


Article

Bleaching Effect of Ozonized Substances on Resin Composite: A New Potentiality for Ozone Therapy in Dentistry

Simone Gallo ^{1,*}, Marco Colombo ^{2,*}, Claudio Poggio ², Andrea Scribante ^{1,3,*}, Matilda Saracino ³
and Riccardo Beltrami ²

- ¹ Unit of Orthodontics and Paediatric Dentistry, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, 27100 Pavia, Italy
- ² Unit of Restorative Dentistry, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, 27100 Pavia, Italy
- ³ Unit of Dental Hygiene, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences, University of Pavia, 27100 Pavia, Italy
- * Correspondence: simone.gallo02@universitadipavia.it (S.G.); marco.colombo@unipv.it (M.C.); andrea.scribante@unipv.it (A.S.)

Abstract: Composite resins are commonly used in dentistry for direct restorations. The color stability of these materials still represents a major concern for both the clinician and the patient. In recent years, ozone therapy has been extensively used in dentistry to manage wounds healing, dental caries, oral lichen planus, gingivitis and periodontitis, halitosis, osteonecrosis of the jaw, post-surgical pain, plaque and biofilms, root canal treatment, dentin hypersensitivity, temporomandibular joint disorders, and teeth whitening. To date, several studies have evaluated the bleaching effect exerted by ozone on natural teeth, but no studies have been conducted to determine the effect on the color of composite resins. The aim of the present study is to determine whether ozonized oils/gels could determine a color change on composite resin. A total of 40 discs of an A3 shade restorative composite were divided into two groups, respectively exposed to a pigmentation treatment consisting of 10 applications of 10 min each of a 1% chlorhexidine-based gel (trial group) and to storage into physiological solution (control group). The samples of both groups were respectively subdivided into four subgroups which underwent four different protocols, three of which were based on the exposure to different ozonized products and the latter representing the control. A colorimetric analysis with the CIELAB method was conducted with the following timing: after 24 h of storage in physiological solution (before the experimental procedures) (T0), after the subdivision into groups A and B (corresponding to the pigmentation for samples of group A and storage in physiological solution for samples of group B) (T1), and after subdivision into subgroups 1–4 (corresponding to the application of the ozonized products vs. control) (T2). No statistically significant difference was found between the samples at T0. The 20 samples of group A, exposed to the colorant agent, underwent a color change from T0 to T1, whereas the 20 samples exposed to the physiologic solution did not undergo any significant color change. A positive but moderate influence was assessed for E and L values, whereas no significant change occurred for A and B values. Therefore, dental ozonized oils/gels could be valuable in restorative dentistry as bleaching agents of resin composites exposed to discoloration, an alternative to the traditional hydrogen peroxide and carbamide peroxide, but further studies are required to confirm these findings.

Keywords: ozone; ozone therapy; resin composite; composite materials; bleaching; chlorhexidine; restoration; dentistry



Citation: Gallo, S.; Colombo, M.; Poggio, C.; Scribante, A.; Saracino, M.; Beltrami, R. Bleaching Effect of Ozonized Substances on Resin Composite: A New Potentiality for Ozone Therapy in Dentistry. *Appl. Sci.* **2023**, *13*, 2149. <https://doi.org/10.3390/app13042149>

Academic Editor: Bruno Chrcanovic

Received: 20 November 2022

Revised: 1 February 2023

Accepted: 6 February 2023

Published: 7 February 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Composite resins are extensively used in dentistry for both posterior and anterior restorations. Despite being more aesthetic with respect to previous materials, resin composite restorations still suffer from several shortcomings [1,2]. In addition to polymerization

shrinkage and secondary caries, plaque accumulation and color stability are some of the most relevant drawbacks [3]. Discoloration of composite resins can be caused by intrinsic or extrinsic factors. In light-curing composite resins, camphorquinone is generally used as the photoinitiator. However, if curing is inadequate, unconverted camphorquinone could cause a yellowish discoloration. Moreover, additional components of the photoinitiator system, e.g., tertiary aromatic or aliphatic amines acting as synergists/accelerators, also tend to cause yellow or brown discoloration under the influence of light or heat [4]. These internal discolorations are permanent and depend on intrinsic factors such as polymer quality, filler type, and quantity, as well as the synergist added to the photoinitiator system [5–7]. In addition to that, the discoloration can derive from a superficial degradation or a slight penetration and adsorption of staining agents at the superficial layer of composite resins [8]. The resin's affinity for extrinsic stains is influenced by its conversion rate and physico-chemical characteristics, with the water sorption rate being of particular importance [9,10]. Additional important factors affecting stainability are surface roughness, surface integrity, and polishing technique. Previous studies concerning color stability have shown that drinks (such as coffee, tea, red wine, and cola) and mouth rinses have different degrees of staining effect on auto- and light-cured composite resin restorative materials; the staining potential of these substances differs on the basis of their composition and properties [11–13].

