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ORIGINAL RESEARCH

The antimicrobial effect and pain control of ozonized gel versus gaseous ozone in the management of primary molars caries: randomized clinical trial with *in vivo* microbiological evaluation

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Abstract

Background: Pediatric dentistry has been radically revolutionized in recent years with the atraumatic approach (atraumatic restorative treatment (ART)), which is able to preserve the vitality of the tooth, with a higher attention to the child's well-being and emotional comfort. In this scenario, the use of ozone with its decontaminating power and bacterial load reduction, can play a decisive role in the application of the principles of selective caries removal (SCR). The aim of this study was to evaluate and compare the efficacy of ozonized gel (GeliO3) and gaseous ozone (healOzone X4) by analysing colony forming unit (CFU) microbial load of *Streptococcus mutans* (*S. mutans*) after caries removal, together with patient sensitivity and compliance. **Methods**: 16 patients aged 4–12 years were enrolled and randomly assigned to gel or gas treatment. Baseline dental and periodontal indices, such as Gingival Index (GI), Plaque Index (PI), International Caries Detection and Assessment System (ICDAS), and Basic Erosive Wear Examination, were assessed. After manual caries removal with an excavator, microbiological samples were collected with a sterile paper cone at T0 (before treatment), T1 (after ozone application for 30 seconds), and T2 (after an additional 30 seconds application). Schiff Air Index (SAI) and Face, Legs, Activity, Cry and Consolability (FLACC) were recorded at T0 and at T2 to evaluate patients' compliance and dental sensitivity of the clinical procedure. Data underwent statistical analysis (significance: $p < 0.05$). **Results**: Significantly lower microbial loads were found after treatment in both groups ($p < 0.05$) with no significant between-group differences ($p > 0.05$). SAI and FLACC showed no significant inter- and intragroup differences at any time point ($p > 0.05$). **Conclusions**: GeliO3 and healOzone X4 demonstrated comparable antimicrobial effect against *S. mutans*. The procedures had no emotional impact on paediatric patients. **Clinical Trial Registration**: clinicaltrials.gov (NCT06641323).

Keywords

Pediatric dentistry; *Streptococcus mutans*; Dental caries; Antimicrobial agents; Ozone; Ozonized gel

1. Introduction

Dental caries is one of the most common preventable chronic diseases worldwide, affecting approximately 50% of children. If not treated in time, it can affect not only chewing function, but also speech and psychosocial environment, reducing the quality of life of the child and family [1, 2]. Early childhood caries (ECC) is a manifestation of carious lesions in preschool children (under six years of age). It is used to define the presence of a primary tooth with one or more compromised surfaces (cavitated or non-cavitated), missing teeth or filled teeth due to caries. The presence of ECC has a negative impact on growth, development, nutrition, and oral health-related quality of life [3, 4].

Improper brushing technique, poor hygiene, frequent and prolonged consumption of sugary foods and beverages, socioeconomic status of the family, and lack of access to dental care create a favourable environment for the proliferation of cariogenic bacteria, which are the main risk factors for the development of carious lesions [5, 6].

Caries management has changed significantly over the last decade. New techniques aim to preserve healthy tissue as the goal of minimally invasive dentistry. To choose the correct treatment approach, it is important to understand the stage of the carious lesion. Non-cavitated carious lesions can be treated with sealants or infiltrating resins, whereas cavitated lesions can be treated with selective removal of dentin or complete removal and restoration of the cavity with restorative material [7]. In the 1980s, atraumatic restorative treatment (ART) was introduced for children living in economically underdeveloped areas [8].

Among the treatments proposed to halt caries progression and chemically or mechanically reduce biofilm formation, ozone appears to be a viable alternative. Ozone is a natural compound that acts as a powerful oxidant and is used for its antimicrobial properties, to promote healing processes and reduce inflammation [9]. Ozone is available in various formulations and is used in both medical and dental settings, mainly for accelerating wound healing, in the treatment of gingivitis and periodontitis, removal of plaque and biofilm, in post-operative pain management, for dentin hypersensitivity, tooth whitening, temporomandibular joint disorders, in root canal therapy, and in mandibular osteonecrosis. Thus, ozone has also emerged as an innovative solution for the treatment of caries and other oral affections [10–18].

Several studies have investigated the effect of ozone in the form of ozonized water [19], ozone gas [20], and ozonized oil [21] on *S. mutans*. These studies showed that ozone reduces plaque accumulation and has an antimicrobial effect on gram-positive and gram-negative bacteria, particularly inactivating *S. mutans*. However, two studies showed that ozone could not significantly reduce the number of microorganisms present on infected dentin [22] and had no effect on enamel remineralisation [23]. Dental visits, whether for pain or emergencies, along with associated therapeutic procedures, are often a source of anxiety in children. Conservative dentistry has moved in recent years towards more conservative and atraumatic approaches, trying to reduce the use of anaesthesia, rotary instruments, and drills. Some studies have evaluated the use of ozone as an adjunct to various therapeutic procedures. The use of ozone, regardless of the surgical protocol, has been shown to be a simple, painless technique with no adverse effects. These characteristics may help to increase comfort and improve compliance in children [24].

With these premises, the primary aim of this study was to compare the efficacy of a gaseous ozone device with an ozonized gel in reducing the bacterial load of *S. mutans* in carious lesions of primary molars in paediatric patients. The secondary objective of the study was to assess the dental and periodontal status of the patients and their compliance with treatment, using the International Caries Detection and Assessment System (ICDAS), Gingival Index (GI), Plaque Index (PI), Schiff Air Index (SAI), Basic Erosive Wear Examination (BEWE), and Face, Legs, Activity, Cry and Consolability (FLACC).

