Ozone therapy in Dentistry - Where we are and where we are going to?

Dr. Fadi Sabbah
D.D.S. Associate, Texas Institute for Advanced Dental Studies Vice-president, ISCO3. Beirut – Lebanon

Dr. Carlos Goes Nogales
D.D.S. Universidade de São Paulo, Faculdade de Odontologia, Departamento de Endodontia, São Paulo, SP, Brasil

Dr. Eric Zaremski
D.D.S. private practitioner Smile Marin, 1000 S Eliseo Dr #202, Greenbrae, CA 94904, USA

Dr. Gregorio Martinez-Sanchez
Ph.D. Pharmacy Doctor. President, ISCO3

Abstract

There has been very little research to support clinical practices in dental ozone therapies. The protocols used in dental ozone therapy should fulfill the general guidelines and requirements commonly recognized by healthcare professionals and authorities as evidence-based medicine. To meet these criteria, both ozone dental research and the clinical practice of dentistry should converge. The positive results and advantages of dental ozone therapy should define standard parameters that the dental ozone equipment manufacturers need to follow to develop ozone systems that meet the requirements of both dentists and researchers.

The aim of this paper is to review the available published research and to compare it to what the majority of practicing dentists are applying in their practice. Four databases (PubMed; Ovid Medline; Cochrane; ISCO3) were used to search for articles covering the use of ozone in dentistry. Using the key words “ozone in dentistry” on PubMed (last accessed November 2017) retrieved 295 articles. Articles not related to dental ozone and general reviews were excluded (70). The resulting sample size of 225 articles, as well as the retrieved analysis results, are highly indicative to be able to draw conclusions and to formulate future recommendations.

To our knowledge, this is the first attempt to perform such an analysis. It is not the aim of this paper to critique the published research or the clinical practice of dental ozone therapy. The aim is to elicit the required modifications to research protocols and to evaluate whether dental ozone manufacturers provide the required equipment.

This review study used different groupings to evaluate the results. In the “ozone gas only group”, a clear deviation between research and clinical practice was noted. In all the other groups, the results were, in general, more congruent. Most of the current dental ozone research has focused on the antimicrobial effects of ozone, using either just ozone gas only, ozonated water only or ozonated oils only.

It is highly recommended that dental ozone research change its path. Clinicians have expectations that research will support their clinical uses of ozone. We need to meet the expectations of clinicians through adopting new and different studies. We need to create research that goes past the conventional and well-studied antimicrobial potential of ozone and by using ozone both in gas and water, not separately, plus ozonated oils when applicable...
1. Introduction

Since the early pioneering days of the Swiss dentist Dr. E Fisch (1899-1966), ozone application in dentistry has evolved and now is being used by a growing number of dentists worldwide. Due to the high disinfection and oxidation properties of ozone, scientists studied the use of ozone in various applications, mainly in water treatment, where the bulk of the fundamental ozone science as we know it today emerged. The promising results from the use of ozone in water treatment encouraged the expansion of its use to other applications, e.g. air and surface treatments, which are more relevant to healthcare and medical professionals.

In parallel to the use of ozone in industry in the early twentieth century, scientists and physicians also introduced the use of ozone in medical and dental applications. It was not until the last two decades of the 20th century that researchers studied the biological effects of ozone more in depth. Then, the clinical guidelines became more relevant and precise. This better understanding of ozone reactions and its biological effects led clinicians and researchers to re-study its use in dental medicine as well.

During this period, several national and international medical ozone associations were formed, the International Scientific Committee of Ozone therapy (ISCO3) was established, and a multitude of scientific congresses and courses were held. Ozone therapy is now legally practiced in several countries around the world. ISCO3 published the “Madrid Declaration on Ozone Therapy” which is considered an international reference for both clinicians and legal authorities.

Even though there has been a steady increase of healthcare professionals using ozone, ozone therapy has not yet reached the point where it is considered a mainstream treatment modality. Consequently, it is not reimbursed by social security programs or by private insurance companies. Two large issues that pose major obstacles to ozone therapy advancement are the shortage of public research funds and the reticence of pharmaceutical companies to invest in non-patentable modalities. However, some of the many indications of ozone therapy are now classified as evidence-based medicine. Some high quality published clinical trials, meta-analyses, and the gathered clinical experience have proved ozone's efficacy in certain medical applications.

With the collaborative contribution and coordination of both research and clinical practice, the well-studied medical indications of ozone have reached the level of evidence-based science. Very clear guidelines and protocols were established and are constantly updated with new scientifically supported findings.