In recent years, ozone therapy has been extensively used in dentistry to manage wounds healing, dental caries, oral lichen planus, gingivitis and periodontitis, halitosis, osteonecrosis of the jaw, post-surgical pain, plaque and biofilms, root canal treatment, dentin hypersensitivity, temporomandibular joint disorders, and teeth whitening [14,15].

Different *in vitro* studies have demonstrated an excellent anti-bacterial action for ozonized products against different microorganisms, such as bacteria, viruses, protozoa, and fungi [16,17]. Moreover, ozone has shown an immunomodulatory, anti-hypoxic, biosynthetic, and anti-inflammatory action [18]. One of the most relevant applications of ozone in dentistry is represented by its use for the treatment of periodontal conditions, i.e., gingivitis and periodontitis. Scaling and root planning (SRP) is regarded as the gold standard non-surgical treatment, whose goal is to remove dental plaque and calculus and also to smooth the root surfaces infected by pathogenic microorganisms [19]. Besides this conventional mechanical therapy, local chemical agents can be used as antimicrobial substances, and these should be favored to systemic treatments such as antibiotics which are characterized by a systemic effect besides the risk of emergence of resistance. In light of these considerations, the application of ozone as an antimicrobial agent has been demonstrated to be very useful. Broad-spectrum antiseptics such as chlorhexidine have been generally considered, but some shortcomings are associated with this latter substance, such as tooth discoloration. Conversely, ozone guarantees a broad antimicrobial spectrum as well but with lower toxicity. It has been shown *in vitro* that the exposition of periodontopathogens to ozone causes the oxidation of the phospholipids and lipoproteins of the bacterial cell envelope, causing the destruction of the cell integrity, thus allowing ozone to infiltrate the microorganisms and oxidizing glycoproteins and glycolipids, with a final block of the enzymatic functions of bacterial metabolism [19].

Despite this wide research related to the use of ozonized products in periodontology and dental hygiene, to the best of our knowledge, no evaluations have been conducted until now to evaluate the efficacy of ozone as a bleaching agent towards pigmented resin composites. Based on these considerations, the purpose of the current study is to determine whether ozonized oils/gels could be valuable agents to determine a color change in composite resin following their exogenous pigmentation.

2. Materials and Methods

2.1. Sample Preparation

Sample size calculation (Alpha = 0.05; Power = 95%) for two independent study groups and a continuous primary endpoint was performed using a sample size calcula-

ture ([ClinCalc.com](https://clincalc.com/stats/samplesize.aspx); <https://clincalc.com/stats/samplesize.aspx>, accessed on 15 September 2022). Concerning the primary outcome (delta E), an expected mean of 1.28 was hypothesized, with a standard deviation of 0.06, and the expected difference between the means was supposed to be 0.14. These values were obtained by previous published literature evaluating the same outcomes of the present research and where teeth had been submitted to analog experimental procedures but with different bleaching agents [20]. A total of 5 specimens were requested for each group.

Forty discs of composite (size: 6 mm diameter, 2 mm height) were made after photopolymerization of an A3 shade restorative composite (G-aenial, GC Italia, Vimodrone, Milan, Italy). The samples were set by inserting the composite into a stainless-steel mold (external Ø 8 mm, internal Ø 6 mm, height 4 mm) put on a dark opaque paper background, with a polyester matrix strip (Mylar strip, Henry Schein, Melville, NY, USA) interposed. This arrangement was chosen to obtain a smooth surface under the composite but also to avoid light reflection from the bottom, thus minimizing the artificial hardening of this area [21]. Every mold was lightly overfilled, and another polyester matrix strip was placed on the top to avoid the interference of oxygen with the polymerization of the outset layer of the resin [22]; with the aim of extruding the excess composite and obtaining a flat surface, a glass slide was pressed against the upper polyester film, and removed before photopolymerization [23]. This latter was conducted for every sample with the LED unit Celalux 2 (Voco, Cuxhaven, Germany), whose cordless curing unit was maintained at full charge before every use, and its correct irradiance was confirmed with a radiometer (SDS Kerr, Orange, CA, USA). The distal end of the light guide was positioned perpendicular to the surface of the matrix strip, concentrically to the mold, before starting the photopolymerization of the samples, which was conducted on their only external (top) side [24]. The only standard light polymerization mode was used, consisting of an output irradiance of 1000 mW/cm² for 40 s.