The first null hypothesis of this study was that there would be no significant difference in *S. mutans* bacterial load between the two groups before and after the treatments administered. The second null hypothesis was that there would be no significant differences in the secondary outcomes of FLACC and SAI between the two groups.

2. Materials and methods

2.1 Study design

The study was designed as a single-blind, randomised clinical trial with *in vivo* microbiological evaluation. All procedures in this study conformed to the Declaration of Helsinki (1964) and its subsequent amendments, and it was approved by the Unit Internal Review Board (2024-1009). The protocol has been registered on clinicaltrials.gov (NCT06641323).

2.2 Participants

The study started in November 2024 and ended in April 2025, enrolling 16 patients. Paediatric patients were recruited for the study from the Unit of Orthodontics and Paediatric Dentistry, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy, and screened according to the inclusion criteria: children aged 4–12 years, presence of at least one viable primary vital molar with a carious lesion requiring conservative treatment, and no previous treatment for the selected carious lesion. Exclusion criteria were: lack of cooperation during clinical procedures, non-viable primary molars or with traumatic lesions, and early loss of primary molars due to extensive carious lesions or extraction.

2.3 Interventions and outcomes

Patients were visited and parents or legal guardians were asked to sign the informed consent form before participation in the study. After initial sampling, patients were randomly assigned to two groups: Gel group, with GeliO3 ozonized gel (Bioemmei Srl, Vicenza, Italy) and Gas group, with healOzone X4 (dental ozone generator, CurOzone GmbH, Wiesbaden, HE, Germany) as fundamental part of the modified ART Technique to reduce the bacterial load for the treatment of dental caries in primary teeth [25]. A primary molar presenting caries requiring conservative restoration was chosen per each patient. The following indices were calculated before the beginning of the study: International Caries Detection and Assessment System (ICDAS) [26], Gingival Index (GI) [27], Plaque Index (PI) [28], Schiff Air Index (SAI) [29], Basic Erosive Wear Examination (BEWE) [30], and Face, Legs, Activity, Cry and Consolability (FLACC) [31].

After isolation with rubber dam, to avoid contamination with saliva and to ensure a dry and sterile working area, a microbrush (Microbrush Plus Superfine 467/6, Microbrush International, St. Louis, Missouri, USA) with sterile saline solution was used on the carious lesion to remove debris and obtain a clean operative field. Selective removal of carious dentin was performed using a minimally invasive approach with a manual excavator (SCR excavator Deppeler—Drop Ex-I EX09 + ADEP BC). A sterile paper cone number 50 (Dentsply Sirona—Headquarters: Charlotte, North Carolina, USA) was used to collect a microbiological sample to measure the initial bacterial load (T0). The cone was placed in contact with the carious lesion for 10 seconds to ensure accurate collection of material. The sample was immediately sealed in an Eppendorf container, previously bagged and sterilised (Fig. 1).

The products tested in the study are listed in Table 1.

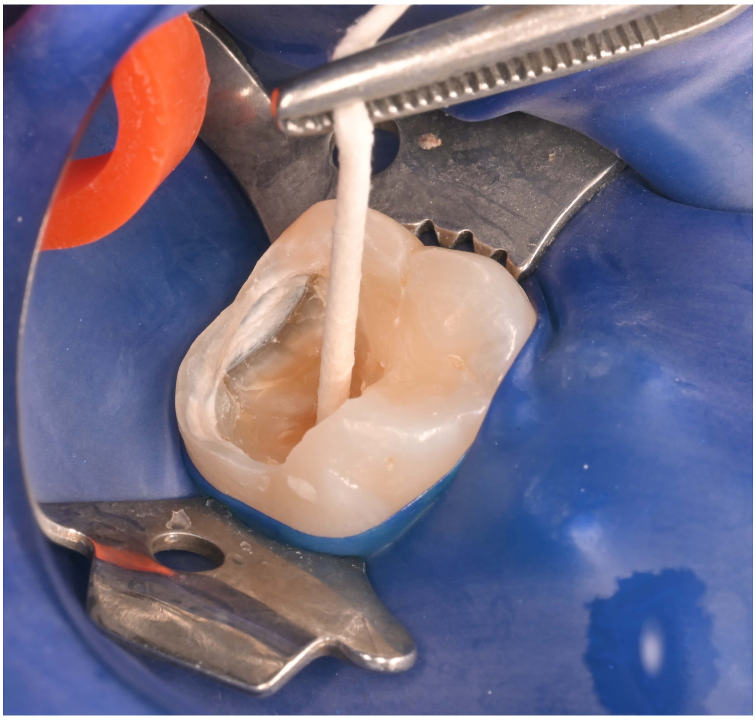

FIGURE 1. Collection of the microbiological sample from the carious lesion.

The first ozone application was performed in both groups. GeliO3 (using a microbrush) and healOzone X4 (using a new silicone cap of 3 mm diameter mounted on the handpiece of the machine for every patient) were applied directly to the carious lesion for 30 seconds (Fig. 2). After treatment, the area was rinsed with sterile saline solution for 10–15 s and dried with compressed air, allowing a second microbiological sample to be collected using another sterile paper cone (T1), which was then sealed and labelled as described above. The treatment procedure was then repeated for the next 30 seconds (total of 60 seconds), after which the surface was rinsed with sterile saline solution for 10–15 s and dried with compressed air. At the end of this second application, a third microbiological sample was taken (T2). According to previous literature, all samples collected during the three phases were stored in a freezer at −17 °C to maintain microbiological integrity [32, 33].