Dental ozone therapy constitutes the number one topical administration in medicine. Research and clinical applications in medical ozone therapy can be a reference to follow for dental
research and its applications. Even though there are published general reviews of available literature with conflicting findings and recommendations, there is a need to examine whether dentists are following the research methodology and if the protocols used are similar. There is also a need to evaluate the technical specifications of commercially available dental ozone systems. Such comparative analyses have not been carried out yet. We believe that the findings of these analyses will aid in highlighting any potential deviation that may exist between research and clinical practice and help direct future research. It is not within the objectives of this paper to evaluate or critique published literature, nor to assess the suitability of the protocols used by practicing dentists. The goals are both to reach a synergistic and common path in dental ozone therapy leading to evidence-based medicine as well as to provide standard requirements for the manufacture of ozone generators to meet the needs of dental practice. This would set more definitive guidelines to apply in general dental practices.

2. Material and Method

2.1 Dental ozone published articles: Data collection

Four databases (PubMed; Ovid Medline; Cochrane; ISCO3) were used to search for articles covering the use of ozone in dentistry. Using the key words “ozone in dentistry” on PubMed (last accessed November 2017) retrieved 295 articles, some of which were not related to dental ozone uses. In addition, selected unpublished articles collected over the years from scientific ozone meetings were also used in this study. In total, 225 articles were included. This sample size of 225 articles, as well as the retrieved analysis results, are highly indicative to be able to develop conclusions and future recommendations as needed.

All 225 articles were used in this comparative analysis, irrespective of ozone form (gas, ozonated water or ozonated oil) or fields of application (in vitro, pre-clinical and clinical studies) were all included. Whenever possible, full text articles were retrieved. Otherwise abstracts were used – Included in Table 1.

As mentioned earlier, the aim is not to critique the studies, but to look at the “Materials and Methods” and the applied ozone parameters and compare them to those applied by the dentists in their practice. Percent of results (G/S Good/Significant, G/NS Good/non-significant, NS non-significant) was stabilized as criterion of clinical efficiency.
Table 1. Frequency distribution of articles by group classification pre-clinical- in vitro-clinical.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Articles</th>
<th>Type of article</th>
<th>Stage of the investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Abstracts</td>
<td>Full Text</td>
</tr>
<tr>
<td>I. O$_3$ Gas</td>
<td>118</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>II. O$_3$ Water</td>
<td>53</td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>III. O$_3$ Oil</td>
<td>30</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>IV. O$_3$ Gas-Water</td>
<td>14</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>V. O$_3$ Topical-Systemic</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>VI. O$_3$ Injection</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>225</td>
<td>82</td>
<td>143</td>
</tr>
</tbody>
</table>

Sidebar I. Classification of articles.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ozone mode of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Ozone Gas</td>
<td>Ozone gas only was used in the research</td>
</tr>
<tr>
<td>II. Ozonated Water</td>
<td>Ozonated water only was used in the research</td>
</tr>
<tr>
<td>III. Ozonated Oil</td>
<td>Ozonated oil only was used in the research</td>
</tr>
<tr>
<td>IV. Ozone Gas-Water</td>
<td>Combined or Separate administration of ozone gas and ozonated water were used, plus ozonated oil when applicable</td>
</tr>
<tr>
<td>V. Ozone Topical-Systemic</td>
<td>Topical and/or Systemic ozone administration was used</td>
</tr>
<tr>
<td>VI. Ozone Injection</td>
<td>Ozone gas and/or ozonated water was injected sub-cutaneous or intra-articular</td>
</tr>
</tbody>
</table>

Classification of articles – Appendix: Reviewed Articles List. Articles were classified into six groups as listed in sidebar I.

2.2 Retrieved Data

Articles from each classification group were categorized according to different criteria: 1) country and period of research (Table 2), 2) field of research i.e. caries, periodontics, endodontic, soft tissue lesions, etc. (Table 3), 3) Ozone equipment/generator and technical specifications (Table 4).
Table 2. Classification of manuscript according to: County and period of research.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td>13</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>20</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
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<td>Germany</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>17</td>
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<tr>
<td>Brazil</td>
<td>-</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>16</td>
<td></td>
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<td></td>
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<tr>
<td>Italy</td>
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<td>7</td>
<td>3</td>
<td>2</td>
<td>14</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>India</td>
<td>-</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>14</td>
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<td></td>
<td></td>
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<tr>
<td>Japan</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>-</td>
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<td>2</td>
<td>-</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuba</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
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<td>2</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>countries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. Total number of revised manuscript was 225.

Table 3. Classification of manuscript according to: Fields of research.