Fine and superfine discs (Sof-Lex Pop On; 3M ESPE, St. Paul, MN, USA) were used in order to finish the composite surfaces. After finishing, samples were measured in order to confirm their original size; in case of the reduction of the original height (2 mm), samples were excluded. Finally, the samples were kept for 48 h in complete darkness, at 37 °C and 100% humidity (physiological solution). This amount of time was considered since polymerization goes on at a slow rate, even following the moment of exposure to the curing light [25].

2.2. Experimental Procedure

The 40 samples were divided into the two following groups:

- Group A: 20 samples underwent a pigmentation treatment consisting of 10 applications of 10 min each of a 1% chlorhexidine-based gel (Corsodyl, GSK, Brentford, UK), known for its pigmental effect;
- Group B: 20 samples were left for further 24 h in physiological solution

The samples of the groups were respectively subdivided into 4 subgroups of 5 samples each and underwent different treatments according to the following protocol:

- Group A1 and B1: two applications of 60 min each of ozonized gel (Gelio3, Bioemmei Srl, 36100 Vicenza, Italy) containing a bio-ozonized olive oil (20 mEq O₂/kg), hydrated silica, and arnica;
- Group A2 and B2: two applications of 60 min each of ozonized oil (Gelio3, Bioemmei Srl, 36100 Vicenza, Italy) containing a bio-ozonized olive oil (20 mEq O₂/kg);
- Group A3 and B3: two applications of 60 min each of ozonized oil (Gelio3, Bioemmei Srl, 36100 Vicenza, Italy) containing a bio-ozonized olive oil (20 mEq O₂/kg) and regenerative substances;
- Group A4 and B4 (control groups): storage in physiological solution.

At the end of the procedure, samples were rinsed with physiological solution, gently air-dried, and stored in distilled water at 37 °C.

2.3. Colorimetric Analysis

Samples were analyzed in three different moments: after 24 h of storage in physiological solution (before the experimental procedures) (T0), after the subdivision into groups A and B (corresponding to the pigmentation for samples of group A and storage in physiological solution for samples of group B) (T1), and after subdivision into subgroups 1–4 (corresponding to the application of the ozonized products vs. control) (T2).

The colorimetric analysis has been performed using the CIELAB method [26]. This system is related to human color perception in all 3 dimensions or directions of the color space. The formula used is the following:

$$DE^*_{ab} = [(DL^*)^2 + (Da^*)^2 + (Db^*)^2]^{1/2} \tag{1}$$

E* represents color variation; L* is the lightness variable, proportional to Munsell’s value; a* and b* are chromaticity coordinates. The L*a*b* space consists of a lightness L* coordinate, coordinate a*, indicating where the color falls along the red/purple—green/blue axis, and coordinate b*, indicating where the color falls along the blue/purple-yellow axis.

The colorimetric analysis has been performed using a spectrophotometer for dental use (Vita Easyshade® V, Vita Zahnfabrik, Germany) which digitally provided the parameters mentioned above. In order to standardize the parameter’s analysis and to reduce the influence of external light, the tip of the spectrophotometer has been placed in contact with the samples with an incident angle of 90°. The experiment was conducted in a shaded room with neither artificial light nor external light at direct impact.

2.4. Statistical Analysis

For each variable, descriptive statistics were calculated (mean, median, maximum, and minimum). Kolmogorov Smirnov test was applied to assess the normality of the data. Friedman test and Dunn test were subsequently applied to calculate the inferential statistics, considering a p-value of 0.05. The software R (Core Team, R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analysis.

3. Results

Data relative to values E, L, A, and B for the two groups and the four subgroups are shown in Tables 1–5.

Table 1. Median (min, max) for the values E, L, A, and B for the two groups at each experimental time.

Group		E	L	A	B
Group A—Corsodyl	T0	4.61 (3.7; 5.8)	−4.08 (−5.6; 4)	−0.73 (−1.8; −0.6)	−1.04 (−2.5; −0.1)
	T1	5.99 (5.2; 6.9)	−5.93 (−6.9; −5.2)	−2.12 (−3.8; −1.2)	0.61 (−1.6; 2)
	T2	4.88 (3.8; 5.8)	−4.77 (−5.8; −3.7)	−0.39 (−1.9; 2)	−1.23 (−2.5; 0)
Group B—Control	T0	4.25 (3.6; 5.1)	−4.21 (−4.9; −3.6)	0.31 (−1.4; 1.1)	−0.85 (−1.8; 1.3)
	T1	4.61 (3.8; 5.1)	−4.61 (−5.1; −3.8)	−0.17 (−1.3; 1.3)	−0.62 (−1.4; 0.5)
	T2	5.27 (4.8; 6.1)	−5.27 (−6.1; −4.8)	0.39 (−0.5; 1.3)	−0.04 (−1.5; 1.3)

According to the data assessed, no statistically significant difference was found between the 40 samples at T0. The 20 samples of group A, exposed to the colorant agent, underwent a color change from T0 to T1, whereas the 20 samples exposed to the physiologic solution did not undergo any significant color change.

