < 0.001). SEM investigation demonstrated better adaptation of the sealants in ozone-pretreated groups. Clearfil Protect Bond did not improve the marginal seal of Fissurit F (p > 0.05). CONCLUSION: Ozone pretreatment favorably affected the marginal sealing ability of the tested fissure sealants.

Influence of ozone application on the repair strength of silorane-based and ormocer-based composites.

METHODS: 160 cavities were created in methacrylate cylinders. Half of the cavities were filled with a silorane-based composite, whereas the other half was filled with an ormocer-based composite. After storage (1 week, deionized water, 37 degrees C) the specimens of each restorative material were divided into two main experimental groups: in Group 1 the specimens were subjected to a 60-second ozone gas application; in Group 2 no pretreatment was performed (control). The corresponding adhesive of each restorative material was applied as the intermediate repair agent in both groups. Repair cylinders were then built up with the homologous material. Half of the specimens in each group were subjected to thermocycling (5,000 cycles, 5 degrees C-55 degrees C, dwell time 30 seconds, transfer time 5 seconds) prior to testing, whereas the other specimens were immediately tested. The repair strength was
assessed with a shear test. The two-way ANOVA with pretreatment and thermocycling as the main factors was used to analyze the shear bond strength data within each restorative material. RESULTS: The pretreatment, the thermocycling and their interaction did not significantly affect the repair strength of either tested materials (P > 0.05). The ozone treatment did not significantly affect the repair strength. The silorane-based composite showed lower repair strengths compared to those of the ormocer-based composite. The immediate repair strengths in the ozone-treated and control groups were respectively, 28.1 (13.8) MPa and 28.8 (8.8) MPa for the silorane-based composite and 31.5 (9.3) MPa and 35.6 (10.6) MPa for the ormocer-based composite. After thermocycling, the repair strengths in the ozone-treated and control groups were 27.7 (9.7) MPa and 29.5 (11.1 ) MPa for the silorane-based composite and 31.4 (6.0) MPa and 34.2 (4.5) MPa for the ormocer-based composite. Mixed failures occurred most frequently in all experimental groups. In conclusion, ozone did not affect the repair strength of the tested silorane-based and ormocer-based composites.

Effect of bleaching versus repolishing on colour and surface topography of stained resin composite.
Abd Elhamid M. Mosallam R.
[Journal Article]
UI: 21133937
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Abd Elhamid, M. Mosallam, R.
[View Annotation(s)]
[View Abstract]
AB BACKGROUND: The aim of this study was to examine the effect of ozonated gel, carbamide peroxide bleaching agent and polishing paste on the colour and surface topography of stained resin composite. METHODS: Ninety disc-shaped resin composite specimens were used in this study. They were randomly divided into three groups of 30 specimens each according to the immersion solutions used, i.e., tea, cola or artificial saliva. Each group was further subdivided into three equal subgroups (n = 10) according to the type of whitening treatment applied; 30% carbamide peroxide, ozonated KY gel or polishing paste. Colour and surface roughness was measured at baseline, after immersion, also following whitening procedures by using a stereomicroscope. The results were recorded, tabulated and statistically analysed. RESULTS: The colour values of artificial saliva displayed the highest statistically significant mean colour difference compared to cola and tea. However, there was no statistically significant difference in surface roughness. Also, a superior whitening effect was demonstrated with ozonated gel. Ozonated gel showed statistically significant lowest roughness compared to both carbamide peroxide and polishing paste. CONCLUSIONS: Immersion solutions have a positive influence on the colour of resin composite. Also, ozonated gel is an
efficient bleaching agent with the least adverse effect on surface roughness. Copyright 2010 Australian Dental Association.

My Projects

Shear bond strength to enamel after power bleaching activated by different sources.
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[Comparative Study. Journal Article. Randomized Controlled Trial]
UI: 21069109
Authors Full Name
Can-Karabulut, Deniz C. Karabulut, Baris.
[View Annotation(s)]
[View Abstract]
AB The purpose of the present study was to evaluate enamel bond strength of a composite resin material after hydrogen peroxide bleaching, activated by a diode laser (LaserSmile), an ozone device (HealOzone), a light-emitting diode (BT Cool whitening system), and a quartz-Plus. Fifty extracted caries-free permanent incisors were used in this study. Thirty-eight percent hydrogen peroxide gel was applied to sound, flattened labial enamel surfaces and activated by different sources. Enamel surfaces that had received no treatment were used as control samples. Bonding agent was applied according to the manufacturer's instructions and the adhesion test was performed according to ISO/TS 11405. Statistical analysis showed significant influence of the different activation technique of hydrogen peroxide on shear bond strength to enamel (ANOVA, LSD, P < 0.05). The data in this in vitro explorative study suggest the activation of hydrogen peroxide by different sources may further affect the shear bond strength of subsequent composite resin restoration to enamel. Within the limitations of this in vitro study, further studies examining the structural changes of activated hydrogen peroxide-treated enamel are needed. Due to the different activation methods; duration of light irradiation effects, longer time periods may be needed before application of adhesive restorations to enamel, compared with non-activated bleaching.

My Projects

The use of ozone to lighten teeth. An experimental study.
Tessier J. Rodriguez PN. Lifshitz F. Friedman SM. Lanata EJ.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 21053679
Authors Full Name
Tessier, Jeronimo. Rodriguez, Patricia N. Lifshitz, Fima. Friedman, Silvia M. Lanata, Eduardo J.
[View Annotation(s)]
[View Abstract]
AB Tooth-whitening agents are available for therapeutic use in the dental office or at home. However, whitening more severe stains, such as those caused by systemic ingestion of
tetracycline, constitutes a challenge. The aim of this study was to evaluate, in an experimental model of growing rats, the efficacy of using ozone to lighten tetracycline-stained incisors. At weaning, male Wistar rats (n=40) were randomly assigned to one of three groups. Two control groups, C2, and C60 (n=8, each) were used to document the usual age-related color. The third group (n=24) received 0.25 g% of oxytetracycline (0) until 60 days of age. These rats were subsequently divided into three further groups: O0, O3 and O5 (n=8, each). These rats were anesthetized; O3 and O5 groups received ozone application to the lower incisors for 3 (group O3) or 5 minutes (group O5), respectively; while O0 did not receive the ozone treatment. Teeth were then photographed and the incisors from the control (C60) and treatment groups (O0, O3 and O5) were cut, and compared to a standard color guide (there were eight shades numbered 0 to 7, lightest to darkest) to assess the hue visually. The teeth were then placed in phosphoric acid to quantify the color by spectrophotometry. The data (mean +/- SD) were analyzed by One-Way Analysis of Variance (ANOVA) followed by Tukey's test or Dunnett test. The visual observation, analyzed blindly by one investigator showed that O3 and O5 groups had diminished yellowing of the teeth as compared to the untreated O0 group (P < 0.001). The color quantified by spectrophotometry also detected significant differences among groups (O3 < O0, P < 0.01; O5 < O0, P < 0.001 and O5 < O3, P < 0.01). C21 and C60 were significantly different among groups (P < 0.001). This is the first experimental study to show that ozone can be successfully used for lightening the yellowish tinge of tetracycline-stained rat incisors. Further studies are required for its potential use in the dental clinic.

Effects of ozone and ND:YAG laser pretreatment on bond strength of self-etch adhesives to coronal and root dentin.
[Journal Article]
UI: 20932187
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB OBJECTIVES: The aim of this study was to evaluate the effects of two different disinfection treatments--ozone and Nd:YAG laser application--on shear-bond strength (SBS) of self-etch adhesives to coronal and root dentin. MATERIALS AND METHODS: Sixty extracted human canines were ground flat, exposing the coronal and root dentin surfaces, and randomly divided into three groups. The surfaces were untreated (Control) or treated with ozone (HealOzone, KaVo) or Nd:YAG laser (Fidelis III, Fotona). Coronal and root dentins of 10 teeth of each group were treated with a two-step self-etch adhesive (Clearfil SE Bond, Kuraray Medical; SE), whereas the remaining 10 teeth were treated with a one-step self-etch adhesive (Clearfil Tri-S Bond, Kuraray Medical; S3). A resin composite
(Clearfil Majesty Esthetics, Kuraray Medical) was then placed, and SBS was tested with a universal testing machine. Failure modes were determined under a stereomicroscope. The mean SBS values of each group were calculated, and data were subjected to statistical analysis \((p = 0.05)\). RESULTS: For the coronal dentin, Control/SE showed significantly higher values than Control/S3, Ozone/S3, and Nd:YAG/S3. Although Ozone/SE showed significantly higher values than Nd:YAG/S3 \((p < 0.05)\), the differences within the other groups were not significant for the root dentin \((p > 0.05)\). Comparison of two dentin substrates in each group did not show any significant difference except for Control/SE, in which coronal dentin showed higher SBS. The failure modes of all groups were mainly adhesive.

CONCLUSION: Pretreatments with Ozone or Nd:YAG laser did not impair the SBS of both of the self-etch adhesives used to coronal and root dentin.

**My Projects**

The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: a clinical and microbiologic study.

Kshitish D. Laxman VK.

Indian Journal of Dental Research. 21(3):341-8, 2010 Jul-Sep. [Comparative Study. Journal Article. Randomized Controlled Trial]

UI: 20930341

Authors Full Name
Kshitish, Durga. Laxman, Vandana K.
[View Annotation(s)]
[View Abstract]

AB BACKGROUND: The development of periodontal disease has been thought to be associated with several restricted members of the oral anaerobic species, such as black-pigmented Porphyromonas species and Actinobacillus actinomycetemcomitans (Aa), in the subgingival environment. Apart from bacteria, certain viruses and fungi that are associated with periodontal disease are also present in the subgingival plaque . MATERIALS AND METHODS: A randomized, double-blind, crossover split-mouth design was performed. A total of 16 patients suffering from generalized chronic periodontitis were selected for the study. The study period of 18 days was divided into two time-intervals, i.e. baseline (0 days) to 7 th day, with a washout period of 4 days followed by a second time interval of 7 days. The use of ozone and chlorhexidine gluconate (CHX) irrigation was randomized. Both the patient and the clinician evaluating the clinical parameters were blinded regarding the type of irrigation used. RESULTS: The interpretation of clinical and microbial data is from baseline to 7 th day. A higher percentage of plaque index (12%), gingival index (29%) and bleeding index (26%) reduction was observed using ozone irrigation as compared to chlorhexidine. The percentile reduction of Aa (25%) using ozone was appreciable as compared to no change in Aa occurrence using chlorhexidine. By using O 3 and chlorhexidine, there was no antibacterial effect on Porphyromonas gingivalis (Pg) and Tannerella forsythensis. The antifungal effect of ozone from baseline (37%) to 7 th day
(12.5%) was pronounced during the study period, unlike CHX, which did not demonstrate any antifungal effect. CONCLUSION: Ozone may be considered as an alternative management strategy due to its powerful ability to inactivate microorganisms. Also, there is growing evidence that ozone can be employed as a useful therapeutic agent in both dentistry and medicine.

Production of reactive oxygen species from photosensitizers activated with visible light sources available in dental offices.
Bouillaguet S. Wataha J.C. Zapata O. Campo M. Lange N. Schrenzel J.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 20001322
Authors Full Name
AB OBJECTIVES: The aim of this study was to assess the ability of commonly available red- or blue-light dental sources to generate reactive oxygen species (ROS) from photosensitive chemicals that might be useful for photodynamic antimicrobial chemotherapy (PACT). BACKGROUND: Although the use of red diode lasers is well documented, there is limited information on how useful blue-light sources might be for PACT in dental contexts. MATERIALS AND METHODS: A diode laser (Periowave; see Table 1 for material and equipment sources) emitting red light (660-675 nm) was used to activate toluidine blue; riboflavin and pheophorbide-a polylysine (pheophorbide-a-PLL) were photoactivated using an Optilux 501 curing unit emitting blue light (380-500 nm). Ozone gas (generated by OzoTop, Tip Top Tips, Rolle, Switzerland), sodium hypochlorite, and hydrogen peroxide were used for comparison. ROS production was estimated using an iodine-triiodide colorimetric assay, and ROS levels were plotted versus concentration of chemicals to determine each chemical's efficiency in ROS production. One-way ANOVA with Tukey post hoc analysis (alpha = 0.05) was used to compare the efficiencies of ROS production for the various chemicals. RESULTS: Sodium hypochlorite, hydrogen peroxide, and ozone gas produced ROS spontaneously, whereas pheophorbide-a-PLL, riboflavin, and toluidine blue required light exposure. The efficiency of ROS production was higher for pheophorbide-a-PLL and toluidine blue than for ozone gas or riboflavin (p < 0.05). Hydrogen peroxide was the least efficient ROS producer. CONCLUSIONS: The results of the current study support the use of blue- or red-light-absorbing photosensitizers as candidates to produce ROS for clinical applications. Blue-light photosensitizers were as efficient as red-light photosensitizers in producing ROS and more efficient than the oxidant chemicals currently used for dental disinfection.

Does ozone water affect the bond strengths of orthodontic brackets?
AB BACKGROUND: Ozone water can be used to eliminate microorganisms from the water systems in dental offices. OBJECTIVES: To determine if ozone water diminishes the bond strength of orthodontic adhesives. METHODS: One hundred and twenty bovine mandibular incisors were randomly divided into four equal groups. The teeth were cleaned with pumice and washed either with tap water (Groups 1 and 3) or with ozone water (Groups 2 and 4) before bonding stainless steel orthodontics brackets to the teeth with either a composite resin (Groups 1 and 2; Transbond XT, 3M Unitek, Monrovia, CA, USA) or a resin-modified glass ionomer cement (Groups 3 and 4; Fuji Ortho LC, GC America Corporation, Tokyo, Japan). The manufacturers’ recommendations for bonding were followed. All samples were subjected to thermal cycling and the shear bond strengths were determined with a universal testing machine. The Adhesive Remnant Index (ARI) was used to score the amount of resin remaining on the teeth after debonding the brackets. RESULTS: There were no statistical differences in the shear bond strengths of the brackets debonded from enamel washed with either ozone water or tap water or between the groups bonded with the two adhesive resins (p = 0.595). The ARIs in Groups 2 and 3 were significantly different from the ARIs in Groups 3 and 4 (p = 0.030). CONCLUSION: Ozone water did not alter the bond strength of brackets bonded with composite resins, but it did alter the sites of resin fracture when Fuji Ortho LC was used.
Oxide has a pzc approximately 5-6 and its water solubility is at the order of 1-2 micromolar. It is often subjected to chemical- and heat treatments in order to increase the anatase- and rutile crystallinity, to modify the surface topography and to decrease the water solubility. In this work, we prepared sol-gel derived titanium and smooth PVD titanium surfaces, and analysed their oxide and protein deposition properties in human blood plasma before and after annealing at 100-500 degrees C or upon UVO-treatment for up to 96 hours. The blood plasma results show that complement deposition vanished irreversibly after heat treatment at 250-300 degrees C for 30 minutes or after UVO exposure for 24 hours or longer. XPS and infrared spectroscopy indicated change of surface water/hydroxyl binding upon the heat- and UVO treatments, and increased Ti oxidation. XRD analysis confirmed an increased crystallinity and both control (untreated) and annealed smooth titanium displayed low XRD-signals indicating some nanocrystallinity, with predominantly anatase phase. The current results show that the behaviour of titanium dioxide in blood contact can be controlled through relatively simple means, such as mild heating and illumination in UV-light, which both likely irreversibly change the stoichiometry and structure of the outmost layers of titanium dioxide and its OH/H(2)O binding characteristics. (c) 2010 Elsevier Ltd. All rights reserved.

**Leave decay in my cavity? You must be kidding!**
Authors Full Name
Knight, Geoffrey M. McIntyre, John M. Craig, Graham G. Mulyani. [View Annotation(s)]
My Projects

**Shear bond strength of epoxy resin-based endodontic sealers to bovine dentin after ozone application.**
Authors Full Name
Bojar, Witold. Czarnecka, Beata. Prylinski, Mariusz. Walory, Jaroslaw. [View Annotation(s)]
[View Abstract]
AB The idea of using ozone to disinfect root canals is of recent origin. The wide acceptance of epoxy resin-based sealers lead us to investigate whether ozone can influence the adhesion to the dentin. In this study, we tested the shear bond strength of AH Plus and EZ Fill. Forty freshly extracted bovine teeth were randomly divided into 5 groups. 16 of these samples were treated with ozone for 60 seconds (HealOzone, Kavo). 8 samples were conditioned with the G Bond bonding system. The groups tested were: (1) AH Plus, (2) AH Plus and ozone, (3) EZ Fill, (4) EZ Fill and ozone, (5) AH Plus and G Bond. 48 hours after being prepared the specimens were tested for shear bond strength. Statistical analysis showed significant differences between materials (AH Plus...
EZ Fill and significant, positive influence of ozone and bonding agent on the shear bond strength.

