

The influence of ozone on the effectiveness of the vital pulp therapy with Biodentine™*

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ABSTRACT

Introduction: The aim of the present study was to clinically evaluate the influence of ozone on the vital pulp therapy (VPT) in human mature teeth. Vital pulp therapy – pulp capping helps to salvage caries-exposed pulp and prevent the need for further endodontic treatment. There are *in vitro* and *in vivo* studies using extracted teeth that indicate that Biodentine is an interesting alternative to mineral trioxide aggregate (MTA). It is believed that unfavourable outcomes of VPT are caused by infection, either due to residual bacteria in the carious cavity or caused by bacterial microleakage. Ozone is a strong oxidant with a beneficial antimicrobial effect.

Materials and methods: Ninety-one teeth were randomly assigned to 1 of 2 groups. Forty-one teeth were treated with ozone and Biodentine (O₃ & B) – the experimental group and 50

teeth were treated with Biodentine only (B) – the control group. Clinical evaluations were performed at 3, 6, and 12 months after direct pulp capping.

Results: There was no statistically significant difference in success rates between the 2 groups. However, the success rates in both groups were high, ranging from 88.2% to 76.9% ($p = 0.34$) in the experimental and control groups, respectively.

Conclusions: Vitality pulp treatment with Biodentine combined with ozone and without ozone was found to be equivalent in terms of therapy efficacy. Biodentine was found to be a highly effective pulp capping material in VPT. There is a need for further *in vivo* studies to evaluate whether *in vitro* results of other studies translate to clinical practice.

Keywords: Biodentine; direct pulp capping; ozone.

INTRODUCTION

The contemporary concept of dental treatment is that the best root canal filling is healthy pulp tissue. Vital pulp therapy (VPT) helps to salvage carious, accidentally or traumatically exposed pulp and prevent the need for further endodontic treatment. Vital pulp therapy promotes the healing of pulp tissue, preserves vital pulp [1, 2, 3] and facilitates the formation of reparative dentin from odontoblasts [4, 5, 6] which is called a dental bridge.

According to the American Association of Endodontists Guide to Clinical Endodontics, the indication for direct pulp capping are as follows:

1. occurrence of mechanical exposure of clinically vital and asymptomatic pulp,
2. controlled bleeding at the exposure site,
3. possibility of direct contact of the capping material with vital pulp tissue after exposure,
4. occurrence of exposure during dental dam isolation of the tooth,
5. maintenance of adequate seal of the coronal restoration, and
6. indication to the patient of possible future endodontic treatment.

Success depends on a good understanding of pulp biology, the use of appropriate materials and sound technical procedures.

The correct diagnosis – irreversible pulpitis – is very important for success. The condition of the pulp should be asymptomatic. The inflammatory response should be minimal [7]. The presence of microorganisms with subsequent infection, the size of the pulpal exposure [8], the time of examination [9], the quality of the dentin bridge, and the type of pulp capping material used [10] are the criteria that determine the prognosis and success of VPT. A careful decision on pulp therapy should be made based on the patient's history, clinical findings, and the ability to restore the tooth [11].

Calcium hydroxide has played an important role in VPT for almost 100 years. It has become a gold standard in pulp capping. However, its use in VPT is controversial due to its drawbacks [12]:

- a) it loses its antibacterial capacity when it comes in contact with tissue fluid due to a decrease in its acidic pH,
- b) calcium hydroxide is not a good material for sealing against bacterial penetration because bacteria can easily penetrate any remaining calcium hydroxide after its initial antibacterial action has passed, and
- c) it is completely dependent on the overlying restorative material to prevent bacterial penetration into the pulp.

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In the early 1990s, mineral trioxide aggregate (MTA) was introduced to dentistry by Torabinejad and Pariookh as a new alternative to calcium hydroxide. Mineral trioxide aggregate has several advantages:

- a) biocompatibility,
- b) close adaptation to adjacent dentin tissues preventing bacterial leakage, and
- c) hydrophilic nature and requires moisture to cure, making it a suitable material for procedures such as pulpotomy [13].