The carious cavity was restored using the modified ART technique [34]: after decontamination with ozone, etching was performed with 37% orthophosphoric acid (Ultra-Etch, Ultradent Products, Inc., 505 West Ultradent Drive, South Jordan, UT, USA) considering 30 seconds applications for enamel and 15 seconds for dentin; after washing and drying the surface, a universal adhesive (Scotchbond Universal, 3M Unitek, Monrovia, CA, USA) was applied and the tooth was restored with Activa (PULPDENT, 80 Oakland Street, Watertown, MA 02472, USA) bioactive restorative material [35].

The samples were then sent to the laboratory for analysis. Using an ultrasonic cleaner (USC-TH, VWR International S.r.l., Milan, Italy), the samples were sonicated in 100 microL of sterile phosphate-buffered saline (PBS) for 1 min at 40 Hz, then vortexed for a few seconds and diluted by serial 1:10 dilutions in Mitis Salivarius supplemented with bacitracin (MSB) bacterial culture medium. Each dilution was plated on an MSB-agar plate and incubated overnight at 37 °C under microaerophilic conditions, with reduced oxygen levels and 5% CO_2 supplementation. On the following day, the colonies were counted and the number of bacteria was expressed as colony forming units (CFU) per mL [36] (Fig. 3). MSB-agar is a selective medium for *S. mutans* that inhibits the growth of most non-mutans streptococci and other oral bacteria. Colonies were identified based on their typical morphology, which included small, raised, rough colonies with a frosted-glass or granular appearance that adhered to the agar surface. These phenotypic features are consistent with those described in the literature [37, 38]. This approach is widely used in clinical and experimental studies for the presumptive identification of *S. mutans*.

The primary outcome of the study was the bacterial count of *S. mutans*, measured in CFU, after the use of ozone in gas (healOzone X4) or gel (GeliO3).

The secondary objectives of GI, PI, BEWE and ICDAS were calculated on all tooth elements, while SAI and FLACC were calculated only on the element selected for the study:

- The ICDAS was developed to provide clinicians with an evidence-based detection system for the standardized diagnosis of carious lesions [26].

- The GI was used to assess the condition of the gingiva and record qualitative changes [27].

- PI was used to score the thickness of plaque deposits on all surfaces of all teeth [28].

- SAI was used to quantify the pain in response to the evaporative stimulus [29].

- BEWE was adopted to assess the degree of tooth wear [30].

- The FLACC scale, first published in 1997, is a system used in children from 2 months to 7 years of age and was used to measure pain intensity by assessing five parameters (face, legs, activity, consolability and crying) [31].

The FLACC and SAI scores were measured before and

TABLE 1. Products tested in the study with their composition and mode of application.

Product	Manufacturer	Composition	Mode of application
GeliO3	Bioemmei Srl, Vicenza, Italy	Ozonized olea europaea fruit oil, silica dimethyl silylate, aroma, helianthus annuus seed oil, arnica montana lower extract.	It is commercially available in 1 mL disposable pouches. The ozonated gel is removed from the pouch and applied directly to the surface of the carious lesion using a microbrush.
healOzone X4	CurOzone GmbH, Hassen, Germany	A high-frequency electrical discharge converts oxygen into ozone.	The application cap is placed on the tooth to be treated; when the flowmeter registers the vacuum, the generator is activated, which converts the oxygen contained in the canister into ozone by means of an electrical discharge. A controlled amount of ozone is released at a concentration of 32 g/m^3, which is then reintroduced into a closed system and converted back into oxygen.

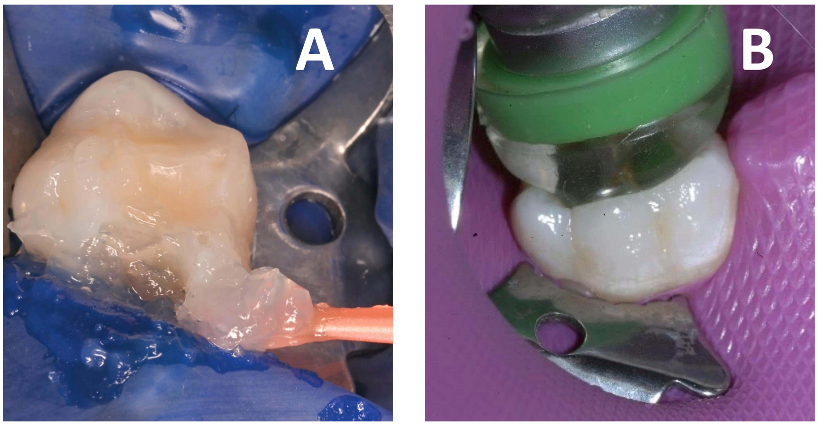

FIGURE 2. Ozone applications. (A) Application of ozonized gel (GeliO3), and (B) ozonized gas (healOzone).