Enamel and dentin bond strength following gaseous ozone application.
Cadenaro M. Delise C. Antoniollo F. Navarra OC. Di Lenarda R. Breschi L.
[Comparative Study. Journal Article. Randomized Controlled Trial]
UI: 19701509
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB PURPOSE: To evaluate the effects of gaseous ozone application on enamel and dentin bond strength produced by two self-etching adhesive systems. MATERIALS AND METHODS: The shear bond strength test was conducted to assess adhesion on enamel (protocol 1), while the microtensile bond strength test was performed on dentin (protocol 2). Protocol 1: 96 bovine incisors were randomly divided into 4 groups, and enamel surfaces were bonded in accordance with the following treatments: (1E) ozone + Clearfil Protect Bond; (2E) Clearfil Protect Bond (control); (3E) ozone + Xeno III; (4E) Xeno III (control). Ozone gas was applied for 80 s. Shear bond strength was measured with a universal testing machine. Protocol 2: 40 noncarious human molars were selected. Middle/deep dentin was exposed and bonded in accordance with the following treatments: (1D) ozone+Clearfil Protect Bond; (2D) Clearfil Protect Bond (control); (3D) ozone + Xeno III; (4D) Xeno III (control). Four-mm-thick buildups were built on the adhesives, then specimens were sectioned in accordance with the nontrimming technique. Specimens were stressed until failure occurred, and failure modes were analyzed. Shear bond and microtensile bond strength data were analyzed using two-way ANOVA and Tukey's post-hoc test. RESULTS: No statistical differences were found between ozone treated specimens and controls, neither on enamel nor on dentin irrespective of the tested adhesive. Clearfil Protect Bond showed higher bond strength to enamel than Xeno III, irrespective of the ozone treatment (p < 0.05). CONCLUSION: The use of ozone gas to disinfect the cavity before placing a restoration had no influence on immediate enamel and dentin bond strength.

Novel preventive treatment options. [Review] [25 refs]
Longbottom C. Ekstrand K. Zero D. Kambara M.
UI: 19494683
Authors Full Name
Longbottom, C. Ekstrand, K. Zero, D. Kambara, M.
A number of novel preventive treatment options which, as with traditional methods, can be differentiated into 3 categories of prevention (primary, secondary and tertiary), have been and are being currently investigated. Those reviewed are either commercially available or appear relatively close to that point. These include: approximal sealants; fluoride applications, including slow-release devices; measures to help remineralize demineralized tissue, including 3 different methods of delivering amorphous calcium phosphate; measures to help modify the biofilm to reduce the cariogenic challenge, including ozone therapy and probiotics; measures to increase enamel resistance to demineralization, including laser treatment of enamel, and a novel 'hybrid' technique for the treatment of primary molar caries which involves 'overlapping' of secondary and tertiary prevention--the Hall technique. Although many of these techniques show considerable promise and dentists should be aware of these developments and follow their progress, the evidence for each of these novel preventive treatment options is currently insufficient to make widespread recommendations. Changes in dental practice should be explored to see how oral health can be best supported through novel preventive systems. Further research is also required involving double-blind randomized controlled trials in order to bring further benefits of more effective caries control to patients. Implementation in practice should follow promptly as new techniques are shown to be clinically valuable for individual patients. Copyright 2009 S. Karger AG, Basel [References: 25]
containing 5 mL of brain heart infusion broth. Then 10-microL suspension was inoculated onto blood agar plates. The colonies of bacteria were counted and data were analyzed statistically using Kruskal-Wallis 1-way analysis of variance and Mann-Whitney U tests. RESULTS: There were statistically significant differences between all groups (P < .05). The saline group had the highest number of remaining microorganisms. CONCLUSION: Both KTP laser and gaseous ozone have a significant antibacterial effect on infected root canals, with the gaseous ozone being more effective than the KTP laser.

Ozonated water improves lipopolysaccharide-induced responses of an odontoblast-like cell line.
Authors Full Name
AB INTRODUCTION: It is important to develop an antimicrobial agent without any damage on dental pulp. In the present study, we examined whether pretreatment of bacterial lipopolysaccharides (LPS) with ozonated water (O(3)aq) improves LPS-induced responses of rat odontoblastic cell line, KN-3. METHODS: After the pretreatment of LPS with O(3)aq, effects of LPS and O(3)aq-treated LPS on cell viability; calcification ability; expression of cyclooxygenase 2 (COX-2), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-alpha); and activation of p38 of KN-3 cells were examined. RESULTS: The formation of mineralized nodules by KN-3 cells was suppressed by LPS, whereas that suppression was inhibited by the pretreatment of LPS with ozonated water. We also found that LPS-induced expression of COX-2, IL-6, and TNF-alpha and p38 activation were markedly suppressed when LPS was pretreated with ozonated water. Furthermore, expression of COX-2, IL-6, and TNF-alpha by LPS were mainly induced through p38 activation. CONCLUSION: These results suggest that odontoblastic cells exhibit inflammatory responses against LPS and that ozonated water has the ability to improve LPS-induced inflammatory responses and suppression of odontoblastic properties of KN-3 cells through direct inhibition of LPS.

The influence of Healozone on microleakage and fissure penetration of different sealing materials.
Authors Full Name
The preventive effect of sealing materials depends on ability to penetrate into the fissures, and microleakage absence, resulting with better clinical success. The aim of present study was to investigate the influence of ozone on microleakage and penetration of nanoparticle fissure sealing resin and flowable composite, and quantitative and qualitative analysis of microleakage. Forty extracted non carious third molars were randomly divided in 4 groups. Group A: KaVo HealOzone and Grandio Seal; Group B: Kavo HealOzone and X-Flow; Group C: Grandio Seal; Group D: X-Flow. The teeth were thermocycled, immersed in 5% methylene dye for 24 hours, and sectioned with precision saw. The total of 149 slices were analysed with stereomicroscope for microleakage and sealant penetration. According to qualitative scores, there is a significant difference between groups C and D, group C showing better results. According to quantitative scores, there are no statistical differences between the groups. The treatment of the enamel with KaVo HealOzone after etching does not affect either microleakage or penetration proportion of flowable composite or sealing resin. There is no statistically significant difference in a degree of penetration between different groups of sealing materials. Groups of materials with flowable composite in combination with an adhesive system show a good degree of penetration into the fissure and low microleakage meaning that they can be used as a fissure sealing materials.

27. [ ]

Antibacterial effect of ozone on cariogenic bacterial species.
Johansson E. Claesson R. van Dijken J W V.
[Comparative Study. Journal Article]
UI: 19342147
Authors Full Name
Johansson, E. Claesson, R. van Dijken, J W V.
[View Annotation(s)]
[View Abstract]
AB OBJECTIVE: The aim was to evaluate the antibacterial effect of ozone on cariogenic bacterial species with and without the presence of saliva and a possible effect on the salivary proteins.
METHODS: Suspensions of Actinomyces naeslundii (ACTCC 12104(T)), Lactobacilli casei (N CTC 151) and Streptococcus mutans (NCTC 10449), in salt buffer or in saliva, were exposed to ozone gas delivered by the ozone generator HealOzone 2130C. Aliquots of the suspensions were taken after 10, 30 and 60s ozone exposures and cultivated on agar plates. Initial number of bacteria per ml was 8.0 x 10(7) (SD 2.2 x 10(7)) (A. naeslundii), 1.0 x 10(8) (SD 3.1 x 10(6)) (L. casei) and 1.0 x 10(8) (SD 7.0 x 10(5)) (S. mutans), respectively. The proteins were separated by SDS electrophoresis and visualized by silver staining. RESULTS: In salt buffer 92%, 73% and 64% of the initial numbers of A. naeslundii, S. mutans and L. casei, respectively, were killed already after 10s ozone exposure, while approximately 99.9% of the bacteria were dead after a 60s
exposure. After 10 and 30s, but not after 60s exposure to ozone, S. mutans and L. casei were less efficiently killed in saliva compared to the salt buffer. Various saliva proteins were degraded by ozone after a 60s exposure. CONCLUSIONS: The cariogenic species S. mutans, L. casei and A. naeslundii were almost eliminated following 60s of ozone treatment. This killing was reduced in the presence of saliva although increasing the ozone application time to 60s overcame these reductants in saliva. Detection of altered salivary proteins indicates that saliva components constitute additional targets for ozone.

Comment on "The application of ozone in dentistry: A systematic review of the literature":
Lynch E.

Efficacy of calcium hydroxide, Er:YAG laser or gaseous ozone against Enterococcus faecalis in root canals.

AB PURPOSE: To evaluate the efficacy of Ca(OH)2, Er:YAG laser or gaseous ozone (either alone or combined with instrumentation and various irrigants) against Enterococcus faecalis in root canals. METHODS: 180 extracted, human, single-rooted teeth were divided into four groups of 45 teeth each. In Group 1 root canal enlargement up to ISO-size 60 (MAF) was performed, whereas only initial shaping (MAF ISO-size 40) was carried out in Groups 2 to 4. After sterilization all teeth were inoculated with E. faecalis and incubated for 3 days, followed by evaluation of CFU. Subsequently, root canal enlargement up to ISO-size 60 was performed in Groups 2 to 4 using NaCl solution (0.9%) in Group 2, NaOCl (1%) in Group 3 and CHX (0.2%) in Group 4. Finally, each group of 45 teeth was subdivided into three groups (n= 15 each) either applying Ca(OH)2 for 7 days, using Er:YAG laser radiation for 30 seconds or gaseous ozone for 120 seconds, followed by final evaluation of CFU. RESULTS: Both in Groups 1 and 2 the median reduction of bacteria after application of Ca(OH)2 (factor 10(4) each) and ozone (in Group 1: factor 5 x 10(3); in Group 2: factor 5 x 10(4)), respectively, was significantly higher than after Er:YAG
laser treatment (factor 102 each, Mann-Whitney test). The antibacterial efficacy was significantly increased by the additional use of NaOCl or CHX as irrigants in all subgroups (Groups 3 and 4) compared to corresponding subgroups of Group 1 (Mann-Whitney test).

Ozone application in dentistry.

Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model.

Abstract: AB AIM: To assess the antimicrobial efficacy of aqueous (1.25-20 microg mL(-1)) and gaseous ozone (1-53 g m(-3)) as an alternative antiseptic against endodontic pathogens in suspension and a biofilm model. METHODOLOGY: Enterococcus faecalis, Candida albicans, Peptostreptococcus micros and Pseudomonas aeruginosa were grown in planctonic culture or in mono-species biofilms in root canals for 3 weeks. Cultures were exposed to ozone, sodium hypochlorite (NaOCl; 5.25%, 2.25%), chlorhexidine digluconate (CHX; 2%), hydrogen peroxide (H(2)O(2); 3%) and phosphate buffered saline (control) for 1 min and the remaining colony forming units counted. Ozone gas was applied to the biofilms in two experimental settings, resembling canal areas either difficult (setting 1) or easy (setting 2) to reach. Time-course experiments up to 10 min were included. To compare the tested samples, data were analysed by one-way anova. RESULTS: Concentrations of gaseous ozone down to 1 g m(-3) almost and aqueous ozone down to 5 microg mL(-1) completely eliminated the suspended microorganisms as did NaOCl and CHX. Hydrogen peroxide and lower aqueous ozone concentrations were less effective. Aqueous and gaseous ozone were dose- and strain-dependently effective against the biofilm microorganisms. Total elimination was achieved by high-concentrated ozone gas (setting 2) and by NaOCl after 1 min or a lower gas concentration (4 g m(-3)) after at least 2.5 min. High-concentrated aqueous ozone (20 microg mL(-1)) and CHX almost completely eliminated the biofilm cells, whilst H(2)O(2) was less effective. CONCLUSION: High-concentrated gaseous and aqueous ozone was dose-, strain-
and time-dependently effective against the tested microorganisms in suspension and the biofilm test model.

My Projects

The inability of Streptococcus mutans and Lactobacillus acidophilus to form a biofilm in vitro on dentine pretreated with ozone.
UI: 19133951
Authors Full Name
Knight, G M. McIntyre, J M. Craig, G G. Mulyani, Zilm, P S.
[View Annotation(s)]
[Abstract]
[Reference]

AB BACKGROUND: The use of ozone therapy in the treatment of dental caries is equivocal. The aim of this study was to use an in vitro model to determine the effects of prior ozone application to dentine on biofilm formation and to measure any associated reduction in bacteria viability. METHODS: Twenty dentine discs were bonded to the bases of 5 mL polycarbonate screw top vials. Ten dentine discs were infused with ozone for 40 seconds, 10 samples remained untreated as a control. The vials were filled with nutrient medium, sterilized and placed into the outflow from a continuous chemostat culture of Streptococcus mutans and Lactobacillus acidophilus for four weeks. At the conclusion of the experiment bacterial growth was monitored by taking optical density readings of the growth medium in each vial and the outer surface of the dentine specimens were examined by scanning electron microscopy as shown by SEM analysis. RESULTS: Ozone infusion prevented biofilm formation on all the treated samples while there was substantial biofilm present on the control specimens. While the average optical density of the control specimens was almost twice that of the ozone infused dentine (0.710 for the control with a SD of 0.288
and 0.446 for the ozonated samples with a SD of 0.371), the results were not significant (p > 0.05). CONCLUSIONS: This preliminary study has shown that the infusion of ozone into non-carious dentine prevented biofilm formation in vitro from S. mutans and L. acidophilus over a four-week period. The possibility exists that ozone treatment may alter the surface wettability of dentine through reaction with organic constituents.

**My Projects**

**In vitro reduction of mutans streptococci by means of ozone gas application.**
Castillo A. Galindo-Moreno P. Avila G. Valderrama M. Liebana J. Baca P.
[Journal Article. Research Support, Non-U.S. Gov't]
UI: 19093059

Authors Full Name

AB OBJECTIVE: To evaluate, in vitro, the antimicrobial effect of ozone gas on strains of reference mutans streptococci and strains isolated from children at a high risk of developing caries.

METHOD AND MATERIALS: A series of dilutions was obtained from a total of 41 strains, and the effect of ozone was determined for applications of 10, 20, and 40 seconds, as well as a control, in terms of the reduction of viable bacteria.

RESULTS: Ozone application for 10 and 20 seconds produced a significant reduction in the number of bacteria, inversely proportional to the bacterial concentration. When exposure lasted 40 seconds, no viable bacteria were obtained.

CONCLUSIONS: Both time of application and initial bacterial concentration bear an influence on the antimicrobial effect of ozone on mutans streptococci.

**My Projects**

**Effect of ozone treatment on different cariogenic microorganisms in vitro.**
Fagrell TG. Dietz W. Lingstrom P. Steiniger F. Noren JG.
[Journal Article]
UI: 18973084

Authors Full Name

AB Ozone treatment has been presented and discussed in the literature, as one of the "new" ways to treat dental caries. The aim of this paper was to study the in vitro effect of ozone on some common oral microorganisms related to dental caries using scanning electron microscopy (SEM). The effect of ozone was tested on three different strains of mutans streptococci and one Lactobacillus strain. After exposure of bacteria to ozone
treatment for 0 to 60 sec, cultivation on different chair side strips and agar plates took place. Preparation and performance of scanning electron analyses in a field emission scanning electron microscope at 10 kV was then carried out. It was found that gaseous ozone treatment for 20 seconds or more was effective to kill the different microorganisms in vitro. Treatment of 20, 40 and 60 seconds of ozone prevented the bacteria to grow on the different media. Treatment times shorter than 20 seconds resulted in varying results with a limited effect on bacterial growth for treatments of 5-10 sec, respectively. The difference between ozone-treated and untreated specimens was macroscopically readily discernable. None of the strains treated with ozone for 60 seconds showed any bacterial growth. Only samples with untreated bacteria could be found in the SEM analyses in form of large and high colonies. This study presents a clear result of the bactericide effect of ozon (in vitro) on four different strains of bacteria associated with dental caries.

Evidence-based caries reversal using ozone.
Lynch E.

Effect of ozone gas application on the mechanical properties of dental adhesives bonded to dentin.
Magni E. Ferrari M. Hickel R. Huth KC. Ilie N.

AB OBJECTIVES: To assess the effect of ozone gas on the mechanical properties of different classes of dental adhesives.