<table>
<thead>
<tr>
<th>Field</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries</td>
<td>46</td>
<td>20.5</td>
</tr>
<tr>
<td>Materials</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Endodontic</td>
<td>34</td>
<td>15</td>
</tr>
<tr>
<td>Surgery</td>
<td>29</td>
<td>12.5</td>
</tr>
<tr>
<td>General</td>
<td>34</td>
<td>14.5</td>
</tr>
<tr>
<td>Periodontics</td>
<td>17</td>
<td>7.5</td>
</tr>
<tr>
<td>Soft Tissue Lesions</td>
<td>8</td>
<td>3.5</td>
</tr>
<tr>
<td>Dental unit water lines (DUWL)</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>Temporomandibular joint (TMJ)</td>
<td>6</td>
<td>2.5</td>
</tr>
<tr>
<td>Whitening</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Orthodontics</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>4</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. Total number of revised manuscript was 225.

Table 4. Frequency distribution of articles by ozone generators and specifications.

<table>
<thead>
<tr>
<th>Generator</th>
<th>N</th>
<th>%</th>
<th>O₃ (µg/mL)</th>
<th>O₂</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healozone</td>
<td>75</td>
<td>65</td>
<td>4.2</td>
<td>Ambient air</td>
<td>650 mL/min</td>
</tr>
<tr>
<td>Ozonytron</td>
<td>18</td>
<td>14.5</td>
<td>Unclear</td>
<td>Air/Pure O₂</td>
<td>Unclear</td>
</tr>
<tr>
<td>Prozone</td>
<td>12</td>
<td>10.5</td>
<td>0.2</td>
<td>Ambient air</td>
<td>2 L/min</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>11</td>
<td>Not specified</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Clinical Expertise: Dental professionals’ Ozone Clinical protocols – Parameters

Due to logistical and time constraints, the collection of clinical protocols and fields of application of ozone therapy applied by the majority of worldwide dental professionals was limited to direct contacts with individual dentists, associations, educators and trainers. Information pertaining to ozone concentration, oxygen source and modes of application were gathered in order to draw a general guideline that is mostly followed by the dental professionals. The majority of dentists use ozone gas and water (mostly combined), few others use ozonated water only. The following are the reported concentrations used:

i. Ozone gas: Concentration 10-100 µg/mL. Flow rate: 30-1 000 mL/min. Application time: 30 s to 5 min.

ii. Ozonated water: Concentration 4-20 µg/mL; Volume: Unclear.

iii. Ozonated oils peroxide value (PV): Unclear.
3. Results

3.1. Group Gas only

Table 6. Frequency and relative frequency distribution of articles by country, year of publication, results and type of ozone gas generator

<table>
<thead>
<tr>
<th>Country</th>
<th>1999</th>
<th>2000</th>
<th>2005</th>
<th>2010</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>N</th>
<th>%</th>
<th>Results (%)</th>
<th>Generator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G/S</td>
<td>G/NS</td>
</tr>
<tr>
<td>1. Turkey</td>
<td>-</td>
<td>4</td>
<td>20</td>
<td>8</td>
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<td></td>
<td>26.5</td>
<td>69</td>
<td>69</td>
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<tr>
<td>2. U.K.</td>
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<td>7.5</td>
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<td>6. Switzerland</td>
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<td>1</td>
<td>6</td>
<td></td>
<td>5</td>
<td>20</td>
<td>80</td>
<td>80</td>
<td>17%</td>
</tr>
<tr>
<td>7. Brazil</td>
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<td>3</td>
<td>4</td>
<td></td>
<td>3.5</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>8. Egypt</td>
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<td>-</td>
<td>3</td>
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<td>66.5</td>
<td>66.5</td>
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<td>9. Australia</td>
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<tr>
<td>10. Croatia</td>
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<td>2.5</td>
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<td>100</td>
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<td>20</td>
</tr>
<tr>
<td>11. Portugal</td>
<td>-</td>
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<td>-</td>
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<td></td>
<td>2.5</td>
<td>100</td>
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<td>20</td>
</tr>
<tr>
<td>12. U.A.E.</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td></td>
<td>1.5</td>
<td>100</td>
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<tr>
<td>Total</td>
<td>23</td>
<td>27</td>
<td>36</td>
<td>18</td>
<td>10</td>
<td>4</td>
<td></td>
<td>67%</td>
<td>33%</td>
<td>74%</td>
<td>17%</td>
</tr>
<tr>
<td>Not listed: countries with 1 article only (14 manuscript)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65%</td>
<td>15%</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts 118 (include 14 manuscripts of the non-listed countries) 1) G/S Good/Significant, G/NS Good/non-significant, N/S non-significant. 2) Type of generator: H: Healozone; Oz: Ozonytron; P: Prozone. U.K., United Kingdom; U.A.E., United Arab Emirates, * Including the not listed article.