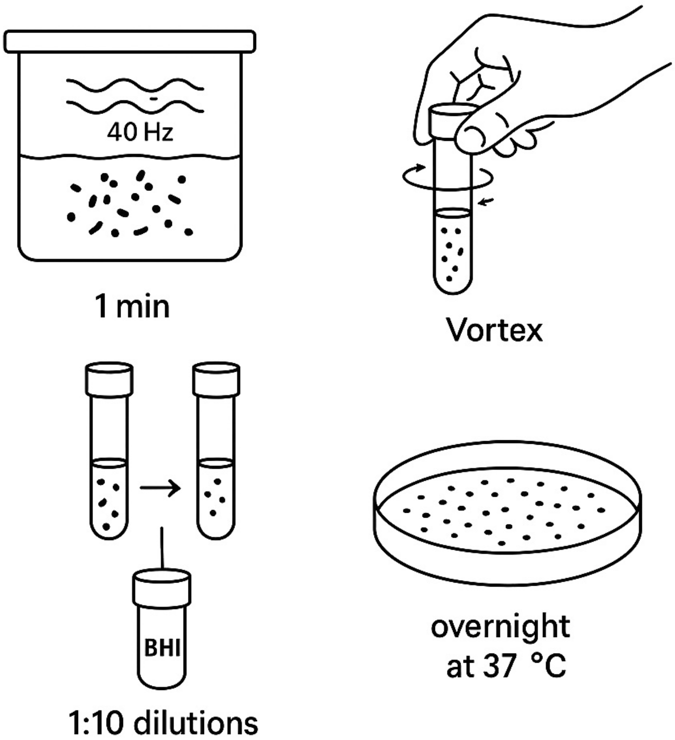

FIGURE 3. Graphical representation of microbiological analysis of the study samples.

after ozone treatment, particularly after the restoration was completed, to assess patient compliance during the procedure and to check for any increase in dentin sensitivity.

2.4 Sample size

The sample size was calculated based on the number of CFU of *S. mutans* detected in the caries samples. The ability to detect a clinically relevant difference of 46.43 CFU, with an expected mean of 68.70 and standard deviation of 32.24 [39], requires 8 patients per group (with 16 patients in total), with alpha = 0.05 and power = 80%.

2.5 Randomisation and blinding

Using a block randomisation table, the data analyst generated a randomisation sequence considering a permuted block of 16 patients. Study participants were allocated to the two different groups by an operator using opaque, sealed envelopes containing the pre-printed allocation sheets. Patients and operators were not blinded, but the data analyst was.

2.6 Statistical analysis

Data analysis was performed using R software (R version 3.1.3, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) by calculating descriptive statistics for each variable, including: mean, standard deviation, median, minimum, and maximum values measured for each group. For inferential statistics, the normality of the distributions was first assessed using the Kolmogorov-Smirnov test. The Kruskal-Wallis test followed by Dunn's *post-hoc* test was used for multiple comparisons between the two groups. Effect sizes were determined with Cohen's d. For all tests, the significance level was set at $p < 0.05$.

3. Results

3.1 Baseline data

The study enrolled 16 patients, starting in November 2024 to April 2025. All patients met the inclusion criteria and agreed to participate in the study, none were excluded from the analysis, and all received the assigned treatment. The sample characteristics are shown in Table 2.

The Consolidated Standards of Reporting Trials (CONSORT) flow chart of the study is shown in Fig. 4. The teeth included in the study were 16 vital primary molars with carious lesions requiring conservative restoration, 8 were treated with GeliO3 ozonized gel and the remaining 8 were treated with healOzone X4 ozonized gas.

The clinical data for the periodontal indices (GI and PI), the ICDAS data and the BEWE index were recorded only before ozone treatment in both groups. The data presented in the following tables are for all dental elements in the arch.

Table 3 shows the baseline clinical data of the oral conditions of the study sample. No significant differences were found in periodontal conditions ($p > 0.05$). There were also no significant differences between the two groups in the extent of carious lesions assessed by ICDAS and the degree of enamel wear assessed by BEWE ($p > 0.05$).

TABLE 2. Baseline data of the study sample.

Demographic characteristics	Age (yr)	
	Mean ± SD	Range
Study sample		
Male (n = 10)	7.50 ± 2.54	4–12
Female (n = 6)	6.16 ± 1.47	4–8
Group Gas		
Male (n = 8)	8.25 ± 2.25	5–12
Female (n = 0)	0.00 ± 0.00	0–0
Group Gel		
Male (n = 2)	4.50 ± 0.71	4–5
Female (n = 6)	6.50 ± 1.64	4–8

SD: Standard Deviation.

3.2 Microbiological analysis

Descriptive and inferential statistics are presented below. Concerning the bacterial loads in terms of CFUs presented in Table 4, a reduction in bacterial loads was shown in both groups, with a significant within-group difference between T0–T1 and T0–T2 values ($p < 0.05$). In terms of between-group differences, no significant differences were found for any of the time frames ($p > 0.05$). Microbiological data indicate a reduction in bacterial load following the application of ozone in both the forms.

The same considerations can be done for the percentage of decrease (%) of CFUs, which are shown in Table 5.

Fig. 5 provides a visual representation of bacterial loads and percentage reduction in the two groups.

3.3 Clinical outcomes

In addition to microbiological analysis, the study aimed to evaluate secondary indices. For the SAI and the FLACC scale, data were collected both before and after ozone treatment in both groups to assess whether there was a reduction in sensitivity in the patient. Table 6 shows data from the SAI, which shows that there was no reduction in pain in response to the evaporative stimulus between groups, and no significant differences between groups before and after treatment ($p >$ 0.05).

The same considerations can be made regarding the present data on the FLACC scale present in Table 7.