METHODS: Extracted molars were sectioned perpendicularly to their long axis for obtaining dentin slices to be bonded with one of the following adhesives: Prime&Bond NT (Dentsply), Excite (Ivoclar-Vivadent), Syntac/Heliobond (Ivoclar-Vivadent) and Silorane System Adhesive (3 M-ESPE). Prior to bonding, the slices were sectioned in two halves: one was treated with ozone gas for 120 s (4.2g/m³; HealOzone, KaVo) and the other served as control. The Vicker's hardness (VH), elastic modulus (E), elastic work (We/Wtot) and the creep (Cr) of the materials were measured with an automatic micro hardness indenter (Fischer) 30 min and 24 h after bonding procedure. Data were analyzed with the multivariate ANOVA followed by the Tukey's test and
RESULTS: The adhesive and the time of testing were significant factors affecting the investigated parameters ($p<0.001$; $\eta^2>0.06$). Dentin treatment, though significant, exerted a minimal effect on mechanical properties ($\eta^2<0.01$). Ozone did not consistently affect the mechanical properties at both, 30 min and 24 h measurements, as all variations were below 10%. Small differences between ozone and control resulted only in the $Cr$ and $We/\text{Wtot}$ of Excite and in the $VH$ of the Silorane System Adhesive at the 30 min measurement; and in the $We/\text{Wtot}$, $Cr$ and $VH$ of the Syntac/Heliobond system after 24h.

SIGNIFICANCE: Ozone gas did not compromise the mechanical properties of the tested adhesives. Thus, its application on dentin prior to bonding procedures is possible, without impairing the performance of the final restoration.

**My Projects**

**A quantitative approach to the effectiveness of ozone against microbiota organisms colonizing toothbrushes.**

Bezirtzoglou E, Cretoiu SM, Moldoveanu M, Alexopoulos A, Lazar V, Nakou M.


[Journal Article. Research Support, Non-U.S. Gov't]

UI: 18502558

Authors Full Name


AB OBJECTIVES: Toothbrushes are rapidly contaminated with different microorganisms, which colonize the oral cavity and interdental spaces. This can represent a possible cause of infection or reinfection. In this study, the ozone experimental effect upon toothbrushes microflora was estimated microbiologically before and after saturation with ozone gas.

METHODS: Fifty used toothbrushes coming from children and adults were entered our study. Microorganisms were enumerated and identified. Bristles from each brush were soaked in ozone saturated PBS solution for 5, 10, 15, 20 and 30 min and the total microbial population was reassessed.

RESULTS: Counts of microorganisms isolated per brush varied between $10^2$ and $10^7$ CFU. *Candida albicans* was present in used toothbrushes. No obligate anaerobes were isolated. Members of Streptococcaceae family were regularly found (65.2%) belonging to the following species: *Streptococcus pyogenes*, *S. mutans*, *S. mitis*, *S. oralis*, *S. sobrinus*, *S. viridans*, *S. salivarius*, *S. sanguis*, *Aerococcus viridans*. *A. viridans* and *S. mutans* were more frequently isolated on children toothbrushes while *Staphylococcus aureus* and *S. epidermidis* were found on adults brushes. *Escherichia coli*, *Pseudomonas* sp. and *Enterococcus sp.* were also recovered. We found that the ozone treatment decreased gradually the microbial load. However, a bacterial re-growth was effective following short ozonation period. Decontamination was complete after an extended exposure to ozone for 30 min.

CONCLUSIONS: Ozone application was found to remove the toothbrushes bristles.
microbiota following conventional brushing. Maximum decontamination efficacy of ozone treatment was observed after 30 min while exposure for short time periods seems to be inefficient which probably reflect the low dose of ozone used in this study.

**My Projects**

**The disinfecting effect of ozonized oxygen in an infected root canal: an in vitro study.**


**Authors Full Name**

**AB OBJECTIVES:** To determine the disinfecting effect of ozonized oxygen (120 seconds from the HealOzone generator, KaVo) on Enterococcus faecalis, representing bacteria that are difficult to eliminate in the root canals of human teeth, and to compare it with the conventional irrigants: sterile physiologic sodium chloride solution (negative control group), 3% hydrogen peroxide solution, 0.2% chlorhexidine solution, 1.5% sodium hypochlorite solution, and 3% sodium hypochlorite solution (positive control group).

**METHOD AND MATERIALS:** The roots (n = 10 in each group) were sterilized, contaminated with the test microorganisms in a quantitative preparation, rinsed with the test solutions, and dried. The residual concentration of E faecalis was determined through another irrigation stage with the sodium chloride solution. **RESULTS:** The positive control group showed a significantly lower concentration of microorganisms than all the other groups, whereas the negative control group showed a significantly higher concentration compared to the other groups. The test groups showed low concentrations. **CONCLUSION:** Ozonized oxygen appears to be suitable for disinfecting root canal systems in cases where sodium hypochlorite is not indicated.

**Ozone therapy in medicine and dentistry. [Review] [26 refs]**


**Authors Full Name**

**AB AIM:** The purpose of this review is to present the potential for the incorporation of ozone therapy into the practice of dentistry. **BACKGROUND:** Ozone gas has a high oxidation potential and is 1.5 times greater than chloride when used as an antimicrobial agent against bacteria, viruses, fungi, and protozoa. It also has the capacity to stimulate blood circulation and the immune response. Such features justify the current interest in its application in medicine and dentistry and have been indicated for the treatment of 260 different pathologies. It can be used for the treatment of alveolitis as a replacement for
antibiotic therapy, as a mouthwash for reducing the oral microflora, as well as the adherence of microorganisms to tooth surfaces. Ozone has been shown to stimulate remineralization of recent caries-affected teeth after a period of about six to eight weeks. CONCLUSION: The future of ozone therapy must focus on the establishment of safe and well-defined parameters in accordance with randomized, controlled trials to determine the precise indications and guidelines in order to treat various medical and dental pathologies. Scientific support, as suggested by demonstrated studies, for ozone therapy presents a potential for an atraumatic, biologically-based treatment for conditions encountered in dental practice. [References: 26]

The effects of ozone gas application on shear bond strength of orthodontic brackets to enamel.
UI: 18435374
Authors Full Name
AB PURPOSE: To investigate the possibility that ozone may have an adverse effect on the bond strength of orthodontic brackets and to determine the area of residual adhesive on teeth after the debonding of brackets. METHODS: 60 extracted premolars teeth were used in this study. Resin coated APC brackets (3M) were bonded according to the manufacturers' instructions. Bonded teeth were randomly divided into two groups. The teeth in Group 1 were subjected to a 10-second dosage of ozone from the HealOzone unit (Kavo) after etching and to a further 10 seconds of ozone after bonding the brackets using a 5 mm delivery cup. Teeth in Group 2 were used as a control. Debonding was carried out using a testing instrument at a cross-head speed of 1 mm/minute. RESULTS: The Mann-Whitney test revealed no significant differences in shear bond strength between the two groups (P = 0.337). The mean shear bond strength (11.66 MPa) of Group 1 (subjected to ozone) was not significantly different than the mean shear bond strength (10.88 MPa) of Group 2 (not subjected to ozone). A Pearson Chi-square test of the Adhesive Remnant Index (ARI) revealed no significant difference in residual adhesive among the groups tested.

Effectiveness of ozonated water on Candida albicans, Enterococcus faecalis, and endotoxins in root canals.
The aim of this study was to evaluate the effectiveness of ozonated water in the elimination of Candida albicans, Enterococcus faecalis, and endotoxins from root canals. Twenty-four single-rooted human teeth were inoculated with C. albicans and E. faecalis, and 24 specimens were inoculated with Escherichia coli endotoxin. Ozonated water (experimental group) or physiologic solution (control group) was used as irrigant agent. Antimicrobial effectiveness was evaluated by the reduction of microbial counts. Lipopolissacharide complex presence was assessed by limulus amebocyte lysate test and B-lymphocyte stimulation. Data were analyzed by Wilcoxon and Mann-Whitney tests (5%). Ozonated water significantly reduced the number of C. albicans and E. faecalis at the immediate sampling, but increased values were detected after 7 days. Ozonated water did not neutralize endotoxin. It could be concluded that ozonated water was effective against C. albicans and E. faecalis but showed no residual effect. No activity on endotoxin was observed.

Shear bond strength of epoxy resin-based endodontic sealers to bovine dentin after ozone application.
Bojar W. Czarnecka B. Prylinski M. Walory J.
[In Vitro. Journal Article]
UI: 20131749
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB The idea of using ozone to disinfect root canals is of recent origin. The wide acceptance of epoxy resin-based sealers lead us to investigate whether ozone can influence the adhesion to the dentin. In this study, we tested the shear bond strength of AH Plus and EZ Fill. Forty freshly extracted bovine teeth were randomly divided into 5 groups. 16 of these samples were treated with ozone for 60 seconds (HealOzone, Kavo). 8 samples were conditioned with the G Bond bonding system. The groups tested were: (1) AH Plus, (2) AH Plus and ozone, (3) EZ Fill, (4) EZ Fill and ozone, (5) AH Plus and G Bond. 48 hours after being prepared the specimens were tested for shear bond
strength. Statistical analysis showed significant differences between materials (AH Plus > EZ Fill) and significant, positive influence of ozone and bonding agent on the shear bond strength.

49. Periradicular repair after two-visit endodontic treatment using two different intracanal medications compared to single-visit endodontic treatment.
[Comparative Study. Journal Article]
UI: 18278299
Authors Full Name
[View Annotation(s)]
[View Abstract]
AB The number of appointments necessary to treat infected root canals is one of the most controversial issues in endodontics. This study evaluated, in dogs, the response of the periradicular tissues to the endodontic treatment of infected root canals performed in a single visit or in two visits, using different interappointment dressings. Periradicular lesions were induced by inoculating Enterococcus faecalis in the root canals. After confirming that a periradicular lesion developed, the root canals were treated within one or two visits, using either ozonized oil or calcium hydroxide in camphorated paramonochlorophenol (CMCP) as an intracanal medication. After 6 months, the animals were sacrificed and the specimens were processed for histological and histobacteriological analysis. The root canals treated in a single visit showed a success rate of 46%. When a calcium hydroxide/CMCP-based interappointment intracanal medication was used, 74% of the cases were categorized as success. In cases where ozonized oil was used as the intracanal medication, a success rate of 77% was observed. These results of the present study demonstrated that the two-visit treatment offered a higher success rate compared to one-visit therapy. In addition, ozonized oil may potentially be used as an intracanal medication.

50. Ozone air levels adjacent to a dental ozone gas delivery system.
AB OBJ ECTIVE: Ozone (O3) has been suggested as an anti-microbial treatment in dentistry, with an ozone gas delivery system introduced for the treatment of fissure and root caries. The aim of this study was to investigate the sealing capacity of the novel delivery system and its re-suction capacity during accidental displacement of the cup at different stages of ozone delivery. MATERIAL AND METHODS: Ozone leakage was studied in vitro after application on a flat metal surface and on buccal and occlusal tooth surfaces. An ozone analyzer was used to measure ozone gas concentrations adjacent to the delivering cups when adapted to the target surfaces during and after 10-20 s application cycles. The measured levels were compared with the background concentrations in the room. Measurements were performed 1) after complete ozone application cycles, 2) within the cycle before the start of the suction period, and 3) after displacements of the cup during the cycles. RESULTS: Ozone air values varied between 8 and 166 microgram/meter-3 for the flat metal surface and between 0 and 108 microgram/meter-3 for the tooth surfaces. Ozone leakage levels were 7.6 microgram/meter-3 for the flat and and 7.4 microgram/meter-3 and 5.6 microgram/meter-3 for the buccal and occlusal surfaces, respectively, and 5.2 microgram/meter-3 and 9.8 microgram/meter-3 for the premolar and molar surfaces, respectively. Cycles with displacement showed significantly higher leakage levels than continuous complete cycles (p=0.03). CONCLUSIONS: Ozone application cycles with displacements showed significantly higher leakage levels than continuous complete cycles. The largest ozone delivery cups showed the highest leakage values. A change in background levels was seen with similar change in adjacent ozone levels. The overall measured ozone leakage values were low after normally functioning delivery cycles and after repeated displacements. The delivery system can be considered safe.
AB Cleaning of dental instruments is the first line of control in reducing the adherent bioburden. The threat of vCJD and the difficulty in removing prion protein has provided a new challenge for cleaning surgical and dental instruments. Prion proteins are also more resistant to many disinfection and sterilisation techniques. A number of different methods are currently available in primary care for cleaning instruments including manual washing, ultrasonic cleaners and washer disinfectors. Manual cleaning of dental instruments is time-consuming, introduces operator error and the risk of puncture wounds, is not reproducible and does not completely remove debris from instruments. Ultrasonic baths are significantly more effective than hand cleaning alone and are currently used by the majority of dental surgeries (often as an adjunct to manual cleaning). Automated washer-disinfectors appear to provide a validated, reliable and reproducible procedure for disinfection and sterilisation of dental instruments to ensure both the safety of patients and dental staff. Dental instruments that are difficult to clean are frequently contaminated with tissue debris after routine reprocessing and cannot be excluded as a potential transmission risk for infectious agents, including prions. The transmission of vCJD via dentistry is considered to be low risk, however, the Department of Health (DoH) has recently advised dentists to ensure that endodontic reamers and files are treated as single-use as a precautionary basis in order to further reduce any risk of vCJD transmission.

**Ozone therapy in extractive surgery on patients treated with bisphosphonates.**

AB It is certain that oral extractive surgery is a remarkable trigger to avascular osteonecrosis of the jaw in patients treated with pyrophosphate analogous. This acquisition limits the use of endo-oral surgery in those patients, even when they have already developed the lesions. In this study, we present the results obtained in a group of 15 patients deriving from a 33-patient cluster with osteonecrosis of the jaw in treatment at our department with a new protocol based on ozone therapy. The object of this article is to demonstrate how dental extraction becomes possible in a patient with avascular bisphosphonate-related jaw osteonecrosis or in those who simply received pyrophosphate analogous when proper treatment with ozone therapy has been done.
Assessment of the safety of two ozone delivery devices.
Millar BJ, Hodson N.
[Journal Article]
UI: 17030396

Authors Full Name
Millar, Brian J. Hodson, Nicholas.
[View Annotation(s)]
[View Abstract]
AB OBJECTIVES: To evaluate the safety of an ozone gas device designed for use in dentistry. METHODS: Two commercially available ozone applicators, Ozi-cure and HealOzone were used in a clinical simulation using a phantom head while recordings of ozone levels were made in pharyngeal and nasal regions of the patient and near the mouth of the operator. Clinical simulations included ozone application for caries management and endodontic treatment. Recordings were made five times with different levels of suction to assess the effect on ozone levels.
RESULTS: The results with Ozi-cure on caries mode resulted in a peak ozone level in the pharynx of 1.33 +/- 0.52 ppm when no suction was used. The use of suction nearby reduced the ozone level to zero while suction on the opposite side of the mouth reduced the level to 0.22 +/- 0.04 ppm. Used on endodontic mode the peak ozone level in the pharynx was 5.51 +/- 1.63 ppm when no suction was used. The use of suction nearby reduced the ozone level to zero while suction on the opposite side of the mouth reduced the level to 0.84 +/- 0.54 ppm. Recordings in the patient's nasal region gave a peak of 0.22 ppm when using the Ozi-cure on endodontic mode with no suction. At the operator's mouth the ozone level did not exceed 0.01 ppm although the characteristic smell of ozone was detectable. All recordings with HealOzone were zero. Concentrations of 15 ppm were recorded in a simulated tooth cavity with Ozi-cure and >20 ppm with HealOzone. CONCLUSIONS: The Ozi-cure device when used without adequate suction allows ozone to reach a concentration above permitted levels and therefore should not be used. The HealOzone was safe to use.

Dahnhardt JE, Jaeggi T, Lussi A.
[Controlled Clinical Trial. Journal Article]
UI: 17073201

Authors Full Name
Dahnhardt, J E. Jaeggi, T. Lussi, A.
AB PURPOSE: To determine whether the treatment of dental caries with ozone was possible in apprehensive children and to ascertain whether ozone reverses caries in open single-surface lesions. Further, the influence of ozone on laser fluorescence was investigated.

METHODS: 82 lesions in 28 children with at least two open single-surface lesions were assessed. The children were anxious and were judged by the referring dentist as non-treatable. For each test lesion, which was treated with ozone, a control lesion was left without ozone treatment. Hardness and laser fluorescence values were assessed and the changes for hardness and laser fluorescence values in the test lesion were compared with the values in the control lesion after 2, 4, 6, and 8 months.

RESULTS: 94 percent of the children were treatable and 93% lost their dental anxiety. The hardness values improved significantly in the ozone-treated test lesions after 4, 6, and 8 months (P< 0.05) compared with baseline while the control lesions had no significant change in hardness at any recall interval. Comparing the differences between test and control teeth over time, the laser fluorescence values improved, however the improvement was not statistically significant (P> 0.05). The use of ozone resulted in an average reduction of 13% of the laser fluorescence values immediately after the ozone treatment.
their ozonides. Moreover, evidence for the ability of O₃ to induce the release of selected low-molecular-mass salivary biomolecules from macromolecular binding-sites was also obtained. Since many of the oxidation products detectable in O₃-treated samples are identical to those arising from the attack of *OH radical on biofluid components, it appears that at least some of the modifications observed here are attributable to the latter oxidant (derived from O₃*- generated from the single electron reduction of O₃).