3.1.1 Generators used in group Gas only

1. The Healozone\(^6\) generator was used in 65% of the ozone gas only studies - Table 6. For safety reasons, this system generates ozone gas only when a hermetic seal of the treatment area is achieved by using a silicone cap on the delivery handpiece. It generates ozone gas from dry ambient air at a fixed concentration of \(\sim 4.2 \mu\text{g/mL}\) and \(\sim 650 \text{ mL/min}\) flow rate. The application time varied from 10 s to 180 s yielding a total dose of \((0.06 – 8.20) \text{ mg}\) ozone gas. In 57/74 studies (77%) good/significant results were noted, whereas the remaining 23% revealed a combined good/non-significant and non-significant results. Only one in vivo study, which showed good/significant results, used a second version of the Healozone which generates ozone gas from dry ambient air as the first version) or from pure oxygen at a fixed concentration of 32 \(\mu\text{g/mL}\). Application time was 120 s yielding a total ozone dose of \(\sim 128 \text{ mg}\).
2. The Ozonytron was used in 15% of the ozone gas only studies – Table 6. This system generates a plasma phase, including ozone, from the air surrounding the treatment area via an electro-magnetic field. Other models generate ozone from pure oxygen. According to the technical specifications from the manufacturer’s website, as well as the ozone concentration values and measurement units, it is difficult to specify with accuracy the ozone concentration that can be achieved with these units. The claimed ozone gas concentration values, in our opinion, are either exaggerated or misrepresented, a fact confirmed by the following concentration values as indicated in the manufacturer’s website:

“Ozone concentration when using the ozone injector:
- Flowing in with 1 L/min
- Using atmospheric oxygen: 800 ppm to 22 000 ppm
- Using 99.5 % medically pure oxygen from the gas bottle: 3 000 bis 100 000 ppm – (6 – 200) g/L

Converting ppm into µg/mL (refer to sidebar 2), it is clear that there is a misrepresentation of the ozone concentration and the measurement units used.

Sidebar II

<table>
<thead>
<tr>
<th>Converting ppm into µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>800 ppm to 22 000 ppm is equal to about 1.6 µg/mL – 44 µg/mL.</td>
</tr>
<tr>
<td>3 000 ppm to 100 000 ppm is about 6 µg/mL – 200 µg/mL.</td>
</tr>
<tr>
<td>6 – 200 g/L is equal to 6 000 – 200 000 µg/mL.</td>
</tr>
</tbody>
</table>

The majority of the articles citing the use of the ozone injector, the Ozonytron, the ozone concentration could not be easily interpreted as the authors reported it in general terms such as “According to the manufacturer’s instructions”, “The system was operated at 5N intensity in accordance with the manufacturer’s instructions”, “O₃ was delivered at 100 % for 40 s with peristaltic motions, as recommended by the manufacturer”, “Gas Topical application. Activated oxygen ozone) concentration of 30 %”, or “In accordance with the manufacturer’s instructions, at 100% volume for 40 s”.

Of the 17 studies, 65% showed good/significant results and 35% combined good/non-significant and non-significant results.

3. The Prozone was used in 10% of the ozone gas only studies – Table 6. This system generates ozone from ambient air at a fixed ozone concentration of ~0.25 µg/mL and 2 L/min flow rate. Time of application varied between 6 s and 240 s yielding a total applied ozone dose of 0.08 mg – 2 mg. Of the 12 studies, 50% showed good/significant results and 50% combined good/non-significant and non-significant results.

By comparison to medical ozone generators where only pure oxygen is used to generate ozone gas, these dental systems operate on ambient air and produce low ozone concentrations. A reason why most of the research authors used these models is that they are CE medical device certified, which is a prerequisite in the European Union where the vast majority of research was conducted.

This discrepancy between medical and dental ozone systems is unclear. It’s highly recommended that dental ozone manufacturers follow the technical specifications commonly used in medical ozone systems which reference pure oxygen sources and a large spectrum of ozone gas concentrations.
Summary of ozone parameters used in group gas only between research and clinicians:

<table>
<thead>
<tr>
<th>Group O₃ gas only</th>
<th>Research</th>
<th>Dentists</th>
<th>Results %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>G/S</td>
</tr>
<tr>
<td>Concentration (µg/mL)</td>
<td>0.2 – 4.2</td>
<td>10 - 100</td>
<td>67</td>
</tr>
<tr>
<td>Oxygen source</td>
<td>Ambient air</td>
<td>Pure oxygen</td>
<td>-</td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>0.06 – 8.2</td>
<td>3 - 120</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend: Results: G/S, Good/Significant; G/NS, Good/non-significant; NS, non-significant.