4. Discussion

This study aimed to verify the efficacy of ozone by comparing two formulations: ozonized gel (GeliO3) and ozonized gas (healOzone X4). The study focused on carious lesions of vital primary molars requiring conservative treatment to reduce the *S. mutans* microbial load, as this bacterium is the main cause of dental caries. Secondary indices were also analysed during the session using the FLACC scale, SAI, GI, PI, ICDAS and BEWE. The aim was to provide further evidence to support the use of ozone in clinical practice as a fundamental part of the modified ART technique, which is based on SCR, ozone,

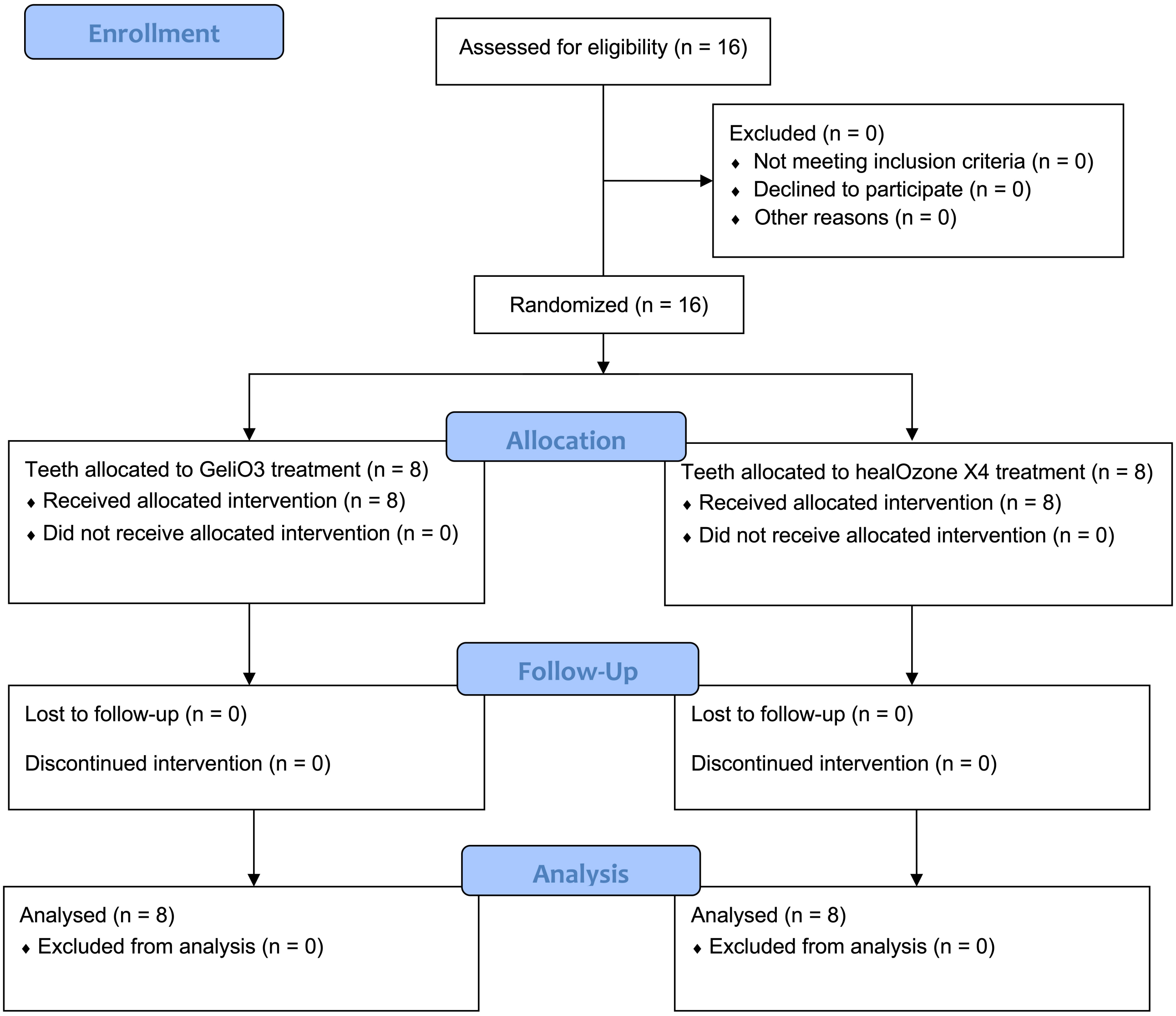

FIGURE 4. CONSORT flow chart of the study.

TABLE 3. Baseline oral assessment.

Group	Mean	SD	Min	Median	Max	Effect size	Significance*
GI							
Gas Before	0.76	0.52	0.00	1.00	2.00	-	A
Gel Before	0.72	0.61	0.00	1.00	2.00	0.08	A
PI							
Gas Before	0.73	0.44	0.00	1.00	1.00	-	A
Gel Before	1.37	0.91	0.00	1.00	3.00	−0.95	A
ICDAS							
Gas Before	3.00	2.64	0.00	5.00	6.00	-	A
Gel Before	1.51	1.90	0.00	1.00	6.00	0.66	A
BEWE							
Gas Before	0.77	0.99	0.00	0.00	3.00	-	A
Gel Before	0.00	0.00	0.00	0.00	0.00	1.56	A

**: means with same letter/s are not significantly different ($p > 0.05$).*
GI: gingival index; PI: plaque index; ICDAS: International Caries Detection and Assessment; BEWE: Basic Erosive Wear Examination; SD: standard deviation; Min: minimum; Max: maximum.

TABLE 4. Descriptive and inferential statistics for the bacterial loads of colony forming units.