**Antimicrobial potential of ozone in an ultrasonic cleaning system against Staphylococcus aureus.**


UI: 16924341

Authors Full Name

AB The aim of this study was to evaluate the antimicrobial potential of ozone applied to 3 different solutions in an ultrasonic cleaning system against Staphylococcus aureus. A total of 120 mL of S. aureus were mixed in 6 L of the experimental solutions (sterile distilled water, vinegar and sterile distilled water + Endozime AWplus) used in a ultrasonic cleaning system (UCS). Ozone was produced by an electric discharge through a current of oxygen and bubbling with flow rate at 7 g/h ozone (1.2%) into the microbial suspensions. Ten mL of each experimental suspension were collected and 5 fold dilutions were made in 9 mL of BHI and incubated at 37 degrees C for 48 h. Bacterial growth was evaluated by turbidity of the culture medium. At the same time, 1 mL of bacterial samples was collected and inoculated in BHIA plates. After incubation at 37 degrees C for 48 h, the number of colony forming units (cfu) per mL on BHIA surface was counted. In dilution test in BHI tubes and in BHIA plates (cfu/mL), bacterial growth was not observed in any of the experimental solutions when ozone was added. Under the tested conditions, it may be concluded that the addition of ozone to a ultrasonic cleaning system containing different experimental solutions resulted in antibacterial activity against S. aureus.

**Antibacterial effect of an ozone device and its comparison with two dentin-bonding systems.**


UI: 16911107

Authors Full Name
Polydorou, Olga. Pelz, Klaus. Hahn, Petra.
AB Microorganisms remaining beneath restorations can cause secondary caries and pulp damage. Because of this, antimicrobial treatment could be useful. The aim of this study was to evaluate the antibacterial effect of the HealOzone device on Streptococcus mutans and to compare it with the already proven activity of two dentin-bonding systems. Thirty-five human molars were divided into 5 groups. Cavities were then cut into the teeth (n = 28 cavities per group). After sterilization, the teeth were left in broth cultures of 10^6 colony-forming units (CFU) ml^-1 of S. mutans at 36 degrees C for 48 h. The appropriate treatment followed (group A, control; group B, Clearfil SE Bond; group C, Clearfil Protect Bond; group D, 40 s of treatment with ozone; and group E, 80 s of treatment with ozone), and the cavities were then filled with composite resin. After 72 h, the restorations were removed, dentin chips were collected with an excavator, and the total number of microorganisms was determined. All treatments significantly reduced the number of S. mutans present compared with the control group. The antimicrobial effect of both bonding systems and treatment with 80 s of ozone was significantly higher than the 40 s ozone treatment. In conclusion, HealOzone and the bonding systems show striking antimicrobial effects against S. mutans.

The use of ozone in dentistry and maxillofacial surgery: a review. [Review] [88 refs]
[]
overview of the current applications of ozone in dentistry and oral surgery. Research was based on peer-reviewed sources found through a Medline/PubMed search and other textbooks, reviews, and journals. [References: 88]

Missing the point.
Holmes J.
[Comment. Letter]

The impact of ozone treatment on enamel physical properties.
Celiberti P. Pazera P. Lussi A.
[Journal Article. Randomized Controlled Trial]

Praise for ozone.
Perregaard L.

An epistemological approach.
Dicran Megighian G.
Difficulty with samples.
Jackson P.  
[Letter]

72. [ ]  
UI: 16444201  
Authors Full Name  
Jackson, P.  
[View Annotation(s)]  
My Projects  

A catalyst for change.  
Cronshaw MA.  
[Comment. Letter]

73. [ ]  
UI: 16444200  
Authors Full Name  
Cronshaw, M A.  
[View Annotation(s)]  
My Projects  

Ozone slayers.  
Johnson ND.  
[Comment. Letter]

74. [ ]  
UI: 16444197  
Authors Full Name  
Johnson, N D.  
[View Annotation(s)]  
My Projects  

The use of ozone in dentistry and medicine. Part 2. Ozone and root caries.  
Baysan A. Lynch E.  
[Journal Article]  
UI: 16393498  
Authors Full Name  
[View Annotation(s)]  
[View Abstract]  

75. [ ]  
AB A previous paper, recently published in Primary Dental Care, gave an overview of the medical uses of ozone and outlined some of its uses in dentistry. The current paper focuses on a description of use of ozone in the management of root caries and considers recent studies in this area. There has been relatively limited research into the non-invasive (pharmaceutical) management of root caries. The best management strategy still remains to be developed. Initial studies have indicated that an application of ozone for a period of either 10 or 20 seconds is capable of clinically reversing
leathery root carious lesions. It is suggested that, subject to confirmation from extensive trials, this simple and non-invasive technique may benefit many patients with root caries throughout the world since this approach to treat root caries can easily be employed in primary care clinics and in the domiciliary treatment of home-bound elderly people and immobile patients in hospices and hospitals.

My Projects

[Ozone therapy with the OzonyTron apparatus]. [Polish]
UI: 16519995 Authors Full Name

AB PURPOSE: OzonyTron is one of the newest devices for the generation of ozone. The aim of this study was to assess the benefits of ozone in some pathologies. MATERIAL AND METHODS: Ozone therapy was used in 20 patients, including eight with lip herpes, five after dental surgery and chiseling of third molars, six with seborrheal inflammation of facial skin and one with mycosis of the toes. RESULTS: Treatment with OzonyTron is noninvasive and painless. The oxidative properties of ozone are exploited to combat microbial infection. Thus, the device is valuable primarily for its antiseptic action. Following ozone therapy, a very good effect was observed in each case.

My Projects

[Experience in medical ozone use for root canal treatment]. [Russian]
UI: 16353031 Authors Full Name
Bezrukova, I V. Petrukhina, N B. Voinov, P A. [View Annotation(s)]

AB The results of clinical and laboratory assessment of effectiveness of PSR-diagnosis are presented. The high efficacy of ozone therapy is revealed. The findings demonstrate the reduction of number in the micro-organisms in root canal: Actinobacillus actinomycetemcomitans from 31.25% to 10.21%; Bacteriodes forsythus from 68.75% to 15.50%; Treponema denticola from 37.5% to 11.4%; Porphyromonas gingivalis from 56.25% to 45%; Prevotella intermedia from 16% to 0%.

My Projects

78. [ ] Effect of ozone on non-cavitated fissure carious lesions in permanent molars. A controlled prospective clinical study. • Abstract
AB PURPOSE: To investigate, with a randomized controlled clinical study, the effect of ozone on non-cavitated initial occlusal fissure caries compared with untreated contra-lateral control lesions (split mouth) considering the patient's current caries risk. METHODS: Forty-one patients with 57 pairs of lesions were enrolled in the study (mean age 7.7 +/- 2.2 years; upper jaw n=29, lower jaw n=28). Gaseous ozone (HealOzone) was applied once for 40 seconds to the randomly assigned test molar of each pair without the use of remineralizing solutions. Lesion progression or reversal was monitored by the laser fluorescence system DIAGNOdent for up to 3 months and the deterioration or improvement compared between the ozone-treated lesions and the untreated control lesions (in pairs). This was done for the whole study population and a subgroup of patients with high current caries risk (lesion pairs n=26). RESULTS: After 3 months, explorative data analysis revealed that the ozone-treated lesions showed significantly more caries reversal or reduced caries progression than the untreated control lesions within the group of patients at high current caries risk (Wilcoxon-Test, P= 0.035). There was no statistical significance examining the whole study population. From the data it can be concluded that ozone application significantly improved non-cavitated initial fissure caries in patients at high caries risk over a 3-month period.
In the early 1900s, a new high-energy device was introduced as a cure-all for a multitude of diseases and conditions that afflicted the population. Dental pain and disease were among those treatable ailments. Treatment consisted of applying direct contact to the body with a handheld, high-voltage Tesla Coil and Geissler tube, which created heat, ozone, and ultraviolet light (UV), termed the "Violet Ray". This treatment was considered mostly a fraud and banned by the Food and Drug Administration (FDA) in 1950.

The use of ozone in dentistry and medicine.
Baysan A. Lynch E.

Effect of ozone on enamel and dentin bond strength.
Schmidlin PR. Zimmermann J. Bindl A.
negative control (Hi-Lite, Shofu), and (IV) untreated enamel and dentin (positive control). Specimens were bonded with a functional 3-step adhesive system (Syntac Classic, Ivoclar Vivadent) and restored with a composite (Tetric Ceram, Ivoclar Vivadent) according to the Ultradent method. After storage in water at 37 degrees C for 24 h, shear bond strength was measured using a Zwick universal testing machine. Data were analyzed using ANOVA and Scheffe’s post hoc analysis. Results: In concordance with the existing literature, bleaching resulted in significantly decreased bond strength (p < 0.05) on enamel specimens. No decrease in shear bond strength was detected for ozone-pretreated specimens compared to untreated controls. CONCLUSION: Despite a possible retention of surface and subsurface oxide-related substances during high-dose ozone application, shear bond strength was not impaired. Thus, adhesive restoration placement should be possible immediately after ozone application for cavity disinfection.

Lesion orientated caries treatment--a classification of carious dentin treatment procedures. [Review] [56 refs]
UI: 15646589
Authors Full Name
Noack, Michael J. Wicht, Michael J. Haak, Rainer.
[View Annotation(s)]
[View Abstract]
AB PURPOSE: To review experimental and marketed techniques for the treatment of infected dentin as a potential substitute for conventional rotary excavation. MATERIALS AND METHODS: A hand and systematic search of Medline (via DIMDI) was performed. Additionally, manufacturers' data about relevant clinical studies were checked. After classification of the identified techniques described, relevant studies are cited to allow an overview of the different treatment options. RESULTS: Excavation, disinfection and sealing techniques for the treatment of infected dentin can be differentiated. Besides several mechanical approaches, chemo-mechanical excavation, enzymatic digestion and photo ablation are discussed. Disinfection techniques can be undertaken with even less invasive approaches like gasiform ozone application, photodynamic therapy or local application of antibacterial materials. Additionally, or alternatively, the sealing of carious dentin is discussed using fluoride-releasing cements, dentin adhesives or antibacterial resin materials. CONCLUSION: Although some of the techniques are still experimental and much clinical research has to be done, many different approaches are so promising, or already established, that hopefully the days of radical excavation with rotary instruments are numbered. [References: 56]

Efficacy of ozone on survival and permeability of oral microorganisms. [Abstract]
In the present study, we examined the effect of ozonated water on oral microorganisms and dental plaque. Almost no microorganisms were detected after being treated with ozonated water (4 mg/l) for 10 s. To estimate the ozonated water-treated Streptococcus mutans, bacterial cells were stained with LIVE/DEAD BacLight Bacterial Viability Kit. Fluorescence microscopic analysis revealed that S. mutans cells were killed instantaneously in ozonated water. Some breakage of ozonated water-treated S. mutans was found by electron microscopy. When the experimental dental plaque was exposed to ozonated water, the number of viable S. mutans remarkably decreased. Ozonated water strongly inhibited the accumulation of experimental dental plaque in vitro. After the dental plaque samples from human subjects were exposed to ozonated water in vitro, almost no viable bacterial cells were detected. These results suggest that ozonated water should be useful in reducing the infections caused by oral microorganisms in dental plaque.

Ozone as Janus: this controversial gas can be either toxic or medically useful. [Review] [76 refs]

Bocci V.
[Journal Article. Research Support, Non-U.S. Gov't. Review]
UI: 15203558

Ozone is an intrinsically toxic gas and its hazardous employment has led to a poor consideration of ozone therapy. The aim of this review is to indicate that a wrong dogma and several misconceptions thwart progress: in reality, properly performed ozone therapy, carried out by expert physicians, can be very useful when orthodox medicine appears inadequate. The unbelievable versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of a pathological state. During the past decade, contrary to all expectations, it has been demonstrated that the judicious application of ozone in chronic infectious diseases, vasculopathies, orthopedics and even dentistry has yielded such striking results that it is deplorable that the medical establishment continues to ignore ozone therapy. [References: 76]
Response to Beggs’ viewpoint.
Leon SP.
Dentistry Today. 23(4):14, 18, 2004 Apr.
[Comment. Letter]

Reliable caries reversal: another paradigm shift?.
Beggs R.
Dentistry Today. 23(2):14-6, 2004 Feb.

Biodegradability of organic by-products after natural organic matter oxidation with ClO2—case study.
Raczyk-Stanislawiak U. Swietlik J. Dabrowska A. Nawrocki J.

**Abstract**
Apart from well-known chlorites and chlorates, chlorine dioxide also generates easily biodegradable carbonyl compounds and short chain carboxylic acids during water disinfection. The main goal of the presented study was to examine the influence of natural organic matter (NOM) oxidation with chlorine dioxide, on the quantity as well as the quality of formed biodegradable by-products. In the experiments conducted at the pilot plant the sand filtered water (MWI) and ozonated/biofiltrated water (BAF) were oxidised with ClO2. The amount of BDOC formed as a result of the oxidation of both waters with ClO2 was compared. The results showed considerable differences in formation of ClO2 oxidation by-products between non-ozonated and ozonated/biofiltered waters. The disinfection of ozonated/biofiltrated water with ClO2 generated comparable amounts of aldehydes and much higher amounts of carboxylic acids than ClO2 oxidation of sand filtered water. These findings are essential for waterworks with ozonation/biofiltration units and ClO2 disinfection implemented.
Clinical reversal of root caries using ozone, double-blind, randomised, controlled 18-month trial.
Holmes J.
[Clinical Trial, Journal Article, Randomized Controlled Trial]
UI: 14697022
Authors Full Name
Holmes, J.
[View Annotation(s)]
[View Abstract]
AB OBJECTIVE: To assess the effect of an ozone delivery system, combined with the daily use of a remineralising patient kit, on the clinical severity of non-cavitated leathery primary root carious lesions (PRCL's), in an older population group. DESIGN: A total of 89 subjects, (age range 60-82, mean +/- SD, 70.8 +/- 6 years), each with two leathery PRCL's, were recruited. The two lesions in each subject were randomly assigned for treatment with ozone or air, in a double-blind design, in a general dental practice. Subjects were recalled at three, six, 12 and 18 months. Lesions were clinically recorded at each visit as soft, leathery or hard, scored with a validated root caries severity index. RESULTS: There were no observed adverse events. After three months, in the ozone-treated group, 61 PRCL's (69%) had become hard and none had deteriorated, whilst in the control group, four PRCL's (4%) had become worse (p<0.01). At the six-month recall, in the ozone group, seven PRCL's (8%) remained leathery, the remaining 82 (92%) PRCL's had become hard, whilst in the control group, 10 PRCL's had become worse (11%) and one had become hard (p<0.01). At 12 and 18 months, 87 Subjects attended. In the ozone group at 12 months, two PRCL's remained leathery, compared to 85 (98%) that had hardened, whilst in the control group 21 (24%) of the PRCL's had progressed from leathery to soft, i.e. became worse, 65 PRCL's (75%) were still leathery, and one remained hard (p<0.01). At 18 months, 87 (100%) of ozone-treated PRCL's had arrested, whilst in the control group, 32 lesions (37%) of the PRCL's had worsened from leathery to soft (p<0.01), 54 (62%) PRCL's remained leathery and only one of the control PRCL's had reversed (p<0.01). CONCLUSIONS: Leathery non-cavitated primary root caries can be arrested non-operatively with ozone and remineralising products. This treatment regime is an effective alternative to conventional “drilling and filling”.
My Projects

Preventive use of ozone, short waves, and laser therapy alone and in combination in early postoperative period after dental implantation]. [Russian]
PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination.


My Projects
AB The main objective of this study was to assess the effect of temperature (4-30 degrees C) on the inactivation kinetics of Cryptosporidium parvum oocysts with sequential disinfection schemes involving the use of chlorine dioxide as the primary disinfectant and free or combined chlorine as the secondary disinfectant in synthetic water. The synergy previously reported for sequential inactivation of C. parvum oocysts with ozone/free chlorine or ozone/combined chlorine did not occur when chlorine dioxide was used instead of ozone, as the primary disinfectant within the temperature range (4-30 degrees C) and the pre-treatment levels investigated. Sequential ozone/chlorine dioxide and chlorine dioxide ozone experiments revealed that the lower level or absence of synergy for chlorine dioxide/free chlorine and chlorine dioxide, monochloramine was likely the result of chlorine dioxide reacting with oocyst chemical groups that are mostly different from those reacting with ozone, free chlorine, or monochloramine. The CT concept was found to be valid for the primary inactivation kinetics of C. parvum oocysts with chlorine dioxide, thus allowing the use of the simpler CT approach for the development of C. parvum inactivation requirements with chlorine dioxide. General consistency was found between the secondary inactivation kinetics of C. parvum oocysts with free chlorine and monochloramine after chlorine dioxide pretreatment obtained in this study with oocyst viability determined by a modified in vitro excystation method and those reported in the literature for the same sequential disinfection schemes based on an animal infectivity assay.
disinfecting efficiency. RESULTS: There was no difference between the efficiencies of five disinfecting methods for Streptococcus sanguis and Staphylococcus epidermidis. But for B. subtilis, the immersion methods have the highest efficiency among the methods and the ozone treatment has the least efficiency. CONCLUSION: 2% glutaraldehyde immersion, spray, 5% Eric immersion, spray are all effective disinfecting methods for impressions and ozone treatment is an effective method in disinfecting the gypsum casts.