Over the past 2 decades, a number of preclinical and human clinical studies have evaluated and compared these materials (calcium hydroxide and MTA). Mineral trioxide aggregate has received attention as an alternative to calcium hydroxide and has become a new gold standard in VPT. On the other hand, MTA has some disadvantages, i.e. long setting time, potential of discoloration and high cost [14]. Therefore, it was necessary to find a new successful agent for pulp capping.

Biodentine™ (Septodont, Saint-Maur-des-Fossés, France) is a new bioactive calcium silicate-based cement, similar to the widely used MTA. Biodentine has similar efficacy and can be considered as an interesting alternative to MTA in VPT [15]. Biodentine has a wide range of clinical indications: pulp capping, pulpotomy, apexogenesis, apexification, revascularization, root perforation, retrograde filling in endodontic surgery as well as a dentin replacement material in restorative dentistry [16]. It is characterized by good sealing ability, adequate compressive strength and short setting time, which provide a significant clinical advantage over other comparable pulp capping materials [17, 18]. Biodentine exhibits bioactivity and is biocompatible [19, 20]. According to the manufacturer, it can also be used as a temporary restoration. All of this has been demonstrated in *in vitro* studies, either on animal teeth or extracted human teeth with healthy pulp. Clinical studies on reversible inflamed teeth are lacking.

It is believed that unfavorable outcomes of VPT are caused by infection due to either residual bacteria in the carious cavity, or new bacteria contaminating the exposed pulp during the procedure. Authors [21, 22, 23, 24, 25] report that the pulp reaction after direct pulp capping is triggered by bacterial microleakage.

Ozone is a natural allotrope of oxygen found in the upper layer of the atmosphere that protects living organisms on Earth from UV radiation. Ozone is a strong oxidizing agent with a beneficial antimicrobial effect. It has been shown to be effective against Gram-positive and Gram-negative bacteria, viruses and fungi [26]. Ozone does not alter the physical properties of enamel or dentin [27, 28, 29]. It has been widely used as a treatment measure for more than 50 pathological processes [30], and in various fields in dentistry, e.g.: cardiology [31], endodontics [32], periodontology [33] and maxillofacial surgery [34]. It is used in a variety of forms, e.g.: gas, ozonated water or oil, aqueous ozone solutions for disinfection, hemostatic effect or to accelerate wound healing [30]. Ozone has been investigated in many studies primarily *in vitro* studies and is presented as a possible alternative antiseptic agent due to its antimicrobial

power [35] and low cytotoxicity [36], but there is little information on the time and concentration to use.

Polydorou et al. demonstrated in an *in vitro* study that 80 s of ozone exposure has a significantly better microbicidal effect than 40 s, but not 100% of the bacterial growth reduction [31]. According to authors of other studies [37, 38], ozone application for 40 s significantly reduced *Streptococcus mutans* count, whereas 60-second exposure almost eliminated cariogenic species such as *S. mutans*, *Lactobacillus casei* and *Actinomyces naeslundii* in carious lesions in roots. Ozone gas should not be inhaled because the bronchial pulmonary system is very sensitive to ozone.

An idea was born to combine VPT using Biodentine as a new effective pulp capping agent with ozone therapy as an effective bactericidal agent to evaluate its influence on treatment results.

MATERIALS AND METHODS

Subject enrollment

This was a prospective study conducted with subjects recruited from the patient pool of the Department of Conservative Dentistry and Endodontics at the Pomeranian Medical University in Szczecin, Poland. Ninety-one patients of both sexes, aged 20–30 years old, generally healthy, with up to ca. 1 mm² of pulp exposure (carious or traumatic) were eligible. Teeth with signs and/or symptoms of irreversible pulpitis or pulp necrosis (history of spontaneous toothache, sinus tract, periodontal inflammation, excessive mobility, furcation/apical radiolucency, radiographic evidence of internal/external resorption) were not included. Teeth with unexposed pulp and uncontrolled pulpal bleeding lasting more than 5 min during the procedure were excluded.

Subjects were treated in accordance with the tenets of the Helsinki Declaration. Patients were thoroughly informed about the experimental rationale, clinical procedures, and possible complications of the procedure. Informed verbal and written consent was then obtained. All treatment procedures were reviewed and approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval number KB – 0012/49/12).

Medication and equipment

In the present study, Biodentine was used as a pulp capping material and an ozone generator – Prozone (W&H, Austria) – was used as an ozone source.