A clear divergence is evident between “ozone gas only” research studies and clinicians reference oxygen source and ozone concentrations. It is noteworthy to mention that dentists follow the same specifications and parameters as used by medical doctors in topical applications, which is also well reflected in medical research covering this particular field of application. Whereas the dental research in “ozone gas only” is somehow following a different path.

It is with hope that future dental ozone studies take into consideration this finding. We would also hope that authors consider following the same guidelines, specifications and parameters as used in medical topical applications. We suspect that the negative results (33%) would be vastly improved. It is also anticipated that dental ozone manufactures provide reliable and improved systems specifically designed for dental applications and be CE certified in order to be safely and legally used in dental ozone studies.

3.2. Group ozonated water only

Table 7. Summary of articles by country, Field, and Results.

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>Country</th>
<th>N</th>
<th>Field</th>
<th>N</th>
<th>Field</th>
<th>N</th>
<th>Results %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>10</td>
<td>Switzerland</td>
<td>4</td>
<td>Periodontics</td>
<td>13</td>
<td>DUWL</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>8</td>
<td>Egypt</td>
<td>3</td>
<td>Surgery</td>
<td>9</td>
<td>Caries</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>7</td>
<td>China</td>
<td>3</td>
<td>Endodontic</td>
<td>7</td>
<td>Soft tissue lesions</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>6</td>
<td>U.K.</td>
<td>2</td>
<td>Materials</td>
<td>6</td>
<td>TMJ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>5</td>
<td>53 articles*</td>
<td>6</td>
<td>General</td>
<td>6</td>
<td>Cytotoxicity</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. * Including the not listed article (countries with 1 article only). DUWL, Dental unit water lines; TMJ, Temporomandibular Joint; UK, United Kingdom. Results: G/S, Good/Significant; G/NS, Good/non-significant; NS, non-significant.

Lower negative results were noted in group “ozonated water only” (15%) – Table 7 - compared to group “ozone gas only” (33%) – Table 6.
Table 8. Ozonated water concentration used in the studies and results.

<table>
<thead>
<tr>
<th>Low concentrations µg/mL</th>
<th>N</th>
<th>%</th>
<th>High concentrations µg/mL</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.1</td>
<td>10</td>
<td>19</td>
<td>4-6</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>6</td>
<td>12</td>
<td>8-15</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>31</td>
<td>16-25</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results %

<table>
<thead>
<tr>
<th>G/S: 50</th>
<th>G/NS-NS: 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. %, percent of the total number of manuscripts analyzed (53). Results: G/S, Good/Significant; G/NS, Good/non-significant; NS, non-significant.

It was further observed that 19% of the total number of studies used less than 0.1 µg/mL concentration, and 12% used less than 2 µg/mL, yielding 50% combined negative results in comparison to 11% in other studies where a higher concentration of (4-25) µg/mL was used.

Unlike the observed discrepancy in ozone parameters between clinicians and research in the group “ozone gas only”, there is a concordance between clinical applications and research findings in the group “ozonated water only”.

It is thus recommended that future research studies use medium to high ozonated water concentrations of (4-25) µg/mL which are commonly used in medical topical applications and by dental clinicians.

3.3 Group ozonated oils

Table 9. Summary of articles by Country and Field.

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>Field</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>7</td>
<td>Surgery</td>
<td>10</td>
</tr>
<tr>
<td>India</td>
<td>6</td>
<td>Antibacterial</td>
<td>6</td>
</tr>
<tr>
<td>Egypt</td>
<td>5</td>
<td>Periodontics</td>
<td>4</td>
</tr>
<tr>
<td>Cuba</td>
<td>4</td>
<td>Endodontic</td>
<td>3</td>
</tr>
<tr>
<td>Brazil</td>
<td>3</td>
<td>Soft tissue lesions</td>
<td>2</td>
</tr>
<tr>
<td>Countries 1 article each</td>
<td>5</td>
<td>Cytotoxicity</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caries</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Materials</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hyper-sensitivity</td>
<td>1</td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. Total number of manuscript analyses was 30.

Table 10. Concentrations and Results.

