Clin Oral Invest (2011) 15:249–256
DOI 10.1007/s00784-009-0370-5

ORIGINAL ARTICLE

Influence of ozone on the composite-to-composite bond

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Received: 12 July 2009 / Accepted: 7 December 2009 / Published online: 7 January 2010

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

Keywords Composite repair · Shear bond strength · Ozone · Thermocycling

Introduction

In the last decades, resin composites have become the main choice for all classes of restorations. Despite the constant effort to improve the properties of these restorative materials, composite restorations cannot be regarded as permanent. Secondary caries and marginal defects have been reported to be the main reasons for restorations' failure [1–3].

Defective restorations have been traditionally removed and replaced. This operative approach requires a cavity preparation more extended than the previous one, with the subsequent sacrifice of healthy dental structure. Therefore, more recently, a minimal invasive treatment has been suggested, which foresees the repair rather than the replacement of failed restorations [4, 5] and allows for an increase of the longevity of the original filling [6].

The excavation of small carious or staining lesions at the tooth-restoration interface does not necessarily imply the complete removal of pathogenic bacteria [7], which could

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be responsible for the reoccurrence of caries [8] with the subsequent failure of the repair procedure. Therefore, additional methods for obtaining the disinfection of the repair site might be considered.

Ozone has been introduced in the dental practice due to its antimicrobial potential against oral pathogens [9–12]. Gasiform ozone has been investigated for the treatment of occlusal [13] and root caries [14], whereas, the aqueous form, due to its biocompatibility [15] and to its anti-inflammatory potential [16], has been suggested as an alternative treatment for periodontal disease. More recently, the application of ozone gas on dental hard tissues prior to adhesive procedures has been proposed [17–20]; thus, it could also be of interest to assess its effect prior to restoration repairs.

The application of ozone at a repair site determines not only the desired disinfecting action of the gas on the adjacent tooth structure, but also the contact of ozone with the surface of the restoration to be repaired. Since the application of a strong oxidizing agent, such as hydrogen peroxide, has been reported to be unfavorable for composite repair procedures [21], some concerns about the use of ozone could also be arisen.

The major aim of this laboratory study was to evaluate the effect of an ozone gas application on immediate and aged composite–repair bonds after the use of different intermediate agents. Thus, the null hypothesis tested is that neither the ozone application nor the intermediate agent or the aging affect the composite–repair bond. The study also evaluated the effect of the ozone application on hardness and elastic modulus of a composite surface. The tested null hypothesis is that the surface treatment with ozone does not affect the tested properties.

Materials and methods

Shear specimens preparation

Three hundred and twenty methacrylate cylinders (Technovit 4004, Heraeus Kulzer, Wehrheim, Germany) were obtained from metallic molds with a height of 20 mm and a diameter of 15 mm. Cylindrical cavities with a depth of 2 mm and a diameter of 6 mm were created on one free surface of each methacrylate cylinder with a metallic bur. Each cavity was filled with a single increment of composite (Tetric EvoCeram, Shade A3, Ivoclar-Vivadent, Schaan, Liechtenstein), which was light-cured for 20 s with a LED curing unit (Bluephase, Ivoclar-Vivadent) at an output intensity of 1,200 mW/cm^2. The same curing unit was used through the study.

After storage (deionized water, 24 h, 37°C), the composite surfaces were ground with 400-grit silicon carbide paper and the specimens were divided into two main experimental groups (*n*=160):

Group 1: The specimens were subjected to a 60-s ozone gas application (2,100 ppm equal to 4.2 g/m^3; HealOzone, KaVo, Biberach, Germany);
Group 2: No pretreatment was performed (control group).

Four subgroups (*n*=40) were then obtained from each group, according to the intermediate agent used for the repair procedure:

Subgroup A: A silane coupling agent (ESPE Sil, 3M ESPE, Seefeld, Germany) was applied on the composite surface and was allowed to dry for 30 s;
Subgroup B: A total-etch two-steps adhesive (Adper Scotchbond 1XT, 3M ESPE, St. Paul, MN, USA) was applied on the composite surface and light-cured for 10 s;
Subgroup C: The application of the total-etch two-steps adhesive (Adper Scotchbond 1 XT, 3M ESPE) was preceded by the application of the silane coupling agent (ESPE Sil, 3M ESPE);
Subgroup D: A flowable composite (Tetric EvoFlow, Shade B3, Ivoclar-Vivadent) was applied in a thin layer on the composite surface and light-cured 20 s.

Repair composite cylinders (Tetric EvoCeram, Shade A2, Ivoclar-Vivadent) with a diameter of 3 mm were then built-up in two 2-mm thick increments on the composite surfaces by means of a silicon mold. Each increment was separately light-cured for 20 s. The specimens were then stored 24 h in deionized water at 37°C.

Chemical compositions, batch numbers, and modes of use of the materials used in the study are reported in Table 1.

Half of the specimens of each subgroup were subjected to thermocycling for 5,000 cycles (temperature changing from 5°C to 55°C in deionized water, dwell time 30 s, transfer time 5 s; Willytec, Dental Research Division, Munich, Germany) prior to testing; whereas, the other specimens were immediately processed for the shear strength test.

Shear strength test

The bonded specimens were introduced in a universal testing machine (MCE 2000ST, quickTest, Langenfeld, Germany), and the composite repair strength was tested with a shear test at a crosshead speed of 0.5 mm/min until failure occurred. The load at failure (Newtons) recorded by the testing machine was divided by the bonding area (square millimeter), and the shear strength was expressed in megapascals.