The device is equipped with a handpiece with a suitable tip “cor” for ozone application in a cavity of the tooth and selection of exposure time period on its display, i.e.: 6, 12, 18, and 24 s. The ozone exposure time was set to 120 s (5 x 24 s). Such a time period was chosen considering the abovementioned research data in order to achieve a better bactericidal effect [31, 37, 38].

Pulp vitality was assessed with the Vitality Scanner (Sybronendo, USA).

Treatment procedure

Teeth with reversible pulpitis (absence of spontaneous unprovoked toothache and signs of periapical and periodontal inflammation) were included. Ninety-one teeth were randomized to 1 of 2 groups. Forty-one teeth were treated with ozone and Biodentine (O₃ & B) – experimental group – and 50 teeth were treated with Biodentine only (B) – control group.

Caries removal was performed under local anesthesia and rubber dam application. The caries excavation of caries and the whole procedure were performed under an operating loupe with a magnification 2.5x (Carl Zeiss Meditec AG, Germany).

After pulp exposure, the entire cavity was rinsed with saline and dried with sterile cotton pellets. Bleeding was controlled with saline irrigation and a sterile cotton pellet was placed on the exposure site. After hemostasis was achieved, as a prerequisite, the procedure was continued. The ozone application of 120 s (5 x 24 s) was performed according to the manufacturer's instructions, i.e. using a suction device. Biodentine was prepared according to the manufacturer's recommendations and the entire cavity was then filled with it. Biodentine was also used as a temporary restoration for a period of 3 months. Periapical radiographs were taken at the end of the procedure. Patients were informed to return to the office in case of spontaneous pain or prolonged provoked pain in the affected tooth. After 3 months (first follow-up), Biodentine was partially removed and the cavity was filled with Estelite Sigma composite resin (Tokuyama Dental Corp, Japan), according to the manufacturer's recommendation,

Clinical evaluation

Clinical evaluations were performed at 3, 6, and 12 months after pulp capping. At each visit, the teeth were clinically evaluated for pulp vitality and periapical radiographs were taken at the 12-month follow-up. Results were described as success or failure.

The teeth with pulpal vitality and no clinical signs and/or symptoms of irreversible pulpitis were considered successes. The teeth with no pulp vitality test response and those with clinical signs and/or symptoms of irreversible pulpitis or pulp necrosis were considered failures. Exceptionally, at a 3-month visit, if the tooth was considered a success, as mentioned above, Biodentine was partially removed and the cavity was restored with Estelite Sigma composite resin.

Statistical analyses

Age and sex data were statistically analyzed using the Mann-Whitney test. To analyze and compare the results according to the treatment methods used, the χ^2 -squared test with Yate's correction factor was performed with a significance threshold of 0.05.

RESULTS

Ninety-one patients (91 teeth) were initially enrolled in this study. Eighteen patients lost motivation to continue the study or changed their telephone number or residence. Seventy-three

patients (73 teeth) completed the study. There was no statistically significant difference in success rates between the 2 groups ($p = 0.34$).

The failed cases (4 teeth in the O₃ & B group and 9 teeth in the B group) occurred between 2–5 weeks after pulp capping. These patients were forced to return to the office before the 3-month follow-up visit due to spontaneous pain in the affected tooth. They were diagnosed with irreversible pulpitis. Root canal treatment was performed in these cases. Figure 1 shows the flow chart of the patient's progress through the study.

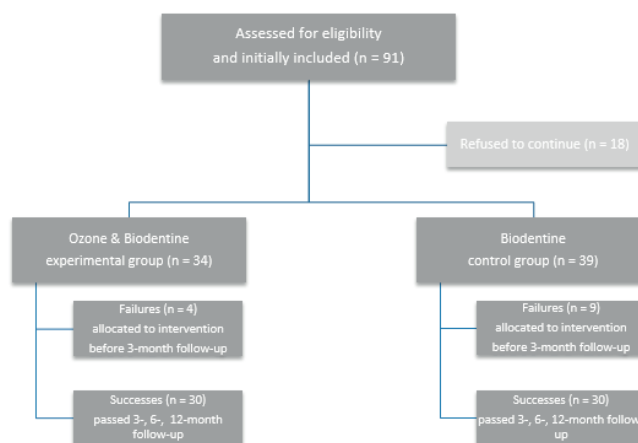


FIGURE 1. A flow chart of a subject's progress through the study

The success rate in the O₃ & B group was 88.2% while in the B group was 76.9%. Statistical analysis showed no significant difference between the groups ($p = 0.34$). The treatment results are shown in Table 1.