**Chemical treatment of machined titanium surfaces. An in vitro study.**
Krozer A. Hall J. Ericsson I.

**Antimicrobial effect of a novel ozone-generating device on microorganisms associated with primary root carious lesions in vitro.**
Baysan A. Whiley RA. Lynch E.
[Clinical Trial. Comparative Study. Journal Article. Randomized Controlled Trial]
UI: 11093025
Authors Full Name
Baysan, A. Whiley, R A. Lynch, E.
[View Annotation(s)]
[View Abstract]
AB The aims of this present study were (1) to assess the antimicrobial effect of ozone from a novel ozone-generating device (Heolozone, USA) [0.052% (v/v) in air delivered at a rate of 13.33 ml.s(-1)] on primary root carious lesions (PRCLs) and (2) to evaluate the efficacy of ozone specifically on Streptococcus mutans and Streptococcus sobrinus. In study 1, 40 soft PRCLs from freshly extracted teeth were randomly divided into two groups to test the antimicrobial effect on PRCLs from exposure to ozonated water for either 10 or 20 s. Half of a lesion was removed using a sterile excavator. Subsequently, the remaining lesion was exposed to the ozonised water for a period of either 10 or 20 s (corresponding to 0.069 or 0.138 ml of ozone, respectively). Using paired Student t tests, a significant (p<0.001) reduction (mean +/- SE) was observed in the ozone-treated groups with either a 10-second (log(10) 3.57+/-.0.37) or 20-second (log(10) 3.77+/-.0.42) ozone application compared with the control groups (log(10) 5.91+/-.15 and log(10) 6.18+/-.0.21, respectively). In study 2, 40 sterile saliva-coated glass beads were randomly divided into two groups for each micro-organism. One glass bead was put into each bijou bottle with 3 ml of Todd-Hewitt broth. S. mutans and S. sobrinus were inoculated anaerobically overnight. Each glass bead was then washed with 2 ml of phosphate-buffered saline. Immediately, 10 s of ozone gas was applied to each glass bead in the test groups. There was a significant (p=0.0001) reduction (mean +/- SE) in ozone-treated samples for S. mutans (log(10) 1.01+/-.27) and S. sobrinus (log(10) 1.09+/-.36) compared with the control samples (log(10) 3.93+/-.07 and log(10) 4.61+/-.13, respectively). This treatment regime is an effective, quick, conservative and simple method to kill micro-organisms in PRCLs. Ozone gas application for a period of 10 s was also capable of reducing the numbers of S. mutans and S. sobrinus on saliva-coated glass beads in vitro.
AB Microbial plaque accumulation on titanium dental implant surfaces can result in an inflammatory condition of the surrounding tissues. Cleaning of such a contaminated surface, in vivo, by means of a solution of amino-alcohol, following surgical exposure, has been proposed. However, the tissue healing following treatment resulted in formation of a fibrous capsule at the tissue-implant interface, i.e. improper implant re-integration. The present experiment was designed to investigate the possible influence of an amino-alcohol solution on machined titanium surface properties. Titanium samples with topography and chemical composition similar to the clinically used Branemark implant surfaces were used in this experimental in-vitro study to investigate the adsorption of amino-alcohol to such surfaces, and the possibilities to chemically remove the adsorbed alcohols in order to recover a pristine titanium surface. The amino-alcohol solution was supplied to the sample surfaces and four different methods were subsequently used in order to remove the adsorbed alcohol molecules. It was shown that rinsing in water, saline solution, and 5% H2O2 did not remove the amino-alcohol from the surface. However, exposure to ozone produced by using a commercial mercury lamp in ambient air resulted in complete removal of the adsorbed amino-alcohol. The results show that the amino-alcohol used forms a stable and dense film at the implant surface in vitro. Presence of such a film most likely prevents re-integration to occur at the implant-tissue interface in vivo.

Influence of ozone on oxidation of dental alloys.
Suzuki T. Oizumi M. Furuya J. Okamoto Y. Rosenstiel SF.
[Comparative Study. Journal Article]
UI: 10371921
Authors Full Name
Suzuki, T. Oizumi, M. Furuya, J. Okamoto, Y. Rosenstiel, S F.
[View Annotation(s)]
[View Abstract]
AB PURPOSE: The purpose of this study was to examine the influence of ozone on the surface of removable partial denture (RPD) alloys to determine its usefulness as a cleaning method for RPDs, since ozone has powerful sterilizing and deodorizing properties.
MATERIALS AND METHODS: Two types of ozone cleaning were used. The quantities of ozone generated by both methods were the same (20 mg/h). In method A, ozone was generated for 10 minutes every 12 hours and in method B, ozone was generated over 24 hours a day. Test specimens of 3 types of dental alloy (Co-Cr, Au-Ag-Pt, and Au-Cu-Ag-Pd) were subjected to different cleaning methods for 7 days and measured in terms of reflectance, surface roughness, and weight. Five different cleaning solutions (three commercial denture cleaners, acid-electrolyzed water with a pH of 2.4, and pure water) were used for comparison with the ozone treatments. RESULTS: No significant changes were detected after treatment of the Co-Cr and Au-Ag-Pt alloys with ozone. Ozone caused a slight change in the Au-Cu-Ag-Pd alloy in terms of reflectance, but the changes were significantly less than those caused by acid-electrolyzed water and one of the commercial denture cleaners. CONCLUSION: Ozone had little influence on the oxidation of dental alloys.
101. [ ] Lukinykh, L M. Kosiuga, S Iu.

[Changes in the quantitative composition of the microbial flora of dental deposits during the intensification of oral hygiene]. [Russian]
[Comparative Study. English Abstract. Journal Article]
UI: 10067405
Authors Full Name

AB The efficacy of hygienic treatment of the oral cavity in combination with ozone therapy is assessed. This combination mechanically removes soft dental deposit and decreases bacterial contamination.

Microbial contamination of dental unit waterlines: the scientific argument.
[Journal Article]
UI: 9779119
Authors Full Name

AB The quality of dental unit water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit. The unique feature of dental chair water lines is the capacity for rapid development of a biofilm on the dental water supply lines combined with the generation of potentially contaminated aerosols. The biofilm, which is derived from bacteria in the incoming water and is intrinsically resistant to most biocides, then becomes the primary reservoir for continued contamination of the system. Dental water may become heavily contaminated with opportunistic respiratory pathogens such as Legionella and Mycobacterium spp. The significance of such exposure to patients and the dental team is discussed. There is at the present time, no evidence of a widespread public health problem from exposure to dental unit water. Nevertheless, the goal of infection control is to minimise the risk from exposure to potential pathogens and to create a safe working environment in which to treat patients. This paper evaluates the range of currently available infection control methods and prevention strategies which are designed to reduce the impact of the biofilm on dental water contamination, and are suitable for use in general practice. Bacterial load in dental unit water can be kept at or below recommended guidelines for drinking water (less than 200 colony forming units/ml) using a combination of readily available measures and strict adherence to maintenance protocols. Sterile water should be employed for all surgical treatments.

102. [ ] biocides, then becomes the primary reservoir for continued contamination of the system. Dental water may become heavily contaminated with opportunistic respiratory pathogens such as Legionella and Mycobacterium spp. The significance of such exposure to patients and the dental team is discussed. There is at the present time, no evidence of a widespread public health problem from exposure to dental unit water. Nevertheless, the goal of infection control is to minimise the risk from exposure to potential pathogens and to create a safe working environment in which to treat patients. This paper evaluates the range of currently available infection control methods and prevention strategies which are designed to reduce the impact of the biofilm on dental water contamination, and are suitable for use in general practice. Bacterial load in dental unit water can be kept at or below recommended guidelines for drinking water (less than 200 colony forming units/ml) using a combination of readily available measures and strict adherence to maintenance protocols. Sterile water should be employed for all surgical treatments.

In vitro testing of a denture cleaning method using ozone.
The purpose of this study was to compare the microbicidal effect of gaseous ozone with that of ozonated water in order to determine its usefulness as a method for disinfecting dentures. Although a large number of research studies have been done on the bactericidal effect of ozone, little is known about its microbicidal effects on oral microorganisms. Therefore, we tested the effect of ozone on three standard strains of oral microorganisms: Streptococcus mutans (strain IID 973), Staphylococcus aureus (strain 209-P), and Candida albicans (strain LAM 14322). When the gaseous ozone injection method was used, the numbers of cells of all three strains decreased to $1/10^5$ at 1 min, and by 3 min they were below the detection limit. Thus, the microbicidal effect of gaseous ozone was ascertained in a short time. In contrast, when ozonated water at 1 ppm and 3 ppm was used, C. albicans decreased to 1/10. A 700 mg/h ozone production level was needed to prepare 1 ppm ozonated water, whereas 20 mg/h of ozone was required by the gaseous ozone generator. These findings indicate that direct exposure to gaseous ozone seems to be a more effective microbicide compared with ozonated water, and that gaseous ozone can be clinically useful for disinfection of dentures.

Disinfection of removable dentures using ozone.
105. [ ] [Comparative Study. Journal Article] 
UI: 9281158
Authors Full Name
Lazutikov, O V. Lunev, B V.
[View Annotation(s)]
My Projects

[The use of ozonized solutions in the combined treatment of odontogenic putrefactive-necrotic phlegmons of the maxillofacial area and neck]. [Russian]

106. [ ] [Comparative Study. Journal Article] 
UI: 9281153
Authors Full Name
Kiniapina, I D. Durnovo, E A.
[View Annotation(s)]
My Projects

[The efficacy of using ozone in the combined treatment of disseminated odontogenic phlegmons of the maxillofacial area]. [Russian]

107. [ ] [Review. Journal Article] 
UI: 8744790
Authors Full Name
Kaiser, H E.
[View Annotation(s)]
My Projects

Introduction: human pathology within the broad scope of comparative pathology. [Review] [78 refs]
UI: 8744790
Authors Full Name
Kaiser, H E.
[View Annotation(s)]
[View Abstract] 
AB Pathologic integration is the basic phenomenon of comparative pathology. Since man evolved as earth's most influential species, he was unequally influenced the progression and prevention of diseases in himself and other species. This has both positive and negative ramifications. Positive influences have been life-style, the prolongation of life under healthy conditions and medical progress as seen in the treatment of diabetes mellitus, dental hygiene and other factors, such as the decrease of infectious and parasitic diseases, which are still dominating factors in developing nations. Negative influences are side effects of medical treatments, the appearance of occupational, and certain recreational diseases. These are the pathologic effects of man's life-style to which car accidents, smoking and other factors can be added. Different species are affected by environmental changes such as pollution, ozone, acidic rain, polluted food, and transmission of different diseases from one species to another. Interspecies-specifically the direct influence of man in the extermination of other species, or the indirect influence such as through pollutants in the environment producing chain reactions in different species, can be distinguished. The physical environment has been changed as can be seen in air pollution in large cities, the damage to the ozone layer and the increase of malignant melanoma in certain regions of western Australia. The industrialized nations are
dominated by non-infectious diseases such as atherosclerosis and neoplasms, whereas in the developing nations parasitic and infectious diseases stand in the fore-front. Particular diseases like acquired immunodeficiency syndrome increase in both types of nations. These diseases may have developed from other species, e.g. the plague which was originally a disease of rodents, especially rats where it was transmitted by the flea, Xenopsylla cheopis, Rothschild. The principle of foremost importance is the disruption of biologic integration of normal processes leading to different types of pathologic progression. A typical problem affecting man and many other fellow species is crowding. Man's pathology and the pathology of other species exhibit continued integration which is the central problem for understanding diseases where similar functions are performed by various structures, such as is the case in gaseous exchange, or differences in size and life span. The broad spectrum of comparative pathology which centers around human pathology provides a source of increased knowledge for a better understanding of diseases. The present issue is based on the two symposia organized by the International Society for the Study of Comparative Oncology during the Fifth International Conference of Anticancer Research, 17-22 October 1995, Corfu, Greece. [References: 78]

[Water disinfection of dental treatment units using ozone]. [German]
Filippi A. Tilkes F. Beck EG. Kirschner H.
[English Abstract. Journal Article]
UI: 1817912
Authors Full Name
Filippi, A. Tilkes, F. Beck, E G. Kirschner, H.
[View Annotation(s)]
[View Abstract]
AB The disinfecting effect of ozonized water has been investigated. Under the precondition that the dental chair had been thoroughly sanitized, the system showed a good disinfecting effect. Finally, for reasons of practical medical treatment, the ozone concentration in air and, for reasons of hygiene in drinking water, the ozone concentrations in water were determined under various conditions. In addition, the influence of continuous-flow water heaters is discussed. The problem of continuous-flow water heaters regarding their effects on the colonisation of water by microbes proves not to be significant. The exposure of patients to disinfectants is discussed.

[Clinical studies of therapeutic results from ozonized water for gingivitis and periodontitis]. [German]
Brauner A.
[Comparative Study. Journal Article]
UI: 1872060
Authors Full Name
Brauner, A.
[View Annotation(s)]
My Projects
AB Ozone is a potent disinfecting agent which has been used successfully in water treatment, as well as to cure wounds. It can also be used in dental clinics, in laboratories, and in hospitals to clean and disinfect work surfaces and instruments. In this paper we have carried out a study of the antimicrobial activity of ozonized water. Antimicrobial activity in ozonized water on bacterial suspensions and contaminated materials was meaningful and depended fundamentally on concentration and time of exposure. On buccal flora, one rinse alone had no effect, but various successive rinses led to substantial reductions in the number of colonies of bacteria. Ozone had a similar effect, although more pronounced, on the flora of the hands. Ozonized water placed in an open dish kept up antimicrobial activity for the first 20 minutes, but after 30 minutes this activity decreased substantially.
impression material, chiefly viewed from the variations of viscosity and molecular weight. 8. Theoretical discussion and summary.


[Journal Article]
UI: 5292167

Authors Full Name
Higashi, S. Yasuda, S. Takamatsu, H. Mimura, Y. Miyasaka, S.

[View Annotation(s)]
My Projects

[Lethal effect of ozone on certain aerobic bacteria strains in a model of the dental pulp chamber]. [French]


[In Vitro. Journal Article]
UI: 552092

Authors Full Name
Deltour, G. Vincent, J. Lartigau, G.

[View Annotation(s)]
My Projects

[Ozone in endodontic therapy]. [Romanian]


[Journal Article]
UI: 5271770

Authors Full Name
Haimovici, A. Lacatusu, S. Irjicianu, A. Joan, E.

[View Annotation(s)]
My Projects

[Ozone therapy in odontostomatology, especially in treatments of infected root canals]. [French]


[Case Reports. Journal Article]
UI: 5221045

Authors Full Name
Sandhaus, S.

[View Annotation(s)]
My Projects

[Sterilization of minute endodontic material by the combination of ethylene oxide and ozone. Experimental evaluation of its effectiveness]. [French]


[Journal Article]
UI: 5216427

Authors Full Name
Brunel, A. Vannier, R. Archinet, F.
118. Effects of morphine and nalorphine upon tooth pulp thresholds of dogs in the alert and drowsy state.
HENG J E. DOMINO E F.

DAUSCH H.
Deutsche Zahnarztliche Zeitschrift. 6(8):421-7, 1951 Apr 15.

121. Experiences with the use of chlorine gas and ozone in the treatment of root gangrene and dental granuloma.
SCHWAN L. BAMFASTE M.


Source

Institute of Preventive Dentistry and Oral Microbiology, School of Dental Medicine, University of Basel, Hebelstrasse 3, 4056, Basel, Switzerland, I.Hauser-Gerspach@unibas.ch.

Abstract

Dental implants are prone to bacterial colonization which may result in bone destruction and implant loss. Treatments of peri-implant disease aim to reduce bacterial adherence while leaving the implant surface intact for attachment of bone-regenerating host cells. The aims of this study were to investigate the antimicrobial efficacy of gaseous ozone on bacteria adhered to various titanium and zirconia surfaces and to evaluate adhesion of osteoblast-like MG-63 cells to ozone-treated surfaces. Saliva-coated titanium (SLA and polished) and zirconia (acid etched and polished) disks served as substrates for the adherence of Streptococcus sanguinis DSM20068 and Porphyromonas gingivalis ATCC33277. The test specimens were treated with gaseous ozone (140 ppm; 33 mL/s) for 6 and 24 s. Bacteria were resuspended using ultrasonication, serially diluted and cultured. MG-63 cell adhesion was analyzed with reference to cell attachment, morphology, spreading, and proliferation. Surface topography as well as cell morphology of the test specimens were inspected by SEM. The highest bacterial adherence was found on titanium SLA whereas the other surfaces revealed 50-75% less adherent bacteria. P. gingivalis was eliminated by ozone from all surfaces within 24 s to below the detection limit (≥99.94% reduction). S. sanguinis was more resistant and showed the highest reduction on zirconia substrates (>90% reduction). Ozone treatment did not affect the surface structures of the test specimens and did not influence osteoblastic cell adhesion and proliferation negatively. Titanium (polished) and zirconia (acid etched and polished) had a lower colonization potential and may be suitable material for implant abutments. Gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium and zirconia without affecting adhesion and proliferation of osteoblastic cells. This in vitro study may provide a solid basis for clinical studies on gaseous ozone treatment of peri-implantitis and revealed an essential base for sufficient tissue regeneration.