<table>
<thead>
<tr>
<th>Concentration mEq O₂/kg</th>
<th>N</th>
<th>Results % [G/S]</th>
</tr>
</thead>
<tbody>
<tr>
<td>~ 1 300</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>590</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>0.025-0.5%</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Not specified</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. G/S, Good significant. Total number of manuscript analyses was 30.
The major positive result noted in ozonated oils studies is strongly suggestive of their usefulness in dental applications, as was also observed in topical medical applications. In the majority of the dental studies, the lack of ozonated oils concentration specification was observed. The role and responsibility of ozonated oils producers to label their products with the peroxide value (in mEq O₂/kg or in mmol O₂/kg) is vital in order to compare different ozonated vegetable oils and the concentrations best suited for dental applications.

### 3.4 Group ozone gas and ozonated water

A striking observation is the very low number of studies where both ozone gas and ozonated water were used. In medical topical applications studies, it’s only normal to use both gas and water, and the positive results observed in the 14 dental studies is proof that both gas and water should be used instead of only gas or only water.

In our opinion, the common practice in clinical applications of the combined use of gas and water when applicable should be the norm in future dental research studies, as well as the use of medium to high ozone concentrations.

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>C/S¹</th>
<th>Field</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>3</td>
<td>C</td>
<td>Endodontic</td>
<td>5</td>
</tr>
<tr>
<td>Brazil</td>
<td>3</td>
<td>S</td>
<td>Materials</td>
<td>2</td>
</tr>
<tr>
<td>Turkey</td>
<td>3</td>
<td>S</td>
<td>Cytotoxicity</td>
<td>2</td>
</tr>
<tr>
<td>Germany</td>
<td>2</td>
<td>S</td>
<td>TMJ</td>
<td>1</td>
</tr>
<tr>
<td>Cuba</td>
<td>1</td>
<td>C</td>
<td>Caries</td>
<td>1</td>
</tr>
<tr>
<td>USA</td>
<td>1</td>
<td>C</td>
<td>Soft tissue lesions</td>
<td>1</td>
</tr>
<tr>
<td>Poland</td>
<td>1</td>
<td>S</td>
<td>Periodontics</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surgery</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. ¹C/S: Combined or Separate use of ozone gas and/or water. TMJ, Temporomandibular Joint.

### Table 12. Concentrations and Results.

<table>
<thead>
<tr>
<th>Concentration µg/mL</th>
<th>N</th>
<th>Results %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone gas</td>
<td></td>
<td>GS</td>
</tr>
<tr>
<td>Ozonated water</td>
<td></td>
<td>93</td>
</tr>
<tr>
<td>4.2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>0.2-53</td>
<td>1-20</td>
<td>2</td>
</tr>
<tr>
<td>6-20</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>60</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Not specified</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. Results: G/S, Good/Significant; G/NS, Good/non-significant. Total number of manuscript analyses was 14.
3.5. Group Topical – Systemic ozone application

Table 13. Summary of articles by Country, Field, Application Mode and Results.

<table>
<thead>
<tr>
<th>Country</th>
<th>Field</th>
<th>N</th>
<th>Application Mode</th>
<th>Results %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ukraine</td>
<td>Surgery</td>
<td>2</td>
<td>O$_3$ water + IV O$_3$ saline</td>
<td>100</td>
</tr>
<tr>
<td>Egypt</td>
<td>Surgery</td>
<td>1</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Russia</td>
<td>Surgery</td>
<td>1</td>
<td>O$_3$ water + IV O$_3$ saline</td>
<td></td>
</tr>
<tr>
<td>Cuba</td>
<td>TMJ</td>
<td>1</td>
<td>IA vs. Rectal + IA</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>Surgery</td>
<td>1</td>
<td>Intraperitoneal + O$_3$ gas</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. TMJ, Temporomandibular Joint. IV, Intra Venous; IA, Intra articular. Results: GS Good/Significant. Total number of manuscript analyses was 6.

In light of the increasing evidence and research into oral-systemic health links, it is essential that medical and dental health professionals along with researchers, join efforts and conduct more studies in order to elucidate the usefulness of ozone therapy in both oral and systemic chronic inflammatory diseases.

This holistic health approach would greatly benefit patients suffering from chronic oxidative stress where ozone therapy is highly indicated.

In our opinion, there is an urgent need to conduct more studies on ozone therapies. This should propel ozone therapy as a whole to a totally new level of prominence. Especially when we consider that adult chronic periodontitis is one of the most common chronic inflammatory diseases contributing to systemic conditions and disease.

3.6. Group ozone injections

There have been a very large number of published articles in medical ozone therapy with the highest grade of evidence-based medicine to support vertebral-paravertebral ozone injections. The promising positive results seen in these dental studies and considering the comparison to this medical research, should warrant more research into ozone gas injections in temporomandibular joint (TMJ) and related muscles.

As in the case of chronic periodontitis which affects a large number of the population, temporomandibular joint disorders are also considered some of the most common skeletal inflammatory and degenerative conditions.