Group	Time	Mean	SD	Min	Median	Max	Effect size	Significance*
Gas	T0	1971.63	2449.44	33.00	833.00	7040.00	-	A
Gas	T1	136.13	240.85	0.00	0.00	660.00	-	B,C
Gas	T2	12.38	35.00	0.00	0.00	99.00	2.16	C
Gel	T0	1391.50	2845.77	10.00	53.00	8228.00	-	A,B
Gel	T1	156.50	390.45	0.00	0.00	1120.00	-	C
Gel	T2	49.50	127.20	0.00	0.00	363.00	1.20	C

**: means with same letter/s are not significantly different ($p > 0.05$).*
SD: standard deviation; Min: minimum; Max: maximum.

TABLE 5. Descriptive and inferential statistics for the percentage decrease (%) of colony forming units.

Group	Time	Mean	SD	Min	Median	Max	Effect size	Significance*
Gas	T0	100.00	0.00	100.00	100.00	100.00	-	A
Gas	T1	8.92	20.43	0.00	0.00	58.82	-	B
Gas	T2	0.18	0.50	0.00	0.00	1.41	14.31	B
Gel	T0	100.00	0.00	100	100.00	100.00	-	A
Gel	T1	3.23	5.24	0.00	0.00	13.61	-	B
Gel	T2	0.76	1.59	0.00	0.00	4.41	43.59	B

**: means with same letters are not significantly different ($p > 0.05$).*
SD: standard deviation; Min: minimum; Max: maximum.

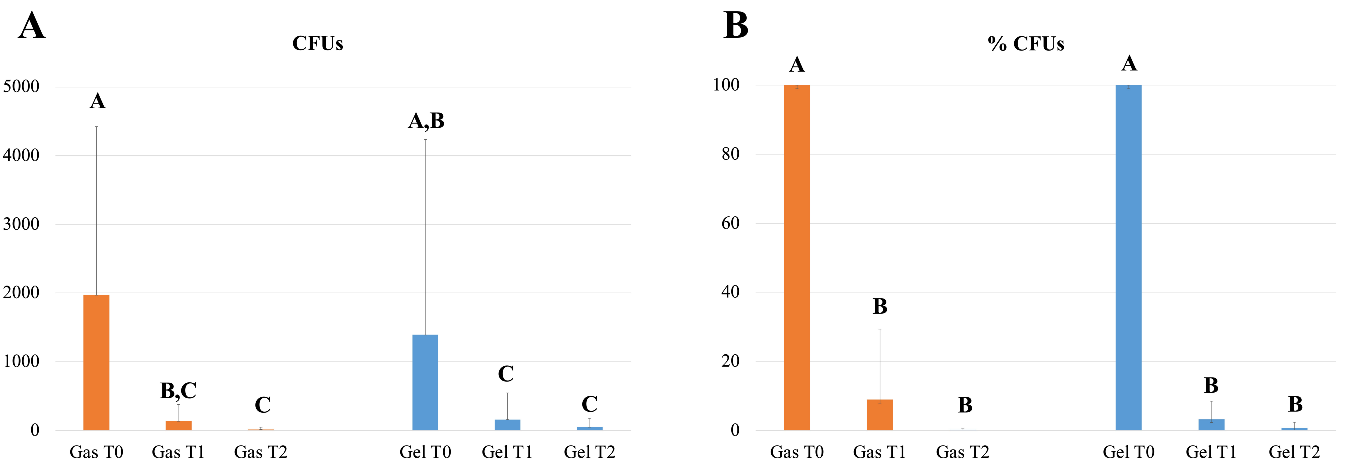

FIGURE 5. Results of the microbiological analysis. Graphical representation of: (A) bacterial loads (CFUs), and (B) their percentage (% CFUs). Means with same superscript letter are not significantly different ($p > 0.05$). CFUs: colony forming units.

TABLE 6. Descriptive and inferential statistics for Schiff Air Index (SAI).

Group	Time	Mean	SD	Min	Median	Max	Effect size	Significance*
Gas	T0	0.62	0.74	0.00	0.50	2.00	-	A
Gas	T2	0.62	0.74	0.00	0.50	2.00	0.00	A
Gel	T0	0.37	0.51	0.00	0.00	1.00	-	A
Gel	T2	0.37	0.51	0.00	0.00	1.00	0.00	A

**: means with same letter/s are not significantly different ($p > 0.05$).*
SD: standard deviation; Min: minimum; Max: maximum.

TABLE 7. Descriptive and inferential statistics for patient compliance (FLACC).

Group	Time	Mean	SD	Min	Median	Max	Effect size	Significance*
Gas	T0	0.25	0.46	0.00	0.00	1.00	-	A
Gas	T2	0.25	0.46	0.00	0.00	1.00	0.00	A
Gel	T0	0.00	0.00	0.00	0.00	0.00	-	A
Gel	T2	0.00	0.00	0.00	0.00	0.00	0.00	A

**: means with same letter/s are not significantly different ($p > 0.05$).*
SD: standard deviation; Min: minimum; Max: maximum.

and bioactive restorative materials for treating dental caries in primary teeth, or as an adjunct to traditional therapies [40–43]. This would allow the reduction or avoidance of the use of rotating instruments and anaesthesia, which would also benefit the comfort of young patients [44]. The microbiological analysis quantified the bacterial load in colony-forming units (CFUs) per mL. The data showed that using ozonized gel or ozonized gas inhibited *S. mutans*. A significant reduction in bacterial count was observed for both products after 30 seconds (from time T0 to T1), and a slight reduction after 60 seconds (from time T1 to T2). The first null hypothesis was partially accepted, as there were no significant differences in the reduction of the *S. mutans* bacterial load between the two groups at any time point.