The effect of dentin hypersensitivity treatments on the shear bond
strength to dentin of a composite material.

Can-Karabulut DC, Karabulut B.

Source
Department of Operative Dentistry, New East University, Mersin 10, Turkey.

Abstract
This study sought to evaluate the influence of a dentin desensitizer and ozone application on the bond strength to dentin of a composite resin material. The dentin desensitizing agent and ozone treatment were applied on the cervical dentin surfaces of extracted, caries-free, erupted third molars. Dentin surfaces that received no treatment were used as control samples. A dentin bonding agent was applied according to the manufacturer's instructions and an adhesion test was performed according to ISO/TS 11405. Statistical analysis showed no significant influence of the different hypersensitivity treatments on shear bond strength to dentin (ANOVA and Tukey's tests, p > 0.05). Within the limitations of this in vitro study, it appears that the short-term use of dentin hypersensitivity treatments like ozone and dentin desensitizers containing gluteraldehyde do not further affect the shear bond strength to dentin of subsequent composite resin restorations.


Management of gingival inflammation in orthodontic patients with ozonated water irrigation--a pilot study.

Dhingra K, Vandana KL.

Source
Department of Periodontics, N.S.V.K Sri Venkateshwara Dental College, Bangalore, India. kunaldhingra@yahoo.co.in

Abstract
OBJECTIVE:
Ozonated water irrigation has recently been tried for its antimicrobial and anti-inflammatory effects in treatment of periodontitis. During orthodontic treatment, gingival inflammation occurs along with increased lactate dehydrogenase (LDH) enzyme levels in gingival crevicular fluid (GCF). Thus, the aim
of this pilot study was to evaluate the clinical effects of a single subgingival irrigation with ozonated water on gingival inflammation in orthodontic patients and also to correlate the clinical effects with LDH enzyme activity in GCF.

**METHODS:**
Fifteen systemically healthy orthodontic patients (seven men and eight women, mean age 17.3 years) with full-mouth brackets were included in this prospective, cross-sectional, clinical and laboratory investigation. Clinical parameters, LDH enzyme activity and GCF volume were measured at baseline (0 day) followed by subgingival irrigation with 0.01 mg l(−1) ozonated water. These parameters were again assessed on 14th and 28th day.

**RESULTS:**
There was significant (P < 0.05) reduction in values of clinical parameters, GCF LDH activity and GCF volume after subgingival irrigation with ozonated water. Also, a significant correlation (r = 0.50, P = 0.01) was observed only between the post-treatment changes of plaque index and LDH values, among the clinical parameters assessed.

**CONCLUSIONS:**
A single subgingival irrigation of 0.01 mg l(−1) ozonated water can effectively reduce the gingival inflammation in orthodontic patients, which is also reflected in the reduction of LDH enzyme levels. However, further randomized controlled trials are required to validate the use of ozone irrigation in orthodontic patients for plaque control measures.


**Effectiveness of ozone against periodontal pathogenic microorganisms.**

Huth KC, Quirling M, Lenzke S, Paschos E, Kamereck K, Brand K, Hickel R, Ilie N.

**Source**
Department of Restorative Dentistry and Periodontology, Ludwig-Maximilians-University Munich, Goethestr, Munich, Germany.

**Abstract**
Ozone has been proposed as an adjunct antiseptic in periodontitis therapy. The aim of this study was to investigate the antimicrobial effectiveness of gaseous/aqueous ozone, in comparison with that of the established antiseptic chlorhexidine digluconate (CHX), against periodontal microorganisms. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, and Parvimonas micra in planktonic or biofilm cultures were exposed, for 1 min, to gaseous ozone, aqueous ozone, CHX, or phosphate-buffered saline (control). None of the agents was able to substantially reduce the A. actinomycetemcomitans count in biofilm cultures. In contrast, P. gingivalis, T. forsythia, and P. micra could be eliminated by 2% CHX or by ozone gas at 53 gm(-3). Significantly greater antimicrobial effects were observed against planktonic cultures than against biofilm-
associated bacteria. The rate of killing was influenced by the species of bacteria, and by the type and concentration of agent. There were no significant differences in the effectiveness of aqueous ozone (20 μg ml(−1) ) or gaseous ozone (≥ 4 gm(−3) ) compared with 2% CHX but they were more effective than 0.2% CHX. Therefore, high-concentrated gaseous and aqueous ozone merit further investigation as antiseptics in periodontitis therapy. A safe system for applying gaseous ozone into the periodontal pocket that avoids inhalation still needs to be developed.

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MICROBIOLOGY

The antimicrobial effect of 0.1 ppm ozonated water on 24-hour plaque microorganisms in situ

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ABSTRACT
Ozone is a known oxidant present in the atmosphere and is commercially produced by simple ozonizer machines. It is a powerful antimicrobial agent in its gaseous and aqueous forms. Ozone readily dissolves in water and retains its antimicrobial property even in the dissolved state. In this study, the effect of 0.1 ppm ozonated water was analyzed on 24-hour supragingival plaque (SP) samples in situ. SP was collected from the two most posterior teeth in the contra-lateral quadrants before and after a 30-second rinse with either distilled water (control group) or 0.1 ppm ozonated water (test group). The plaque was used to count the number of total bacteria, total anaerobic bacteria, Streptococcus mutans, and Candida albicans on selective agar media. The statistical analysis of the number of colony forming units (CFUs) obtained demonstrated a significant antimicrobial effect of ozonated water on the total bacteria (p = 0.01) and anaerobes (p = 0.02). A reduction in the post-rinse CFU count for Streptococcus mutans was also observed, but the effect was not statistically significant (p = 0.07). The Candida species was only grown from one sample. Ozonated water at the 0.1 ppm concentration was effective in reducing the load of 24-hour plaque bacteria, but it did not eliminate them completely.
Descriptors: Ozone; Dental Plaque; Bacteria.

Introduction
Bacteria in a biofilm are more resistant to antimicrobial agents because their organized nature enables them to behave as a quorum. Within its layers, a biofilm has dynamic interactions between its biotic and abiotic components. Dental plaque biofilm is a known etiological factor that causes oral diseases such as dental caries, gingivitis, and periodontitis. Oral microorganisms have also been associated with systemic problems such as pneumonia and cardiovascular diseases. To control the accumulation of dental plaque, antiseptics, antibiotics, oxidizing agents, herbal extracts and enzymes are used as antiplaque agents.
Ozone (O3) in a gaseous or aqueous phase has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa, and viruses. It is an unstable gas capable of oxidizing any biological entity. Its oxidative capacity at 100 ppm, 200 ppm and 400 ppm can also induce serious toxicity due to lipid peroxidation and ultimately cause DNA damage. A low concentration of ozonated water is sufficient to inactivate bacterial cells (0.12–0.19 mg/l) and their spores (2.29 mg/l). It has been shown that Streptococcus mutans, Lactobacilli casei and Actinomyces naeslundii suspended in a salt buffer can be completely killed within 60 seconds following exposure to ozone gas. Ozone readily dissolves and forms ozonated water when introduced into water. Ozonated water is also a powerful oxidizing and antimicrobial agent. In both gaseous and aqueous forms, ozone is potentially effective as a disinfecting agent for the removal of biofilms and their related microorganisms. The powerful disinfecting property of gaseous ozone has been utilized in dentistry to treat primary root caries, occlusal caries, dental hypersensitivity and cervical sensitivity. It is accepted that its application at doses between 90 mg and 120 mg does not affect the physical properties of enamel. Ozonated water has been used in the sterilization of dentures and dental unit water-line systems. Plaque microorganisms have shown vulnerability to ozonated water under in-vitro conditions (4 mg/l for 10 seconds). The main objective of this study was to determine the in situ antimicrobial effect of 0.1 ppm ozonated water on 24-hour plaque microorganisms following a 30-second rinse.

Methodology
The study involved 40 healthy volunteers between the ages of 18 and 40, who had at least 20 permanent teeth that were periodontally healthy. Volunteers who had taken antibiotics or other antibacterial agents less than one month prior to the study were not included. Ethical approval was obtained from the Dental School Ethics Committee, School of Dentistry, University of Malaya.
Sample collection
The study was designed to utilize convenient sampling. The volunteers were randomly distributed and grouped as either Group 1 (20 volunteers) or Group 2 (20 volunteers). The volunteers were asked to not brush their teeth or use any form of oral hygiene for 24 hours before sample collection. Pre- and post-rinse 24-hour supragingival plaque (SP) samples from Group 1 and Group 2 were collected. The pre-rinse samples provided the baseline data for this study. The post-rinse SP samples were collected 20 minutes after a 30-second rinse with either distilled water (Group 1) or 0.1 ppm ozonated water (Group 2). Thus, Group 1 was the control group, and Group 2 was the test group. The pre- and post-rinse SP samples were collected from the buccal and lingual surfaces of the teeth using a sterile stainless steel excavator. To standardize the amount of SP collected, the excavator was used in several gentle scooping motions to avoid contact with the marginal gingiva. Each time a scoop was made, the SP was transferred into a microfuge vial containing sterile reduced transport fluid (RTF). The oxygen content of the RTF was reduced with the addition of tricholoracetic acid (TCA). The pre-rinse SP samples were collected from the three most posterior teeth on the upper right and lower left quadrants, while the post-rinse SP samples were collected from the three most posterior teeth on the upper left and lower right quadrants (contralateral teeth).

Preparation of ozonated water
The ozonated water was freshly prepared using the ORM AW600 ozone gas generator machine (ORM Beauty and Health care Sdn Bhd, Petaling Jaya, Malaysia). Ozone gas produced from this ozonizer was introduced into 1 liter of sterile distilled water for 20 minutes. The concentration of dissolved ozone in the water was measured using an EcoZoneÔ EZ10W portable dissolved ozone meter (Ecosensors Inc., Newark, USA). The concentration of ozonated water used for this study was between 0.08 ppm and 0.1 ppm. The ozonated water was used within 20 minutes after its preparation.

Determination of microbial population
The wet weight of the plaque was calculated by subtracting the weight of the microfuge vial containing the RTF from the weight of the same vial after the addition of the plaque sample. The population of plaque bacteria was expressed as colony forming units per unit of plaque (CFU/mg plaque). Selective agar media was used to culture, isolate and enumerate specific plaque bacteria. The media used were:
• brain heart infusion broth (BHI, Oxoid Limited, Hampshire, United Kingdom),
• BHI agar (Fluka/Sigma-Aldrich Corporation, Bangalore, India),
• Schaedlers agar (SA, Oxoid Limited, Hampshire, United Kingdom),
• Columbia nutrient agar (CNA, Difco/Voigt Global Distribution Inc., Kansas, USA) and
• Sabourauds dextrose agar (SDA, Oxoid Limited, Hampshire, United Kingdom).
BHI broth was used in the preparation of the plaque bacterial suspension. The bacterial plaque suspension was serially diluted five-fold in RTF before inoculation onto the selective agar plates to reduce the bacterial population to a level where the growth could be detected easily. The inoculation of each sample was performed in triplicate in a laminar flow cabinet. The BHI agar was used to culture fastidious Gram positive and Gram negative plaque bacteria aerobically. The SA was used to culture and isolate the anaerobic plaque bacteria. The CNA was mixed with sterile human blood and potassium tellurite to make the media selective for Streptococcus mutans. The SDA was used to selectively grow the Candida species. Following inoculation, all of the agar plates were incubated for 48 hours at 37 °C aerobically, with the exception of the SA plates, which were incubated in an anaerobic jar. The CFU of the pre-rinse and post-rinse SP samples of Group 1 and Group 2 were compared to evaluate the effect of rinsing with ozonated water. The results were statistically analyzed by a one-way analysis of variance (ANOVA) using the Minitab14 software (Minitab, State College, USA). A p value < 0.05 was considered significant.

Results
None of the volunteers expressed discomfort or any kind of adverse reaction to the ozonated water rinse. Three samples were discarded because the media plates were feared to be contaminated. Effect of rinsing with distilled water on microbial components (Group1)
The post-rinse CFU counts for total bacteria, anaerobes and the Streptococcus species showed a difference in a few samples, but many samples were not affected by the distilled water rinse. Only 47% of the BHI agar, 52% of the SA and 58% of the CNA media plates inoculated with post-rinse SP had fewer CFUs than the pre-rinse SP media plates (Table 1). For the few samples that displayed a
difference between pre- and post-rinsing, the effect was determined by the ANOVA test to not be statistically significant. Only one of seventeen Group 1 volunteers showed positive Candida growth.

Effect of rinsing with ozonated water on microbial components (Group 2)
Unlike Group 1, a reduction in the CFU count of SP was observed in all of the post-rinse samples of Group 2 following the ozonated water rinse (Table 1). The pre-rinse and post-rinse CFU values of the total microbes for Group 2 are plotted in Figure 1. The average reduction of the total microbial count observed was 45.3%. This difference was statistically significant (Table 2).

For the anaerobes, the reduction was 51.7% and was shown to be statistically significant (p = 0.02, Table 2). The pre-rinse and post-rinse CFU values of the anaerobes for Group 2 are shown in Figure 2.

The streptococci count was reduced by 56.4% for Group 2. However, ozonated water at 0.1 ppm did not show any significant effect (p = 0.07) on the CFU counts (Table 2). The pre-rinse and post-rinse CFU values of the streptococci for Group 2 are shown in Figure 3.

The Candida species was not isolated from any of the Group 2 samples. Therefore, the effect of ozonated water (0.1 ppm) on the Candida species could not be analyzed.

Discussion
The methodology of this study was generally based on that performed by Pan et al.,24 with some modifications. This pilot study was performed to determine the effect of 0.1 ppm ozonated water on in situ plaque formation and to identify any immediate discomfort or adverse reaction an individual may have to the gas. The commonly employed method of evaluating the effectiveness of oral antiseptics by cleaning the teeth, asking the volunteers to follow a prescribed regimen of mouth rinsing, and then testing the effectiveness after a period of time was not used in this study because ozone machines could not be given to all of the volunteers to prepare fresh ozonated water for rinsing. Taking into consideration the safety of the subjects, this study did not include concentrations of ozonated water higher than 0.1 ppm.

The bacteria grown on selective media from all of the plaque samples were fastidious and required a 24- to 48-hour incubation period. The microbiological tests performed in this study suggested that the ozonated water exhibited some antimicrobial activity on the bacterial population of the 24-hour plaque. Exposure to the ozonated water for 30 seconds reduced the total bacteria population of the 24-hour plaque by 45.3% (Table 2). This reduction may have been due to the activity of the ozonated water, which would have affected the viability of these microorganisms in two ways:
• by directly inactivating the bacterial cells by oxidation and
• by disturbing the normal ecosystem of the plaque by creating an oxygen-rich environment after the dissociation of ozone into oxygen.

A similar observation25 has been reported for peroxycarbonate, which has an active post-rinse oxygen concentration of 11.4% and was shown to reduce the bacterial count in plaque. In a soft-
textured 24-hour plaque, an additional mechanism that can result in the reduction of its bacterial population may be the dislodging effect caused by rinsing.

A few post-rinse samples showed a reduction in the CFU count following rinsing with distilled water (Table 1). However, this observation was erratic, with a few samples showing a reduction in the CFU counts and the remainder of the samples showing either an increase or no difference at all. The reduction in the CFU counts in this group may be attributed to the mechanical dislodging or “washing away” of loose supragingival plaque that formed within the 24-hour period or to the inactivation of viable bacteria by the distilled water. A decrease in the cell viability of bacteria has been reported23 for bacteria grown on agar plates and treated with distilled water. The finding that post-rinse samples isolated from the group treated with distilled water exhibited more CFUs compared to the pre-rinse samples could be due to differences in the bacterial concentrations in the plaque samples collected from the different quadrants of the upper and lower teeth. However, any major discrepancy related to this difference in bacterial concentrations would have been minimized by collecting pre-rinse and post-rinse plaque from the contralateral quadrants.