Future research in this field would help dental clinicians to distinguish between the beneficial effects of the TMJ intra-articular and the para-articular ozone injections, as well as the topical applications as seen in some studies where ozone gas was topically applied over the affected TMJ area (Table 14).

Table 14. Summary of articles by Country, Field, Application Mode and Results.

<table>
<thead>
<tr>
<th>Country</th>
<th>Field</th>
<th>N</th>
<th>Application Mode</th>
<th>Results %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>TMJ</td>
<td>3</td>
<td>O$_3$ gas and/or O$_3$ water</td>
<td>[G/S] 100</td>
</tr>
<tr>
<td>Turkey</td>
<td>Ortho</td>
<td>1</td>
<td>O$_3$ gas</td>
<td></td>
</tr>
</tbody>
</table>

Legend: N, total number of manuscripts. TMJ, Temporomandibular joint. Ortho., Orthodontics. Results: G/S Good/Significant. Total number of manuscript analyses was 4.
4. Discussion

It is indisputable that ozone therapy in dentistry is growing and is being used by an increasing number of dentists worldwide. The clinical experiences and treatment outcomes are adding significant amounts of knowledge. Dental research should follow and support dental professionals in their daily practice of ozone therapy. As well, dental professionals will benefit from the experiences of our medical colleagues.

Most importantly, the discrepancy noted between research and clinicians in ozone gas specifications and oxygen feed sources is significant. A major contributing factor to this discrepancy is the available dental ozone gas systems offered to researchers, most of which operate on ambient air and generate low ozone gas concentrations.

In addition, it is fundamental that ozone dental units be designed with a larger spectrum of gas concentration and be certified for use in research. There is a large choice of commercially available medical units. However the average cost of these systems might be prohibitive for dental use.

In 15% of the ozone gas articles, the concentration and flow rate were not clearly specified, thus of little or no value for clinicians. It is paramount that authors measure the concentrations of the generated gas, and not solely rely on manufacturers' recommendations.

Similarly, the peroxide value (PV) of the ozonated oil products should be tested by researchers in all instances. The majority of ozonated oils articles did not specify the PV, which is essential for clinicians to choose the appropriate concentration according to the clinical case.

We cannot stress enough about the need and importance of future dental research studies incorporating both ozone gas and water, as well as ozonated oils when applicable. These therapies are what the majority of dentists apply in their practice and it might very well improve the overall positive and significant results in research.

Dental ozone research ought to change its course to meet the expectations of clinicians. Research needs to explore new and different studies than the conventional and well-studied antimicrobial potential of ozone. Most importantly the direct ozone application on pulp exposures, whether due to caries, trauma or iatrogenic, and to evaluate the potential of dentin generation and pulp tissue cytotoxicity according to the ozone form and applied dose.

The assistance and cooperation of our medical colleagues is well appreciated, especially in conducting more studies in the field of oral-systemic links.

Lastly, ozone equipment manufacturers have a major role to play in the evolution of dental ozone research and clinical ozone practices.

Acknowledgement
The authors would like to express their gratitude to Nadia Saimua, BSN, quality education manager, American university of Beirut medical center for her technical support.
Appendix: Articles Consulted

Some selected unpublished articles without formal citation (highlighted in yellow) were collected from scientific ozone meetings.

Ozone Gas

7. **Ozone, an effective treatment for DUWL.**
27. Determination of the Performance of Various Root Canal Disinfection Methods after In Situ Carriage. Anca Virtej, Dr. med. dent. 2007 by the American Association of Endodontists. doi:10.1016/j.joen.2006.11.025
29. Assessment of enamel changes during fixed orthodontic treatment with and without ozone– Amna AL-Shamsi. School of Medicine and Dentistry Queen’s University of Belfast (QUB) - PhD thesis 2007
33. Assessment of ozone in root canal therapy – PhD theses


59. Tubular occlusion of simulated hypersensitive dentin by the combined use of ozone and desensitizing agents. RASHA RAAFAT ISSN 0001-6357 Odontologica Scandinavica, 2011.


62. Microbiological effects of gas action depending on the method and the time of application using the ozonytron device. Experimental study Wilczyńska-Borawska M.


95. Remineralization of Three Fissure Sealants with and without Gaseous Ozone on Non-Cavitated Incipient Pit and Fissure CariesMurat UNAL DDS, Journal of Clinical Pediatric Dentistry: Summer 2015, Vol. 39, No. 4


119. Assessment of antibacterial efficacy of ozone therapy in treatment of caries at
the white spot stage. Makeeva IM doi: 10.17116/stomat20179647-10.