As regards the E value, a significant increase was found at T1 in the control group, in the ozonized gel group, in the ozonized oil group, as well as at any time in the group submitted to the ozonized oil with regenerative substances.

As regards the L value, no significant changes were found at any time between the subgroup, except for the ozonized gel group at T0.

Table 2. Median (min, max) for the value E for the two groups at each experimental time.

	Mean	SD	Min	Mdn	Max	Significance *
Control T0	4.56	0.36	4.20	4.50	5.10	A
Control T1	6.08	0.22	5.80	6.00	6.30	B
Control T2	5.48	0.41	4.80	5.70	5.80	A
Ozonized Gel T0	4.00	0.37	3.70	4.00	4.60	A
Ozonized Gel T1	5.82	0.49	5.20	5.80	6.40	B
Ozonized Gel T2	4.48	0.47	3.80	4.70	4.90	A
Ozonized Oil T0	4.86	0.65	3.80	4.90	5.40	A
Ozonized Oil T1	6.24	0.42	5.90	6.10	6.90	B
Ozonized Oil T2	4.94	0.57	4.40	4.90	5.70	A
Ozonized Oil + Regen T1	5.02	0.51	4.40	4.90	5.80	B
Ozonized Oil + Regen T2	5.82	0.37	5.20	5.90	6.10	B
Ozonized Oil + Regen T3	4.62	0.41	4.00	4.90	4.90	B

* Different letters show a statistically significant difference between the groups ($p < 0.05$).

Table 3. Median (min, max) for the value L for the two groups at each experimental time.

	Mean	SD	Min	Mdn	Max	Significance *
Control T0	-4.48	0.40	-5.10	-4.40	-4.10	A
Control T1	-5.92	0.22	-6.20	-5.80	-5.70	A
Control T2	-5.44	0.40	-5.80	-5.60	-4.80	A
Ozonized Gel T0	-2.36	3.57	-4.40	-3.70	4.00	B
Ozonized Gel T1	-5.72	0.44	-6.40	-5.70	-5.20	A
Ozonized Gel T2	-4.32	0.48	-4.80	-4.20	-3.70	A
Ozonized Oil T0	-4.68	0.56	-5.20	-4.80	-3.80	A
Ozonized Oil T1	-6.22	0.43	-6.90	-6.00	-5.90	A
Ozonized Oil T2	-4.78	0.48	-5.50	-4.80	-4.30	A
Ozonized Oil + Regen T1	-4.82	0.48	-5.60	-4.80	-4.30	A
Ozonized Oil + Regen T2	-5.88	0.37	-6.20	-5.90	-5.30	A
Ozonized Oil + Regen T3	-3.02	3.82	-5.00	-4.80	3.80	A

* Different letters show a statistically significant difference between the groups ($p < 0.05$).

Table 4. Median (min, max) for the value A for the two groups at each experimental time.

	Mean	SD	Min	Mdn	Max	Significance *
Control T0	-0.32	0.11	-0.40	-0.40	-0.20	A
Control T1	-2.38	1.07	-3.80	-2.00	-1.30	A
Control T2	-0.34	1.40	-1.60	-0.80	2.00	A
Ozonized Gel T0	-0.18	0.73	-1.00	-0.20	0.60	A
Ozonized Gel T1	-2.08	0.40	-2.60	-2.10	-1.60	A
Ozonized Gel T2	-0.16	1.13	-1.90	0.40	0.70	A
Ozonized Oil T0	-0.96	0.71	-1.70	-1.30	-0.20	A
Ozonized Oil T1	-1.96	0.61	-2.80	-1.90	-1.30	A
Ozonized Oil T2	-0.74	0.89	-1.80	-0.80	0.60	A
Ozonized Oil + Regen T1	-1.48	0.43	-1.80	-1.70	-0.80	A
Ozonized Oil + Regen T2	-2.06	0.59	-2.60	-2.10	-1.20	A
Ozonized Oil + Regen T3	-0.34	0.95	-1.60	0.10	0.50	A

* Different letters show a statistically significant difference between the groups ($p < 0.05$).

Table 5. Median (min, max) for the value B for the two groups at each experimental time.