In contrast to the erratic results obtained by the distilled water–rinsed SP samples, a clear reduction in the CFU counts was observed in all of the ozonated water–rinsed SP samples (Table 2). The average reduction of the anaerobes (51.7%) in the subjects treated with ozonated water was greater than the reduction of the total bacterial load (45.3%). The enhanced effect of ozonated water on the anaerobes could be because the obligate anaerobes are sensitive to and become inactive in oxygen-rich conditions.26

The average reduction observed for the streptococci load (56.4%) was more than that observed for the total bacteria (45.3%) and the anaerobes (51.7%). However, it is interesting to note that this difference was not statistically significant when compared with the CFU count of streptococci following the distilled water rinse. The most plausible reason for this is that Streptococcus showed the least resistance to the distilled water rinse. 10 of 17 streptococci samples treated with distilled water showed a reduction in CFUs, whereas total bacteria (8 of 17 samples) and anaerobes (9 of 17 samples) showed greater resistance to the distilled water rinse. This suggests that streptococci are somewhat sensitive to distilled water and much more sensitive to ozonated water rinsing than other microbes, and therefore, a statistically insignificant result was obtained for the streptococci for the comparison between the distilled and ozonated water rinses. The pre-rinse and post-rinse CFU values of the streptococci for Group 1 are shown in Figure 4.

The results of this study are comparable to those of previous studies on oxidizing agents and antiseptics. Moran et al.,25 studied the effect of a single dose of two mouth rinses containing either peroxyborate or peroxyborate oxidizing agents on salivary bacteria. The methodology of the present study was similar to that of their study except that salivary bacterial counts instead of plaque bacterial counts were assessed in their study after the single rinse. Both peroxyborate and peroxyborate reduced the bacterial count, but the results were not significant when compared to those of a negative control saline rinse. Additionally, both rinses showed an inhibition of plaque accumulation over time.

Conclusion

This study suggests that ozone at a concentration of 0.1 ppm is effective in reducing the plaque microbial load but does not eliminate the entire plaque microbial population. The prevention of plaque accumulation is more desirable than plaque elimination. Therefore, ozonated water rinsing may be an extremely useful addition to tooth brushing and flossing because it is bactericidal, easy to prepare and cost effective. There is also convincing evidence demonstrating its bio-compatibility (at 1.25–20 mg/ml) with human oral epithelial, gingival and periodontal cells.27 The magnitude of bacterial inactivation that it produces, however, needs further investigation. The need for an ideal tool to maintain oral health supports the rationale for further study of its benefits in inhibiting the accumulation and growth of plaque microorganisms.

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Inactivation of template-directed misfolding of infectious prion protein by ozone.

Ding N, Neumann NF, Price LM, Braithwaite SL, Balachandran A, Belosevic M, El-Din MG.

Source
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Abstract
Misfolded prions (PrP(Sc)) are well known for their resistance to conventional decontamination processes. The potential risk of contamination of the water environment, as a result of disposal of specified risk materials (SRM), has raised public concerns. Ozone is commonly utilized in the water industry for inactivation of microbial contaminants and was tested in this study for its ability to inactivate prions (263K hamster scrapie = PrP(Sc)). Treatment variables included initial ozone dose
(7.6 to 25.7 mg/liter), contact time (5 s and 5 min), temperature (4°C and 20°C), and pH (pH 4.4, 6.0, and 8.0). Exposure of dilute suspensions of the infected 263K hamster brain homogenates (IBH) (0.01%) to ozone resulted in the in vitro destruction of the templating properties of PrP(Sc), as measured by the protein misfolding cyclic amplification (PMCA) assay. The highest levels of prion inactivation (≥4 log(10)) were observed with ozone doses of 13.0 mg/liter, at pH 4.4 and 20°C, resulting in a CT (the product of residual ozone concentration and contact time) value as low as 0.59 mg · liter(-1) min. A comparison of ozone CT requirements among various pathogens suggests that prions are more susceptible to ozone degradation than some model bacteria and protozoa and that ozone treatment may be an effective solution for inactivating prions in water and wastewater.


Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice.

Valacchi G, Lim Y, Belmonte G, Miracco C, Zanardi I, Bocci V, Travagli V.

Source

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Abstract

Ozone is well recognized as a bactericidal agent and its beneficial effect on wound healing could be a consequence of this property. Because ozone itself does not penetrate the cells but immediately reacts with polyunsaturated fatty acids, its effects should be the results of oxidative reaction. For this reason, ozonated oils could be a way to deliver ozone messengers to the skin. This paper evaluated the therapeautic effects of three different grades of ozonated sesame oil in acute cutaneous wounds made in the skin of SKH1 mice. Specifically, wound closure rate, histological parameters, and the level of key proteins such as vascular endothelial growth factors and cyclin D1 have been analyzed in relation to the peroxide level present in the ozonated oil. Treatment with moderately ozonated sesame oil--expressed as peroxide value about 1,500)--has a faster wound closure rate in the first 7 days than treatment with oil containing either lower or higher peroxide value, and even with controls. Moreover, under the same treatment, an earlier and higher response of cells involved in wound repair, a higher angiogenesis, as well as an enhanced vascular endothelial growth factors and cyclin D1 expression were observed. The present study shows the validity of ozonated sesame oil in cutaneous wound healing and emphasizes the importance of the ozonation grade.

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Mediators of Inflammation
Although orthodox medicine has provided a variety of topical anti-infective agents, some of them have become scarcely effective owing to antibiotic- and chemotherapeutic-resistant pathogens. For more than a century, ozone has been known to be an excellent disinfectant that nevertheless had to be used with caution for its oxidizing properties. Only during the last decade it has been learned how to tame its great reactivity by precisely dosing its concentration and permanently incorporating the gas into triglycerides where gaseous ozone chemically reacts with unsaturated substrates leading to therapeutically active ozonated derivatives. Today the stability and efficacy of the ozonated oils have been already demonstrated, but owing to a plethora of commercial products, the present paper aims to analyze these derivatives suggesting the strategy to obtain products with the
best characteristics.

1. Introduction

The increase of ageing, obesity, and diabetes in conjunction with inappropriate healthcare programs have emphasized the problem of having to treat almost 1.5 billion people affected by skin and mucosal infections due to bacteria, viruses, protozoa, and dysmetabolism. Pathologies range from the diabetic foot (ulcer with necrosis), bed sores, ulcers after a trauma or burns, chronic viral infections due to either herpes virus I and II, or human papylloma viruses, vaginal infections now frequent also in young girls due to Candida, Trichomonas, and Chlamidia, rectal mucosa infections such as anal ragadis, abscesses with fistula to end with mouth aphthous ulcers. These infections are rarely deadly but are considerably distressing because many patients often suffer of diabetes or vascular diseases with tissue hypoxia, other patients are immunosuppressed drugaddicts, or with concomitant HIV infection. Official medicine provides a variety of drugs that are expensive and often poorly efficacious because infections in hypoxic tissue containmethicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa. Patients are suffering not only because they become uncompliant to frequent medications but they are discouraged by observing a lack of healing [1]. Wound healing is a multiphase process involving blood clotting, inflammation, tissue proliferation, and remodelling [2], but both innate and adoptive immune systems are too
often hindered by the chronic infection naturally difficult to overcome. This is also the reason explaining the failure of growth factors in heavily contaminated ulcers [3, 4].

The judicious use of ozone (O3) appears providential because first of all eliminates the pathogens and then, by releasing oxygen (O2), activates the proliferation of fibroblasts, hence the building of intercellular matrix with consequent proliferation of keratinoblasts and successive healing.

In Section 2, we propose to briefly review the physical chemistry of oil ozonation and all the basic analyses necessary for demonstrating the quality of the obtained products.

In Section 3, it appears useful to inform readers that both skin and mucosae are sensitive to excessive amounts of gaseous O3 as there are clear demonstrations of a variety of alterations linked to a prolonged exposure. In Section 4, we will then clarify the various procedures devised to enhance the disinfectant and healing-promoting properties of O3. Finally, after an extensive analysis of a cornucopia of Mediators of Inflammation proposals, we will try to suggest guidelines for the future medical application of topical ozone and its derivatives (Section 5).

2. Physical Chemistry of Oil Ozonation with a Description of the Analytical Methods for Characterizing the Process

Unsaturated lipid substrates react with insufflated gaseous
O2/O3 mixture leading to therapeutically active ozonated derivatives (Figure 1).

Briefly, the postulated mechanism known as Criegee reaction provides that ozone combines with an unsaturated bond to form an initial, unstable primary ozonide which readily decomposes to form a zwitterions and a carbonyl fragment. In anhydrous environment these substrates combine to give the typical cyclic trioxolane derivative.

However, the word "ozonated" is itself without scientific meaning if it is not associated with how much peroxides are present in the oil. In fact, from a therapeutic point of view, the ozonide compositions have the capacity to deliver active O2 and/or other useful species deep within the lesion without causing primary skin irritation. The few studies concerned with the therapeutic effects of ozonated oils on acute cutaneous wound healing in animal models do not investigate the dose/behaviour response, expressed as the amount of peroxides existing in the ozonated derivative used [5]. Recently, a quantitative evaluation of the therapeutic effect of topically applied ozonated sesame oil on acute cutaneous wound healing in mice as animal model has been developed [6]. The results indicate that both low (<1000) and high doses (>3000), as expressed in terms of peroxide value (see the corresponding section in this paper), delay cutaneous wound healing. Such an evidence is reinforced by a number of results between groups where the middle concentration (about 1500) has the most beneficial effect in
accelerating the wound closure ratio.

From an industrial applicative viewpoint, the overall quality of ozonated derivatives depends upon several parameters, such as: (i) the type and the quality of ozone generators; (ii) the ozonation conditions, in terms of reactors and time, material type and amount, presence of water and/or catalysts; (iii) the efficacy of the ozonizer, in terms of O3 concentration output, gas flow, gas carrier. As for the latter, the use of medical grade O2 instead of air is an important point to be considered; in fact, air feedstock (containing about 78% of nitrogen) used for the ozonation of unsaturated substrates could lead to the production of potentially toxic nitrated by-products [7], and to a significant decrease of the ozonation efficiency [8]. Another important feature is that ozonated oil has to be unequivocally characterized in terms of the species contents as well as the reaction kinetics. For these purposes, the knowledge of the physicochemical properties of ozonated vegetable oils during production has a great importance for their characterization and identification. For determining the quality of ozonated products, spectroscopic techniques, as Fourier-Transformed Infrared (FT-IR) and 1H and 13C-NMR [9], together with analytical methods as peroxide, acidity, and iodine values as well as viscometric determination are usually carried out [10].

2.1. FT-IR Spectroscopy. FT-IR spectroscopy is used to highlight differences in the functional groups during the
oil ozonation, in particular the decrease of the bands corresponding to both C=C and =C.H stretching (e.g., sesame oil at 1654 cm.1 and 3009 cm.1, respectively), and the increase of the band corresponding to ozonide CO stretching (e.g., sesame oil at 1105 cm.1).

Ozonated samples can be analyzed using two different methods.
(1) An adequate aliquot (usually about 2 \( \mu \)L) of sample is deposited between two disks of KBr, avoiding air bubble formation, then the percentage transmittance or other suitable parameters are measured in the range 4000-800 cm.1. Spectra are obtained setting the appropriate scan summations and minimal resolution (generally, 16 at 4 cm.1, resp.).

(2) An adequate aliquot (usually about 2 \( \mu \)L) of sample is dissolved in a suitable solvent (preferably chloroform) and then the solution is settled in the sample holder avoiding air bubble formation, then the transmittance (expressed as a percentage) or other suitable parameters are measured in the range 4000-800 cm.1. Spectra are obtained setting the appropriate scan summations and minimal resolution (generally, 16 at 4 cm.1, resp.).

2.2. NMR Spectroscopy. 1H and 13C NMR spectroscopies are performed to obtain more information about the variation of the functional groups involved in the reaction of ozonation. Both the disappearance of the signals relative to protons and
carbons on the double bond (e.g., in sesame oil 5.29 ppm, and various signals in the range 127.8–130.0 ppm, resp.) and the parallel appearance of a signal on the proton and carbon of 1,2,4-trioxolane (e.g., in sesame oil in the 5.11–5.08 ppm range, and 103.4–104.3 ppm range, resp.) are evidenced. Quantitative analysis can be performed by spectra normalized with respect to the integral areas of the OCH2 protons (glycerol) that remain constant during the whole process.

Spectra will be obtained using suitable instruments by solubilizing the ozonated sample in a proper solvent (preferably CDCl3). Particularly, an adequate aliquot (usually about 100 fL) of sample is solubilised with 750 fL of CDCl3 in a 5mm NMR tube, then the analysis will be performed. To obtain quantitative data, it is sufficient to perform a 1H-NMR, while 13C-NMR essentially provides qualitative informations [9].

2.3. Iodine Value. The iodine value (IV) represents the quantity of iodine (in grams) that will react with the double bonds in 100 grams of sample. IV is determined according to Mediators of Inflammation 3

Palmitate
Linoleate
Oleate Primary ozonides
Secondary ozonides
Criegee ozonides
Figure 1: Representative chemical structures of ozonated derivatives which are formed by chemical reaction of ozone with unsaturated triglycerides. The primary ozonides are transient, unstable species which rearrange in the normal, secondary ozonides also known as Criegee ozonides.

to the Pharmacopoeia monographs. The IV is calculated by means of the following equation:

$$IV = 1.269 \cdot E (n_1 \cdot n_2)$$

$$m$$

, (1)
where \( n_1 \) is the volume in mL of thiosulphate solution (0.1 M) used for carry out a blank test, \( n_2 \) is the volume in mL of thiosulphate solution (0.1M) used for the titration and \( m \) the quantity, in grams, of substance. It is, therefore, a measure of the total number of double bonds present in the sample and for such a reason it is a chemical analysis useful for evaluating the decrease of double bonds during the oil ozonation process, giving information about the 1,2,4-trioxolane formation.

2.4. Acid Value. The acid value (AV) is an index that expresses, in mg, the quantity of potassium hydroxide required to neutralise the free acids presents in 1 g of the substance. The AV is calculated by means of the following equation:

\[
AV = 5.6 \times 10^3 \frac{n}{m}.
\]

(2)

where \( n \) is the volume in mL of titrant and \( m \) the quantity, in grams, of substance.

It is representative of the acidity level of the product and it represents an index of the degradation by-products that could be formed during the ozonation process.

2.5. Peroxide Value. Peroxide value, (PV), is usually used as an indicator of the advancement and/or the control of the ozonation process because of its simplicity, rapidity, and low cost. Moreover, the PV may be adequate for the stability evaluation of vegetable oil ozonides and it appears to be
very important for commercial distribution as well as for the determination of the better storage modalities. However, it had been necessary to standardize the methodology for a validated PV.

In the present paper, a detailed analysis of PV assessments of ozonated lipid derivatives based on both literature data and our laboratory experiments will be presented together with their possible correlations with other techniques. Such a report allows an in-depth acquaintance of the ozonation process of vegetable oils as well as of the related products obtained, allowing to define the quality parameters useful for industrial purposes. Specifically, the peroxide value (PV) represents the quantity of peroxide expressing in milliequivalents of active O2 contained in 1000 g of the sample.

For the PV evaluation, three different methods were adopted.

(a) First official monograph described in Pharmacopoeia (e.g., European Pharmacopoeia, British Pharmacopoeia, United States Pharmacopoeia), which provides the solubilization of sample in 30mL of chloroform/glacial acetic acid (2 : 3), the addition of saturated potassium iodide solution (0.5mL) and the titration after 1 minute with a solution of sodium thiosulphate.

(b) Second method described by Martinez Tellez et al. [11], which always provides the solubilization of
sample in 30mL of chloroform/glacial acetic acid (2 : 3) and the addition of saturated potassium iodide solution (0.5 mL), but the titration is done after 24 hours.

(c) Third method recently proposed [10]. Briefly, 2 g of SO were weighed in a 250mL conical flask and 30mL of chloroform/glacial acetic acid (2 : 3) were added. Then, 3.0mL of saturated potassium iodide solution were added. The flask was stirred at reflux temperature (60°C) for various times (5.180 minutes). After this time, the solution was cooled and 25mL of water were added. Solutions of sodium thiosulphate at the appropriate concentration (0.0001.0.1M) were used for the titration.

In all determinations the PV was calculated by means of the following equation:

\[ PV = 1000 \frac{E (V_1 - V_0) E c}{m}, \quad (3) \]

where V1 is the volume in mL of thiosulphate solution used for the titration, V0 is the volume in mL of thiosulphate solution used for carry out a blank, c the thiosulphate concentration and m the sample quantity (grams).

The ozonation efficiency (expressed as a percentage) represents ratio of the amount of peroxidation due to ozonation process, as estimated by PV value, to the O3 total amount applied to the system. It was calculated by means of
the following equation:

\[ OE = \left( \frac{PV_s \cdot PV_0}{1000} \right)^{\frac{1}{24}} \cdot OAD \]

where \( PV_s \) is the ozonated sample PV, \( PV_0 \) is the PV of untreated sample, and OAD stands for the O3 applied dose (mg/g).