The oxidising and remineralising properties of ozone have been extensively studied in medicine, particularly in dentistry. It is easy to use and non-invasive, and it can reduce overall treatment time [45]. Johansson *et al*. [46] demonstrated that a flow rate of 65 μmol/min of gaseous ozone, produced by the KaVo healOzone™ 2130C ozone generator (Curozone; KaVo Biberach/Riss, BW, Germany), could eliminate almost all microorganisms in a saline buffer suspension within 10 seconds, achieving a 99.9% reduction within 60 seconds. The presence of saliva was found to reduce the antibacterial effect, but this could be overcome by increasing the exposure time to ozone. Therefore, the rubber dam was used in the current study to isolate the surgical field and avoid potential contamination [46].

Unlike the previous study, the present one used a rubber dam to prevent saliva contamination. This meant that there was no need to increase the ozone concentration; the healOzone X4 generator releases a lower concentration of ozone (32 g/m^3) than the generator used in the previous study, yet the treatment time remained the same. Wang *et al*. [47] reported that ozone concentration and treatment time could influence the inhibitory effect on *S. mutans*. In contrast, Sancakli *et al*. [48] stated that 80 seconds is not sufficient to inhibit *S. mutans*, although the article does not specify the ozone concentration used. Thanks to the data obtained from this study, it can be seen that increasing the amount of ozone used or the length of treatment is unnecessary, as there is still a significant reduction in microbial load.

Kapdan *et al*. [49] compared the efficacy of 2% chlorhexidine and gaseous ozone on *S. mutans* in *in vitro* tests, increasing the exposure time to 80 seconds, which is the longest time recommended [50]. The data showed that chlorhexidine was the gold standard for reducing microbial load, while ozone still reduced *S. mutans*, but to a lesser extent. Another study compared the effectiveness of a chlorhexidine gel and an ozone-containing gel. Both gels were found to reduce bacterial load, but there was no difference between the results obtained using the two gels [51]. Unlike the studies mentioned above, this study did not use a chlorhexidine-treated control group; it only compared two ozone-containing products.

The results of the current report are consistent with previous studies regarding the ability of ozone to inhibit Gram-positive bacteria. The study conducted by Beretta and Federici Canova showed that applying gaseous ozone using the healOzone X4 ozone generator with a disposable silicone cup for 60 seconds to deep carious lesions of primary teeth was successful in 93% of cases, thus avoiding the need for endodontic intervention and pulp exposure and contamination [10]. Meanwhile, Nardi *et al*. [21] demonstrated that ozonized olive oil exhibits antimicrobial activity. Microbiological data from our study showed that both gas and gel formulations were effective in reducing the microbial load, thus confirming previous findings [21].

Interestingly, the ozone gas is released from the generator once the disposable silicone cup has taken over the vacuum in order to prevent dispersion [52], whereas the gel is taken from the disposable sachet and applied directly to the carious lesion.

Some studies in the literature highlight the possible resistance of certain microorganisms to ozone, which contradicts previous reports. In a 2012 study, the healOzone device was used on cavities containing *S. mutans* or *Lactobacillus casei* for 60 seconds. After four and eight weeks, there was a significant reduction in *S. mutans*, but not complete removal. Meanwhile, the concentration of *L. casei* remained unaffected, which could indicate a possible resistance [20]. A review of the effects of ozone shows that it has broad antibacterial activity against both Gram-positive and Gram-negative microorganisms. However, the *in vitro* results are controversial, as some strains remain unaffected by ozone concentrations [45]. The data obtained from this study show a significant reduction in *S. mutans*, but not complete removal. This could be due to the timing of the treatment or to the microorganisms' possible resistance to ozone.

Dental treatments require adequate cooperation from paediatric patients. In general, it is advisable to assess the patient profile to determine whether the patient is afraid of the dentist [53], has special needs [54], or is cooperative. Dental visits can cause anxiety and worry, especially in children, who may be negatively influenced by previous dental experiences or parental/family influences [55]. To achieve effective treatment, clinicians must use targeted approaches and preoperative communication to establish trusting relationships with children and their parents/carers [56]. In recent years, research has focused on non-invasive therapies with the aim of eliminating the need for anaesthesia and rotating instruments. This also results in longer treatment times, making the session more stressful for the child [57]. Banerjee *et al.*'s [58] publication listed the main innovative techniques, including partial or gradual removal of the carious lesion and the use of lasers, air abrasion, sound abrasion, and chemomechanical removal of carious dentin. Several studies have demonstrated the effectiveness and minimally invasive nature of ART in paediatric patients. It enables carious lesions to be treated without the use of anaesthesia, since only hand instruments are employed, thus avoiding the use of rotating instruments [59, 60]. Ozone could be a valuable aid, as it can reduce the microbial load within the carious lesion further, thereby promoting the longevity of the restoration. Due to its antibacterial properties, ozone can contribute to improving the child's compliance and overall comfort when used as a fundamental part of the modified ART Technique or as an adjunct to traditional techniques.

Ozone is an unstable compound that tends to revert to its original form, oxygen. This leads to an interaction with composite resins, as ozone is the main factor inhibiting their polymerisation. Ozone also causes dehydration of the dentin itself [61]. Whether ozone affects the adhesion of dentin and the bond strength of composite resins is a topic of much controversy, with conflicting results emerging from various studies. For this reason, it was not considered in this study.

The study was carried out with a single-blind design, meaning that only the statistician was unaware of which treatment had been administered to which patient. The study was not double-blinded because these are two different procedures and it is not possible to blind both the operator and the patient. Consequently, the risk of bias could not be reduced.