	Mean	SD	Min	Mdn	Max	Significance *
Control T0	−0.54	0.28	−0.80	−0.60	−0.20	A
Control T1	0.74	0.88	−0.40	0.50	2.00	A
Control T2	−0.92	0.82	−2.10	−1.00	0.00	A
Ozonized Gel T0	−0.70	0.54	−1.40	−0.60	−0.10	A
Ozonized Gel T1	0.40	1.26	−1.60	1.00	1.60	A
Ozonized Gel T2	−1.34	0.35	−1.70	−1.50	−0.80	A
Ozonized Oil T0	−1.42	0.75	−2.50	−1.60	−0.70	A
Ozonized Oil T1	0.72	0.63	0.00	0.70	1.40	A
Ozonized Oil T2	−1.42	0.79	−2.50	−1.30	−0.50	A
Ozonized Oil + Regen T1	−1.50	0.57	−1.80	−1.80	−0.50	A
Ozonized Oil + Regen T2	0.58	0.65	−0.10	0.30	1.50	A
Ozonized Oil + Regen T3	−1.24	0.48	−1.90	−1.00	−0.80	A

* Different letters show a statistically significant difference between the groups ($p < 0.05$).

No significant changes were found at any time in any subgroup for A and B values.

4. Discussion

The measurement of the color represents a fundamental step in dentistry with the aim of guaranteeing the major similarity between natural teeth and prosthetic or restorative materials. This aspect is more and more expected by patients, especially in aesthetic areas of the mouth. In addition to a visual evaluation, color assessment in dentistry can be realized instrumentally with the aid of spectrophotometers and colorimeters [27]. Instrumental colorimetry has the advantage of avoiding errors in color assessment by the operator, and additionally, it is more precise than the naked eye in finding out slight differences in colored objects on flat surfaces [28]. Colorimeters measure the quantity of light reflected by selected colors (like red, green, and blue), and color measurement is generally expressed using the CIELAB color system, developed by the International Commission on Illumination Colorimetry [29]. According to this method, the color difference value, ΔE , is expressed as the relative color change between repeated color measurements.

The chemical structure of a resin composite and the properties of its particles have a direct influence on the surface texture and the susceptibility to extrinsic staining. Besides material composition, the finishing and polishing can also affect the composite surface quality and are consequently related to the early discoloration of resin composites [8].

As regards tooth bleaching, this is a common clinical procedure that is more and more required by patients for aesthetic purposes. Tooth whitening can be realized both by clinicians in a dental setting as well as by the patients themselves at home. The most common chemical substances used for teeth whitening are hydron peroxide (HP) and carbamide peroxide, whose action is exerted thanks to their oxidizing power on exogenous or endogenous pigments responsible for external and internal tooth discoloration, respectively. Professional bleaching, also called “in-office” bleaching, is realized using a 35 wt% HP solution in water for about 20–30 min. Considering its highly oxidizing power, particular attention should be focused on avoiding the contact of HP with soft tissues, thus requiring the protection of these latter with a liquid dam. Conversely, domiciliary bleaching consists of the application of a 10–20% carbamide peroxide-containing gel inside a mouthguard which is worn by the patient overnight [30].

A relevant point related to tooth bleaching which must be taken into account is the risks associated with this procedure. The risks commonly linked to tooth whitening are increased tooth sensitivity and gingival irritation. Both these side effects mostly depend on the concentration of the specific peroxide bleach component used. In particular, tooth sensitivity usually arises at the end of the treatment and can last for several days after. As regards gingival irritation, it generally begins within a day of treatment and can last for several days as well. Additional risks for the tooth exposed to bleaching agents are

tooth erosion, tooth mineral degradation, increased susceptibility to demineralization, and pulpal damage [31].

The efficacy of ozone therapy on the bleaching of a tooth may depend on the application time, gas flow rate, and on the synergic effect with conventional bleaching agents. A recent systematic review was conducted by Dietrich et al. [32] to determine whether O₃ can improve the clinical performance of tooth bleaching in vital teeth. The authors found that the bleaching effectiveness for the combination of O₃ + hydrogen peroxide (HP) compared to HP was similar and, based on the published literature, concluded that ozone is not superior to the conventional technique using HP on the change of tooth color. Moreover, they also found that ozone did not cause sensitivity when used alone. When it is used in combination with HP, patients tend to report hypersensitivity only when ozone is applied before HP, whereas no sensitivity is perceived when ozone is applied subsequently to it.