2.6. Viscosity Measurement. Viscosity evaluation is a useful technique because it is fast and it could be online, giving an estimation of the double bonds present in the sample. In fact, the greater the ozonation time the higher the product viscosity because of the disappearance of the double bonds. Moreover, its typical trend can be a useful tool in providing a rapid quality control assessment during the entire ozonation process, as well as to decide on the process time for obtaining the desired ozonation level of the sample [9].

3. Cutaneous Responses to Environmental Ozone Exposure

The skin, along with the respiratory tract, is directly exposed to environmental pollutants including O3, an important constituent of photochemical smog. Although numerous studies have documented effects of O3 on the respiratory tract in animals and humans [12.15], only recently some studies characterizing its effect on cutaneous tissue have been published [16.20]. The skin consists of two main
layers, the inner dermis, mainly composed of fibroblasts and connective tissue matrix, and the outer epidermis, which contains keratinocytes that, by progressively differentiating to form enucleate corneocytes, become imbedded in a lipid matrix and together comprise the outermost part of the epidermis, the stratum corneum (SC) [21, 22].

Previous studies have shown that exposure to O₃ results in the depletion of both water soluble and lipophilic antioxidants such as uric acid, ascorbic acid, and tocopherol, and this was accompanied by increase in parameters of both lipid peroxidation and protein modification, primarily in the outermost skin layers [16, 17, 23].

In further studies, we were also able to show that the exposure of hairless mice to O₃ will not only deplete the antioxidant levels and increase oxidative markers but these molecules are able to induce active cell responses.

These effects can be briefly summarized as follows.

1. Induction of Redox Sensitive Transcription Factors. Ozone, like many others environmental challenges, is able to activate transcriptional factors redox sensitive such as Nuclear Factor k B (NFkB). This transcriptional factor acts as an activator for a multitude of proinflammatory genes (IL-8, TNFα, TGFβ) and adhesion molecules (ICAM and VCAM). It has been assessed that O₃ is able to activate NFkB using both in vitro and in vivo systems. Thiele et al. [16], using an immortalized human keratinocytes (HaCaT cells), were able to show that O₃ induced the activation of NFkB by
electrophoretic mobility shift assay (EMSA). Ozone induced a dose dependent activation of the transcription factor. This effect was likely to be mediated by ROS, particularly H2O2, because it was inhibited by the incubation of the cells with lipid soluble antioxidants (tocopherol).

(2) Induction of Heat Shock Protein (HSP) and Inflammatory Markers. As a consequence of the induction of transcription factors, O3 exposure (6 days to 0.8 fÈg/mL for 6 hours/day) induced the expression of proinflammatory markers in skin homogenates such as cyclooxygenase-2 (COX-2). This induction was accompanied by an increase level of heat shock protein (HSP) 32, also known as heme oxygenase-1 (HO-1). In this paper, we were the first to demonstrate the upregulation of HSPs 27, 32 and 70 in homogenized murine skin upon O3 exposure. HSP27 showed the earliest (2 hours) and highest (20-fold) response to O3 compared with the delayed induction (12 hours) of HSP70 and HO-1. HSP27 is expressed predominantly in the suprabasal epidermis in human skin, whereas HSP70 predominates in the dermis compared with the epidermis. These differences in location between HSP27 and HSP70 might explain the different time course of induction of these stress proteins upon O3 exposure. It is therefore possible that the generated bioactive compounds may be responsible for the induction of HSPs as was also shown after UV irradiation.

(3) Induction of Matrix Metalloproteinases (MMPs). Among the multiple systems altered in the skin by environmental
pollutants, MMPs are among the major targets. Indeed, O3 exposure is able to affect their synthesis and/or activity with logical consequences on tissue remodeling and wound healing [23, 24]. Within the MMP family, MMP-2 and MMP-9 are the only members able to degrade type-IV collagen of the basal membranes [25]. MMP-2 is involved in pathological processes such as photoageing and precancerous/cancerous skin lesions after UV exposure; moreover, MMP-2 is capable of cleaving other substrates, in addition to type-IV collagen, including other MMPs and therefore can (indirectly) control extracellular matrix degradation and remodelling.

MMP-9, like MMP-2, plays a role in human skin ageing [26] tumor development [27], as well as in other cutaneous lesions such as psoriasis and dermatitis [28, 29]. In a recent study, we were able to demonstrate that O3, was able to affect MMP activity. Most likely the generation of bioactive molecules can be the cause of such activation. It has been also demonstrated that O3 is able to induce NO production via the activation of iNOS in cutaneous tissues [18]. When produced in excess, NO, may combine with superoxide to form peroxinitrite (derived from other sources) that can activated MMPs especially MMP-9. Thus, the increase of oxidative stress after O3 exposure, plus the interaction between O2 and nitrogen activemolecules might be the main mechanism that leads to the enhanced MMPs activities in skin tissues. This can be also a result from an imbalance
between MMPs and their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) [30].

In fact, the activities of MMPs are regulated by TIMPs, which can be produced by a multitude of cell types present in the cutaneous tissue. While MMP activity is altered by the O3, neither TIMP-1 nor TIMP-2 level expression is affected. The lack of changes in TIMP-1 and 2 levels, combined with the increased activity of MMPs suggest that O3 can cause a net increase in matrix degradation. On the other hand, in a comparative study where normal skin has been exposed for two hours to environmentally realistic levels of ozone, only a moderate state of oxidative stress at level of the stratum corneum has been induced, without producing a visible clinical response [31].

4. Skin Age-Related Responses to Ozone Exposure: Wound Healing

Wound healing is a critical process in the skin and it has known to be affected by oxidative stress and also to decline with increasing age [32]. Although the exact sequence of wound healing is complex, cutaneous wound healing begins with wounding induced signaling factor-based transformation of stationary keratinocytes into cells capable of both replication and migration. Upon transformation, these cells express a host of molecules that promote the invasion of the injured epithelial matrix and reepithelialisation of the wound surface [33]. Delayed wound healing in the elderly has been well described [34].
As mentioned above, O3 exposure is also associated with activation of transcription factor NFkB, which is important to regulate inflammatory responses and eventually entire wound healing. O3 exposure increased levels of Transforming Growth Factor (TGF-β) that is a critical factor in tissue remodeling [35, 36]. We can summarize that while O3 as an oxidant, might stimulate wound healing, it would be detrimental in an engaging environment due to the increased concentration-dependent oxidative stress. Therefore, these aspects have biological as well as practical implications and needed further investigations.

In a recent study, we demonstrated the detrimental effects of O3 on cutaneous wound healing in the aged animals. In fact, when hairless young (8-week-old) and aged mice (18-months-old) with after full thickness excisional wounds were exposed to 0.5 μg/mL O3 for 6 hours per day the rate of wound closure was significantly delayed in the old group. We also showed induction of protein and lipid oxidation assessed as changes in protein oxidation (carbonyls) and lipid peroxidation (4-hydroxynonenal, HNE adducts) in the old mice compared to the young mice during the later stage of cutaneous wound healing. O3 exposure has different effects depending on the age of the mice. In fact, it significantly delayed wound closure in old mice, while in young mice, it led to accelerated trend during the first few days of the exposure. This might be attributed to the antibacterial properties of O3, as it has been shown
that application of ozonation provides fast cleansing of wound surface from pyonecrotic masses, promotes elimination of infection and thus substantially reduces the period of treatment of the patients [37]. Recently, clinical treatments using hyperbaric oxygen therapy demonstrated that increased O2 tension at the wound site increases the formation of granulation tissue, enhances accelerated wound closure and ameliorates impaired dermal wound healing [38]; therefore, accelerated trend of wound closure shown in young population may be due to decreased bacterial infection and/or increased O2 tension by O3 exposure in wound area.

One of the possible driving processes of the effect of O3 on wound healing can be also in this case the modulation of the transcription factor NFkB. Interestingly, the dose effect relationship between level of oxidative stress and NFkB exhibits a biphasic profile: while moderate levels of oxidative stress activate NFkB through an IkB kinase independent mechanism, extremely high levels of oxidative stress have been shown to inhibit NFkB activation by blocking IkBα phosphorylation [39]. One potential explanation for the differential effect in the older animals is that the level of oxidative stress generated by O3 exposure combined with aging causes levels of oxidative stress that inhibits IkBα phosphorylation, thereby resulting in a decline in NFkB activation. This finding is consistent with what mentioned previously that O3 exposure induced skin antioxidants
depletion. This interpretation is also bolstered by data on TGF-β, a crucial modulator of tissue remodeling and is linked to both NFκB status as well as to levels of oxidative stress during entire wound healing process [40]. The reduced TGF-β levels in both air and O3 exposed old mice as well as the lower induction of TGF-β by O3 exposure in the old animals suggests that the noted delays in wound closure might be related to defects in oxidative stress-dependent NFκB status as well as levels of oxidative stress and TGF-β signaling in aged mice during later stage of wound healing.

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5. Topical Application of Ozone in Medicine

To the best of our knowledge, the first application of gaseous O3 was performed during World War I for treating German soldiers affected by gaseous gangrene due to Clostridium anaerobic infections very sensitive to O3 [41, 42]. In 1936, Dr P. Aubourg, by using a metal cannula, was the first to propose the insufflations of gaseous O2/O3 in the rectum to treat chronic colitis, anal ragadis and fistulae. This approach is very empirical and unprecise and today it is mostly used by Cuban physicians. In 1937, a Swiss dentist, E. A. Fisch (1899.1966) had the idea to use it in his practice and, by a twist of fate, he treated Dr. E. Payr (1871.1946) a surgeon who had a painful gangrenous pulpit. Payr was so enthusiastic of the O3 effect to use it in his surgical practice with great advantage [43]. Later on, Werkmeister [44] mastered the use
of gaseous O₃ in several skin ulcers due to atherosclerosis, diabetes and radiotherapy by either enclosing a leg in a polythene-bag (the so-called bagging system) or using an ozone-resistant plastic cup applied in other areas. In the former application the gas was introduced to just inflate the bag containing some distilled water. The system was static but after 20.25 minutes the gas was aspirated and destroyed. The O₃ concentrations varied between a high 80 fÊg/mL in very purulent ulcers and progressively lower concentrations down to 10 fÊg/mL as the ulcers improved because excessive O₃ would be deleterious for healing. As the cup system had an inlet and an outlet, Werkmeister could realize a continuous gas flow with a modest depression that enhanced the vasodilation of the ulcer fs area. With both systems he treated many extensive and otherwise incurable lesions within 50.200 days. It is noteworthy that gaseous O₃ works well only in a water vapour-saturated bag because it must dissolve into superficial water or in the exudate to react proficiently. The normal skin does not undergo any damage during the treatment. Today these procedures are still in use but they are somewhat cumbersome and great care must be exercised to prevent air contamination.

How ozonated oils act remains an open question.

Probably, when the stable triozonide comes into contact with the warm exudate of the wound, it slowly decomposes into different peroxides, which readily dissolves in water, probably generating hydrogen peroxide that can explain the prolonged
disinfectant and stimulatory activity. If it is correct, this reasoning implies that we should have titrated preparations with high, medium, or low ozonide concentrations to be used during the inflammatory septic phase I, regenerating phase II or remodelling phase III, respectively [2]. These phases have been related to the rapidly changing cell types and to the release of cytokines and growth factors that modulate the complex healing process.

An alternative method for treating diabetic foot ulcers is the use of hyperbaric oxygen therapy (HOT) but in such a case one disadvantage is the use of only hyperbaric O2 and another is the need to close the patient in the chamber for two hours. Therapeutic results are far more modest than topical O3 application, particularly when it is contained in a close cabinet with thermostatically-controlled temperature. However this procedure requires considerable idle times and, if an aspirating pump is unavailable, it may contaminate the operating room. For these reason today for cleaning and disinfecting cutaneous and mucosal infections and lesions due to many causes (like, e.g., trauma, ischemia, burns), it appears preferable to use at once freshly ozonated water and then ozonated oil, particularly during the night or at rest conditions.

The process of water ozonation needs of double distilled water and O3 concentrations ranging from 20 up to 100 fEg/mL of gas to have a final yield of 5 up to 25 fEg/mL, respectively. O3 is directly bubbled into the water and the
gas in excess is passed through a dehydrating device and finally through a destructor. Depending upon the water volume and the gas flow, a period of ozonation between 5.20 minutes is sufficient to saturate the water with gaseous O3. In fact, if the water is ultrapure, O3 physically dissolves in the absence of chemical reactions and if kept in a glass bottle closed with a Teflon cap, the concentration halves only after 300 hours at 0.C. However, at 20.C the half-life is about 10 hours [45]. It must be noted that monodistilled water allows a much faster O3 decomposition and it is not practical. It is advised to maintain the bottle at 4.C and to quickly close the bottle at any time, or better to have a valve system to prevent gas losses. It would be useful to device a procedure for maintaining the O3 concentration for longer times and we are investigating a possible procedure. On the other hand, ozonation of either olive or sunflower oils requires a much longer time and the procedure needs to be well-standardized in terms of gas-flow, O3 concentration, oil volume, and temperature. As recently reviewed, at least twenty different vegetable oils have been patented but so far it remains impossible to define their relative cost/benefit [46]. At this stage, after evaluating several physicochemical criteria, stability, efficacy, and cost, it seems that sesame oil has several advantages in comparison to other oils. How and when ozonated water and oils are used? Chronic wounds range from diabetic foot to putrid and deep ulcers due to limb atherosclerosis, or trauma and burns.
Moreover, both immunosuppressive chemotherapy and/or malnutrition cause abscesses, anal fissures and fistulae, bed sores, furunculosis, and osteomyelitis which are difficult to treat and often fail after prolonged treatments. About 7 million patients in the United States are affected with a cost over US$ 25 billion annually. Various types of disinfectants, antibiotics, antifungal, antiprotozoal, and growth factors are scarcely effective because the deranged metabolism and local hypoxia are not modified. Several other approaches such as vacuum therapy [47, 48], maggot therapy [49] and devices for providing topical oxygen therapy in a clinical setting have been proposed and variably used. This last approach has a rationale in the sense that enhanced oxygenation is useful for activating the metabolism and cell proliferation of ischemic tissues [50,52]. However, it has also considerable limitations because it is a cumbersome therapy, with minimal disinfectant activity and modifications of the fundamental pathogenetic mechanisms.

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Another topic of critical interest is the pathologies of the vaginal mucosa. Although rarely deadly (as the toxic shock syndrome due to a forgotten absorbent tampon), a majority of women physically and psychologically frequently suffer from a number of infections due to several pathogens such as Neisseria gonorrhoeae, Trichomonas vaginalis, Candida albicans, Chlamidia trachomatis, Herpes virus type-II (HVII), human papilloma viruses (HPV), human immunodeficiency
virus (HIV), often due to unprotected sexual
intercourses, stress, change in sexual partners and also
physiological hormonal changes during menopausa. About
20 million Americans are affected by the distressing HV-II
and as many 40 million have the genital HPV with warts
and the impending risk of cervix cancer. Moreover, the
further implantation of opportunistic infections complicates
the treatment. It is unfortunate that orthodox medications
are expensive and not so useful because of drug-resistant
pathogens and side effects limiting the compliance. So far
official medicine has not yet entertained the topical use
of O3 and derivatives in therapy because they are not
profitable and no extensive clinical trials have been published
in peer-reviewed journals: the therapy has remained
in practitioners' hands and the results remain anecdotal.
Moreover, the parenteral use of ozone, also known as
ozone therapy, is very useful as adjuvant: it is reasonably
ease to perform in terms of classical ozonated major and
minor autohemotherapy [53]. The latter modality has been
successfully used for eliminating recurrences of HV-I and
II infections. However, topical therapy is essential and it
is carried out by using vaginal irrigation of fresh ozonated
water and application of vaginal ozonated oil pessaries
for the night. During prolonged treatment the ozonated
compounds allows the elimination of any pathogens. So
far no resistance to O3 has been demonstrated. Creams
containing ozonated oils can be used 3-4 times daily
for external genital areas and also for several anorectal affections.

As for the oral infections (aphthae, HV-I, opportunistic superinfections, or acne) the earliest as possible application of ozonated ointments, by minimizing pathogen diffusion and enhancing microcirculation, reduces the swelling, destroys the pathogen, and allows a rapid healing.

Last but not least, clinical trials in tinea pedis as well as onychomycosis [54, 55] have been recently published and have shown the usefulness of ozonated sunflower oil.

6. Conclusions

At the present, especially in young people, venereal infections are increasingly frequent and therefore a suitable, effective medication with ozonated compounds will be a huge economical and social value. Also, elderly people are burdened with a variety of wounds and ulcers, some of which never heal, making life miserable. It is hoped that the present paper will inform official medicine for this advance and will incite to programme suitable clinical trials to show the full efficacy of ozone therapy by evidence-based medicine.

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