TABLE 1. Treatment results in the study and control groups

Results	O3 & B group		B group		p
	n	%	n	%	
Success	30	88.2	30	76.9	0.34
Failure	4	11.8	9	23.1	0.34
Total	34	100.0	39	100.0	

O₃ & B – ozone and Biodentine group (experimental group); B – Biodentine group (control group)

DISCUSSION

The present study was carried out to evaluate the influence of ozone on the efficacy of VPT. To our knowledge, the present study is the first to assess the efficacy of ozone as an adjunct disinfectant in direct pulp capping therapy. The main finding of the study indicates that there is no statistically significant difference between the treatment with the combination of gaseous ozone and Biodentine and the control group using Biodentine alone. However, the rate of positive results of treatment with Biodentine is successful with success rates of 88.2% and 76.9% in experimental and control groups, respectively.

Recently, the material – MTA – has become an alternative to calcium hydroxide in direct pulp capping [39, 40]. The results of those studies showed success rates of 95.5% and 91.3%, respectively. Many other clinical studies evaluating MTA have reported favourable results [41, 42, 43]. In some of them, the success rates were reported to be higher than 90%.

One study has found [15] that MTA and Biodentine were well tolerated by the pulp in histological studies comparing both materials. There was no evidence of inflammation or necrosis. The dentin bridge formed directly under both restorative materials was very similar in histological evaluation. The present clinical study seems to confirm the above results.

The number of studies using Biodentine for pulp capping is very small. Some of them used animal models with healthy pulp, and some of them used extracted human teeth with healthy pulp. This could influence the outcome after direct pulp capping [44, 45]. It should be noted that the results of this clinical and histological evaluation do not reveal the true effects of Biodentine in a clinically relevant context when the pulp is already inflamed due to caries [46]. Therefore, the significance of these studies may have had clinical limitations and further *in vivo* clinical studies should certainly be performed.

This study showed that a 3-month follow-up was sufficient for a preliminary prognosis, as there were no changes in the success rates of both groups with a postoperative follow-up of 3 to 12 months. All failures were observed within 5 weeks of pulp capping. Several studies have confirmed these observations [39, 47, 48, 49].

Some clinical studies [50, 51] have suggested that the age of the patient plays an important role in the performance of direct pulp capping due to the high healing potential of the pulp tissue in young patients, whereas other studies [48, 52] have not confirmed this. On the basis of this study, it is difficult to evaluate the influence of age on the success or failure of pulp capping in both groups due to the short-range age of the subjects (20–30 years). As this was the very first clinical study using ozone in the pulp capping treatment, the authors wanted to obtain the pulp responding to treatment within the teeth with a similar healing potency.

Many studies have suggested that pulp survival is influenced more by the ability of the capping material to protect the pulp from bacterial exposures than its potential bioactivity [7, 23, 53, 54, 55]. The results of the study [15] indicate that Biodentine prevents microleakage due to its excellent sealing properties. This could explain the lack of statistical difference between the groups in the present study.

Because of its mechanical properties, Biodentine seems to be very easy to use clinically (short setting time). An additional advantage of this material is that it can be used as a temporary filling in a whole cavity. All this makes Biodentine less time-consuming material compared to MTA.

The statistical simulation indicates that a fivefold increase in the number of participants in both groups (with results at some success rate) may indicate a significant difference between groups in favor of combination of ozone and Biodentine.

CONCLUSION

Vitality pulp therapy with the combination of ozone and Biodentine and the treatment with Biodentine alone were found to be equivalent in terms of the effectiveness of the therapy.

Biodentine was found to be a highly effective pulp capping material in VPT.

Biodentine is an alternative material to MTA for direct pulp capping, is convenient in handling (short setting time), and could also be used as a temporary cavity filling.

There is a need for further *in vivo* studies with larger numbers of participants to evaluate whether the *in vitro* results of other studies translate to clinical practice.

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