Despite the bleaching effect of ozone on the tooth that has been investigated in the literature, to the best of our knowledge, no research has been conducted until now to determine a potential bleaching effect towards composite resins. These dental materials suffer from the risk of internal and external pigmentation, the latter caused by agents such as fruit juices, tea, coffee, and cola. Previous *in vitro* studies have been conducted to assess the lightening effects of both home-applied and office-applied tooth-bleaching products and polishing procedures on stained composite resins [33]. Villalta and colleagues [34] determined that bleaching improved the color of red wine- and coffee-stained composite resin to a level that was analog to the baseline. Studies comparing the effects of polishing to bleaching stained composite resins have had different results. Garoushi and colleagues [35] found that repolishing was more effective than an in-office bleaching product (40% hydrogen peroxide bleaching gel) for color improvement. Conversely, Türkün and Türkün [8] determined that another in-office bleaching protocol was more effective than simulated office polishing.

The color recovery effect on discolored composite resins has been investigated until now only for bleaching and polishing systems, as reported in the studies mentioned above. On the contrary, no studies have been conducted to assess the bleaching effect of ozone on restorative composite resins. In the present study, a positive but moderate influence was assessed for ozone when evaluating its action on the color change of discolored resins, with a change limited to E and L colorimetric values. In fact, the samples pigmented with chlorhexidine, an antimicrobial agent known for its pigmental action, significantly reported a colorimetric alteration when treated with the ozonized products with respect to the control samples. Independently of the specific formulations, all the ozonized products have shown this beneficial effect, which, therefore, can be directly referred to as the ozone content. The mechanism upon which is based the bleaching effect on natural teeth consists of an oxidative reaction exerted by the bleaching agent towards the exogenous pigments. It can be assumed that an analog mechanism could be exerted by ozone, known for its oxidative potential, both on the enamel and even on restorative composite.

An important consideration is the fact that ozone could be a relevant bleaching agent not only towards resin composites but even towards natural dental enamel, probably without presenting the side effects traditionally related to conventional bleaching agents such as hydrogen peroxide and carbamide peroxide [36]. In fact, traditional tooth whitening agents could cause several side effects on the micro-hardness of human enamel. The reduction of this parameter following exposure to the bleaching agents is explained by the oxidation process of both organic and inorganic components of enamel exposed to bleaching agents. This causes alterations in enamel morphology with porosities and micro-cracks. Additionally, the pH of whitening substances determines the impact that whitening agents have on microhardness reduction. The enamel alterations following whitening procedures depend on the chemical reaction and oxidation process, which is directly related to the concentration and pH of the whitening agents used [37].

The major limitation of the current study is represented by its *in vitro* nature, thus not considering all the factors present *in vivo* (like the oral cavity pH and the saliva

amount), which might exert an effect on the parameters assessed. Moreover, further exogenous pigments could affect the resin composite color, and the bleaching effect of ozone could differ among them. Further studies considering these factors are required to better understand the potentiality of ozone towards the bleaching of both enamel and restorative composites. Moreover, it could be interesting to evaluate whether ozone could have a different influence on the basis of the specific composite considered, e.g., flow composite, bulk composite, etc. Moreover, the bleaching effect of ozone should also be evaluated on other materials commonly used in dentistry, such as ceramics. It could also be interesting to design clinical studies in order to assess, even in vivo, the bleaching properties of ozonized products as well as to deepen the other uses of ozone in dental routine. Finally, in addition to ozonized oils/gels, further ozone administration methods, such as gaseous ozone, deserve to be evaluated as well.

5. Conclusions

In recent years, the desire for a pleasant smile with white teeth represents one of the most common desires of patients. Color is a relevant factor in aesthetics, and a chromatic tooth anomaly is perceived more quickly than any other anatomical dental alteration. This justifies the larger and larger interest in teeth bleaching procedures, usually carried out with carbamide or hydrogen peroxide. Dental ozonized products could be valuable in restorative dentistry as bleaching agents of resin composites exposed to discoloration. Further studies are required to determine the best protocol for using ozone for its bleaching effect. Similarly, other ozone administration methods should be evaluated, as well as the bleaching effect of ozone on other restorative materials commonly used in dentistry.