The clinical dental and periodontal parameters GI, PI, ICDAS, and BEWE were measured only at T0, before treatment, and were recorded on all dental elements. With regard to the periodontal indices, plaque and mild generalised gingival inflammation were present in both groups. The presence of these indices may be related to the fact that children in general do not have optimal oral hygiene due to a high sugar diet or poor oral hygiene habits regarding flossing and brushing. The degree of carious lesion extension (ICDAS) and enamel erosion (BEWE) showed no significant differences between the two groups. The data showed that there were no differences between the groups for these parameters, indicating that the patients were correctly randomised. FLACC and SAI were measured before and after ozone treatment, with data recorded only for the element treated. Statistical analysis showed that there were no significant differences in the two groups treated with ozonized gel or gas in the interval from time T0 (before treatment) to time T1 (after treatment), so the second null hypothesis was accepted.

In terms of practicality and cost, it should be noted that ozonized gel used in the present study is commercially available in single-dose sachets. This facilitates accurate dosing of the product and helps to maintain adequate hygiene during use. Ozonized gas is produced by a generator that can be of various sizes and is applied to the carious lesion using a disposable silicone cup that can create a vacuum. It is important that the generator has adequate control systems to prevent the release of ozone into the environment. In fact, the generator does not produce ozone as soon as the cup is removed; otherwise, there is a risk of inhalation for both the patient and the operator, since ozone is a respiratory irritant, even at low concentrations, and prolonged exposure can cause lung and mucous membrane damage [62].

Despite the statistical significances obtained, the effect size analysis was high not for all statistically significant data, but only for those shown in Tables 4 and 5.

In conclusion, GeliO3 in its professional specific formula could be a really interesting, effective, and practical alternative to healOzone X4 device, and it can also be used to support traditional techniques for treating carious lesions in viable deciduous molars.

This study presents some limitations. Firstly, microbiological samples were collected from the patient and analysed in a laboratory setting, analysis was only performed in relation to *S. mutans*, this is a limitation of the study. All samples were frozen at −17 °C immediately after collection and stored under the same conditions. This protocol was chosen to limit bacterial proliferation and preserve microbial composition. Freezing may potentially affect bacterial viability with a slight reduction in absolute CFU; however, the consistent recovery of colonies from all samples confirms that viable bacteria were retained. Previous studies demonstrated post-freezing recovery of *Streptococcus species* [32, 33], while Phattarataratip *et al.* [63] successfully isolated *S. mutans* from dental plaque samples that were frozen before analysis, following a procedure very similar to the present study. The study compares the efficacy of ozonated gel and ozone gas, so a limitation is the lack of a control group of patients who were not treated with ozone, so we cannot demonstrate that ozone represents an advantage over traditional techniques. Another limitation of this study is that the extent of the carious lesion was not considered; patients with carious lesions extending to the dentin may be less likely to comply with treatment without the use of anaesthesia. Future studies could use a classification of carious lesions to select control and study groups. Evaluating these parameters using larger samples would be interesting. Further studies should be conducted to evaluate the efficacy of the products through regular professional applications to assess the progression of the carious lesion over time, maybe in the No restorative cavity control technique, and the degree of pulp involvement, whether it remains vital or undergoes a process of necrosis in the use associated to the ART technique [64] or traditional technique. In addition, objective measures of compliance and sensitivity should be introduced in place of SAI and FLACC to provide an objective assessment of the degree of pain reduction after ozone application. In the future, it would be interesting to test the efficacy of these ozone-containing products not only against *S. mutans* but also against other bacterial strains, and to verify the effects of ozone on dentin and its interaction with the bond strength of composite resins for the subsequent restoration of the treated tooth.

5. Conclusions

The study showed that the GeliO3 ozonized gel and the healOzone X4 gaseous ozone device were effective in reducing the *S. mutans* microbial load at the level of the carious lesion. No significant differences were found in sensitivity and cooperation parameters assessed with SAI and FLACC; therefore, it seemed that the procedures had no emotional impact on paediatric patients.

ABBREVIATIONS

BEWE, Basic Erosive Wear Examination; FLACC, Face, Legs, Activity, Cry and Consolability; GI, Gingival Index; ICDAS, International Caries Detection and Assessment System; PI, Plaque Index; SAI, Schiff Air Index; ART, atraumatic restorative treatment; SCR, selective caries removal; CFU, colony forming unit; ECC, early childhood caries; PBS, phosphate-buffered saline; MSB, Mitis Salivarius supplemented with bacitracin; CONSORT, CONsolidated Standard Of Reporting Trials; SD, standard deviation; Min, minimum; Mdn, median; Max, maximum; *L. casei*, *Lactobacillus casei*; *Streptococcus spp.*, *Streptococcus species*; *S. mutans*, *Streptococcus mutans*.

AVAILABILITY OF DATA AND MATERIALS

Data are available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

MC and AS—designed the research study. NV—performed the research. MB and FFC—provided help and advice on technical procedures and study protocol. GP and CM—conducted the microbiological analysis. NV and MP—wrote the manuscript. AS—performed data analysis. AS, NV and MP—revised the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Unit Internal Review Board (approval number: 2024-1009). Written informed consent from parents/legal guardians of the patients involved was previously collected.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Andrea Scribante and Maurizio Pascadopoli serve as Editorial Board members of this journal. We declare that Andrea Scribante and Maurizio Pascadopoli had no involvement in the peer review of this article and have no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to FSS.

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