Ozone Water


10. Ozonated saline shows activity against planktonic and biofilm growing Staphylococcus aureus in vitro: A potential irritant

11. for infected wounds. Hayder Al-Saadi’, Inga Potapova, Edward TJ Rochford, Thomas F Moriarty PhD, & Peter Messmer

12. Research Institute Davos, A0 Foundation, Davos Platz. Switzerland International Wound Journal ISSN 1742-4801


19. Application of ozonated water in local augmentation in dental implantology A. Filippi 2005
20. Influence of ozonated oils and water in oral surgery
23. Disinfection of dental unit water with ozone. LAURENCE J. WALSH 2006
30. Effect of ozone water on the inflammation and repair in infected wounds. HUANG Hua-jun, Hospital, Southern Medical University, Guangzhou 510515, China 2010-03
34. Antimicrobial effects of ozonated water on the sanitization of dental instruments contaminated with E. coli, S. aureus, C. albicans, or the spores of B. atrophaeusJulio César et al; SP, Brazil 2010
36. Study on Effects of Different Concentrations of Ozone Solution on the HBV Virus in Dental Unit Waterlines. YANG Xin, The First Central Hospital, Clinical College of Tianjin Medical University, Tianjin 300100, China
38. The antimicrobial effect of 0.1 ppm ozonated water on 24-hour plaque microorganisms in situ. Syed SadatullahBraz Oral Res. 2012 Mar-Apr;262):126-31


47. Ozone and Its Role in Periodontal Therapy - A Review. Dr. Vinutha R.S , BDS 1, Dr. Reema Lakshmanan, MDS 2 Journal of Dental and Medical Sciences IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861. Volume 13, Issue 1 Ver. IX.

48. Disinfection of Dental Instruments Contaminated with Streptococcus mutans Using Ozonated Water Alone or Combined with Ultrasound. Pâmela Maria Moreira Fonseca. International Ozone Association ISSN: 0191-9512 print / 1547-6545 online DOI: 10.1080/01919512.2014.904740


Ozonated Oil


2. Suppressive effects on immune cells and oxidative cytotoxicity of ozonated olive oil. Japan 2005


5. Ozone Therapy in the Treatment of Avascular Bisphosphonate-Related Jaw Osteonecrosis. AllesandroAgrillo, MD, PhD, Claudio Ungari, MD, Fabio Filiaici, MD, Paolo Priore, MD, Giorgio Iannetti, MD, PhD, Rome, Italy The Journal of Craniofacial Surgery, 18 (5) September 2007

6. Role of ozone therapy in the treatment of osteonecrosis of the jaws in multiple myeloma patients. Maria Teresa Petrucci, Cristiano Gallucci, Alessandro Agrillo, Maria Cristina Mustazza, Robin Foà. Hematology, Department of Cellular Biotechnologies and Hematology; Clinica Maxillo-Facial University “La Sapienza” of Rome, Italy Haematologica 2007; 92:1289-1290. DOI: 10.3324/haematol.11096


Ozone Gas-Water


8. Antibacterial Effect of Gaseous and Aqueous Ozone in Root Canals Infected by Enterococcus Faecalis. Recai Zan, Ihsan Hubbezoglu, Zeynep Sumer & Tutku Tunc

9. Ozone: Science & Engineering, 36: 264–268 Copyright © 2014 International Ozone Association ISSN: 0191-9512 print / 1547-


14. Effect of Ozone and CO2 Laser on Dental Caries Progression. K.A. ALI1, R. SEGHI, M. MOHSEN, M. SALAH EL DIN, M. HASAN, and H. ABD EL MAGUID, College of Dentistry, Suez Canal University, Egypt, Columbus, OH, USA, Ohio State University, Columbus, USA, Faculty of Oral and Dental Medicine, Cairo University, Egypt, College of Dentistry, Suez Canal University, Ismailia


Topical - Systemic


2. Ozone influence on the mandible regeneration in rats. V.A. Malanchuk, A.V. Kopchak, V.V. Grigorovsky. Department for Oral and Maxillo-facial Surgery National Medical University, Kiev, Ukraine


4. The Effect of Ozone on the Lipid Peroxidation Processes in Case of Mandible Fractures. N.E. Homutinnikova1, E.A. Durnovo, Nizhny Novgorod State Medical Academy, Department of Surgical Dentistry and Maxillofacial Surgery, Minin sg. 10/1, 603005, Nizhny Novgorod, RUSSIA.


6. Systemic and intra-articular ozone therapy in temporomandibular joint arthritis with rheumatoid arthritis. Méndez-Pérez, Ivonne; Menéndez-Cepero, Silvia
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