Author Contributions: Conceptualization, S.G., C.P. and A.S.; methodology, S.G. and C.P.; software, R.B.; validation, M.C. and A.S.; formal analysis, R.B.; investigation, M.S.; resources, M.C.; data curation, M.S. and R.B.; writing—original draft preparation, S.G. and R.B.; writing—review and editing, C.P. and A.S.; visualization, C.P. and A.S.; supervision, C.P.; project administration, C.P.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon reasonable request at the Corresponding Author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hickel, R.; Heidemann, D.; Staehle, H.J.; Minnig, P.; Wilson, N.H. Direct composite restorations: Extended use in anterior and posterior situations. *Clin. Oral Investig.* **2004**, *8*, 43–44.
2. Mjör, I.A.; Moorhead, J.E.; Dahl, J.E. Reasons for replacement of restorations in permanent teeth in general dental practice. *Int. Dent. J.* **2000**, *50*, 361–366. [[CrossRef](#)] [[PubMed](#)]
3. Stober, T.; Gilde, H.; Lenz, P. Color stability of highly filled composite resin materials for facings. *Dent. Mater.* **2001**, *17*, 87–94. [[CrossRef](#)] [[PubMed](#)]
4. Janda, R.; Roulet, J.F.; Kaminsky, M.; Steffin, G.; Latta, M. Color stability of resin matrix restorative materials as a function of the method of light activation. *Eur. J. Oral Sci.* **2004**, *112*, 280–285. [[CrossRef](#)] [[PubMed](#)]
5. Lee, Y.K.; Lim, B.S.; Rhee, S.H.; Yang, H.C.; Powers, J.M. Color and translucency of A2 shade composite resins after curing, polishing and thermocycling. *Oper. Dent.* **2005**, *30*, 436–442. [[PubMed](#)]
6. Hosoya, Y. Five-year color changes of light-cured composite resins: Influence of light-curing times. *Dent. Mater.* **1999**, *15*, 268–274. [[CrossRef](#)] [[PubMed](#)]
7. Schulze, K.A.; Marshall, S.J.; Gansky, S.A.; Marshall, G.W. Color stability and hardness in dental composites after accelerated aging. *Dent. Mater.* **2003**, *19*, 612–619. [[CrossRef](#)]
8. Türkün, L.Ş.; Türkün, M. Effect of bleaching and repolishing procedures on coffee and tea stain removal from three anterior composite veneering materials. *J. Esthet. Restor. Dent.* **2004**, *16*, 290–301. [[CrossRef](#)]
9. de Gee, A.J.; ten Harkel-Hagenaar, E.; Davidson, C.L. Color dye for identification of incompletely cured composite resins. *J. Prosthet. Dent.* **1984**, *52*, 626–631. [[CrossRef](#)]

10. Um, C.M.; Ruyter, I.E. Staining of resin-based veneering materials with coffee and tea. *Quin. Int.* **1991**, *22*, 377–386.
11. Borges, A.B.; Marsilio, A.L.; Pagani, C.; Rodrigues, J.R. Surface roughness of packable composite resins polished with various systems. *J. Esthet. Restor. Dent.* **2004**, *16*, 42–47. [[CrossRef](#)] [[PubMed](#)]
12. Guler, A.U.; Yilmaz, F.; Kulunk, T.; Guler, E.; Kurt, S. Effects of different drinks on stainability of composite resin provisional restorative materials. *J. Prosthet. Dent.* **2005**, *94*, 118–124. [[CrossRef](#)] [[PubMed](#)]
13. Bagheri, R.; Burrow, M.F.; Tyas, M. Influence of foodsimulating solutions and surface finish on susceptibility to staining of aesthetic restorative materials. *J. Dent.* **2005**, *33*, 389–398. [[CrossRef](#)] [[PubMed](#)]
14. Monzillo, V.; Lallitto, F.; Russo, A.; Poggio, C.; Scribante, A.; Arciola, C.R.; Bertuccio, F.R.; Colombo, M. Ozonized Gel Against Four Candida Species: A Pilot Study and Clinical Perspectives. *Materials* **2020**, *13*, 1731. [[CrossRef](#)]
15. Butera, A.; Gallo, S.; Pascadopoli, M.; Luraghi, G.; Scribante, A. Ozonized Water Administration in Peri-Implant Mucositis Sites: A Randomized Clinical Trial. *Appl. Sci.* **2021**, *11*, 7812. [[CrossRef](#)]
16. Sechi, L.A.; Lezcano, I.; Nunez, N.; Espim, M.; Duprè, I.; Pinna, A.; Molicotti, P.; Fadda, G.; Zanetti, S. Antibacterial activity of ozonized sunflower oil (Oleozon). *J. Appl. Microbiol.* **2001**, *90*, 279–284. [[CrossRef](#)]
17. Lezcano, I.; Nuñez, N.; Espino, M.; Gómez, M. Antibacterial activity of ozonized sunflower oil, oleozon, against *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Ozone Sci. Eng.* **2000**, *22*, 207–214. [[CrossRef](#)]
18. Suh, Y.; Shrey, P.; Re, K.; Gandhi, J.; Joshi, G. Clinical utility of ozone therapy in dental and oral medicine. *Med. Gas. Res.* **2019**, *9*, 163–167.
19. Berezow, A.B.; Darveau, R.P. Microbial shift and periodontitis. *Periodontol.* **2000** **2011**, *55*, 36–47. [[CrossRef](#)]
20. Hafez, R.; Ahmed, D.; Yousry, M.; El-Badrawy, W.; El-Mowafy, O. Effect of in-office bleaching on color and surface roughness of composite restoratives. *Eur. J. Dent.* **2010**, *4*, 118–127. [[CrossRef](#)]
21. Rode, K.M.; Kawano, Y.; Turbino, M.L. Evaluation of curing light distance on resin composite microhardness and polymerization. *Oper. Dent.* **2007**, *32*, 571–578. [[CrossRef](#)]
22. Zorzin, J.; Maier, E.; Harre, S.; Fey, T.; Belli, R.; Lohbauer, U.; Petschelt, A.; Taschner, M. Bulk-fill resin composites: Polymerization properties and extended light curing. *Dent. Mater.* **2015**, *31*, 293–301. [[CrossRef](#)] [[PubMed](#)]
23. Beltrami, R.; Chiesa, M.; Scribante, A.; Allegretti, J.; Poggio, C. Comparison of shear bond strength of universal adhesives on etched and nonetched enamel. *J. Appl. Biomater. Funct. Mater.* **2016**, *14*, e78–e83. [[CrossRef](#)]
24. Poggio, C.; Lombardini, M.; Gaviati, S.; Chiesa, M. Evaluation of Vickers hardness and depth of cure of six composite resins photo-activated with different polymerization modes. *J. Conserv. Dent.* **2012**, *15*, 237. [[CrossRef](#)] [[PubMed](#)]
25. Alrahlah, A.; Silikas, N.; Watts, D.C. Post-cure depth of cure of bulk fill dental resin composites. *Dent. Mater.* **2014**, *30*, 149–154. [[CrossRef](#)] [[PubMed](#)]
26. Gómez-Polo, C.; Portillo Muñoz, M.; Lorenzo Luengo, M.C.; Vicente, P.; Galindo, P.; Martín Casado, A.M. Comparison of the CIELab and CIEDE2000 color difference formulas. *J. Prosthet. Dent.* **2016**, *115*, 65–70. [[CrossRef](#)]
27. Joiner, A. Tooth color: Review of the literature. *J. Dent.* **2004**, *32*, 3–12. [[CrossRef](#)]
28. O'Brien, W.J. *Dental Materials and Their Selection*, 3rd ed.; Quint Pub Inc.: Canada, 2002; p. 28.
29. International Commission on Illumination. *Colorimetry: Official Recommendations of the International Commission on Illumination*, 2nd ed.; Bureau Central de la CIE: Vienna, Austria, 1986.
30. Epple, M.; Meyer, F.; Enax, J. A Critical Review of Modern Concepts for Teeth Whitening. *Dent. J.* **2019**, *7*, 79. [[CrossRef](#)]
31. Carey, C.M. Tooth whitening: What we now know. *J. Evid. Based Dent. Pract.* **2014**, *14*, 70–76. [[CrossRef](#)]
32. Dietrich, L.; de Assis Costa, M.D.M.; Blumenberg, C.; Nascimento, G.G.; Paranhos, L.R.; da Silva, G.R. A meta-analysis of ozone effect on tooth bleaching. *Sci. Rep.* **2021**, *11*, 13177. [[CrossRef](#)]
33. Reinhardt, J.W.; Balbierz, M.M.; Schultz, C.M.; Simentich, B.; Beatty, M.W. Effect of Tooth-Whitening Procedures on Stained Composite Resins. *Oper. Dent.* **2019**, *44*, 65–75. [[CrossRef](#)] [[PubMed](#)]
34. Villalta, P.; Lu, H.; Okte, Z.; Garcia-Godoy, F.; Powers, J.M. Effects of staining and bleaching on color change of dental composite resins. *J. Prosthet. Dent.* **2006**, *95*, 137–142. [[CrossRef](#)] [[PubMed](#)]
35. Garoushi, S.; Lassila, L.; Hatem, M.; Shembesh, M.; Baady, L.; Salim, Z.; Vallittu, P. Influence of staining solutions and whitening procedures on discoloration of hybrid composite resins. *Acta Odontol. Scand.* **2013**, *71*, 144–150. [[CrossRef](#)] [[PubMed](#)]
36. Alkahtani, R.; Stone, S.; German, M.; Waterhouse, P. A review on dental whitening. *J. Dent.* **2020**, *100*, 103423. [[CrossRef](#)]
37. Alqahtani, M.Q. Tooth-bleaching procedures and their controversial effects: A literature review. *Saudi Dent. J.* **2014**, *26*, 33–46. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.