Table 1 Chemical compositions, batch numbers, and modes of application of the materials used in the study

Material	Type	Composition	Mode of use
Tetric EvoCeram (Ivoclar-Vivadent) A3: Batch number L16680 A2: Batch number K56745	Composite	Matrix: dimethacrylates (17–18 wt.%) Filler: barium glass, ytterbium trifluoride, mixed oxide and prepolymer (82–83 wt.%). Total content of inorganic fillers 75–76 wt.%. Mean particle size 550 nm. Additional contents: additives, catalysts, stabilizers, and pigments (<1 wt.%).	Apply a 2-mm thick layer. Light-cure for 20 s.
ESPE Sil (3M ESPE) Batch number 313387	Silane	MPS, ethanol	Apply on the composite surface. Allow to dry 30 s.
Adper Scotchbond 1 XT (3M ESPE) Batch number 6JU	Adhesive	Bis-GMA, HEMA, dimethacrylates, methacrylate functional copolymer (polyacrilic and polyitaconic acids), ethanol, water, silica nanofillers (5 nm; 10 wt.%).	Apply 2–3 consecutive coats for 15 s. Air-thin for 5 s. Light-cure for 10 s.
Tetric EvoFlow (Ivoclar-Vivadent) B3: Batch number J20474	Flowable composite	Matrix: dimethacrylates (38 wt.%) Filler: barium glass, ytterbium trifluoride, highly dispersed silicon dioxide, mixed oxide and copolymer (62 wt.%). Total content of inorganic fillers 57.5 wt.%. Mean particle size 550 nm. Additional contents: additives, catalysts, stabilizers, and pigments (<1 wt.%).	Apply a thin layer. Light-cure for 20 s.

Abbreviations: *MPS* 3-methacryloyloxy-propyltrimethoxy-silane, *bis-GMA* bisphenol A-diglycidylmethacrylate, *HEMA* 2-hydroxyethyl methacrylate

The fractured specimens were observed under an optical loupe at a 2.5× magnification, and the types of failure were classified as follows:

> Adhesive (A): when the failure occurred at the interface between the intermediate agent and the original composite surface or the composite repair surface;
> Cohesive in the composite (CC): when the failure occurred within the original composite filling;
> Cohesive in the repair (CR): when the failure occurred within the repair composite cylinder; and
> Mixed (M): when a combination of two or more of the above described modes was observed.

Statistical analysis of the shear bond strength data

The normality of the shear strength data distribution and the homogeneity of variances among the experimental groups were verified (Kolmogorov–Smirnov test and Levene's test). The one-way analysis of variance (ANOVA) was used to compare the shear bond strengths among the experimental subgroups. In order to assess the main factors affecting the shear strengths, a univariate analysis of variance was performed with the shear strength as the dependant variable and the pretreatment, the intermediate agent and the thermocycling as independent variables. The partial eta-squared statistics (η_p^2) was used in order to assess the actual contribution of each independent variable in determining variations of the dependant variable. The Tukey's test was used for post hoc comparisons when needed. The Weibull analysis of the shear strength data was also performed.

The statistical analyses were handled with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA), and the Weibull's analysis was performed with Microsoft Office Excel 2003 (Microsoft Corp.). The level of significance was set at $p<0.05$.

SEM specimens preparation

One representative fractured specimen for each experimental group was selected and processed for SEM examination. The selected specimens were rinsed in 96% alcohol solution for 1 min and air-dried. They were then mounted on a metallic stub, sputter-coated with gold (Polaron Range SC7620, Quorum Technology, Newhaven, UK), and observed under a scanning electron microscope (JSM 6060 LV, JEOL, Tokyo, Japan) at different magnifications.

Testing of the micromechanical properties of the composite surface

In order to additionally characterize the composite surface with and without the application of ozone gas immediately and after aging, 20 composite specimens (Tetric EvoCeram,

Table 2 Means (SD) and Weibull analysis of the composite repair shear strengths in the experimental groups (n=20)

Intermediate agent	Ozone		Control	
	24h	Thermocycling	24h	Thermocycling
Adhesive	14.1 (6.1)abc	19.2 (7.7)bcde	20.0(8.2)bcde	20.1 (7.0)cde
	m=2.3; σ_0=19.3	m=2.5; σ_0=21.6	m=2.2; σ_0=11.4	m=3.1; σ_0=14.2
Silane	10.1 (4.9)a	12.6 (4.1)ab	17.2 (8.6)abc	19.2 (9.4)bcde
	m=2.7; σ_0=20.9	m=2.8; σ_0=22.4	m=2.9; σ_0=16.6	m=3.7; σ_0=18.8
Silane+Adhesive	14.8 (6.4)abc	17.0 (5.1)abc	18.6 (8.5)bcd	19.9(7.5)bcde
	m=2.8; σ_0=22.4	m=2.8; σ_0=22.8	m=3.0; σ_0=15.8	m=2.7; σ_0=21.7
Flowable composite	20.7 (5.7)cde	18.5 (2.8)bcd	26.5 (6.8)e	24.9 (5.5)de
	m=3.1; σ_0=30.2	m=5.5; σ_0=27.0	m=4.3; σ_0=22. 8	m=7.7; σ_0=19.7

The shear strengths are expressed in megapascals. Different letters indicate statistically significant differences in the composite repair strength among the groups ($p<0.05$). m=Weibull's modulus; σ_0=characteristic strength.

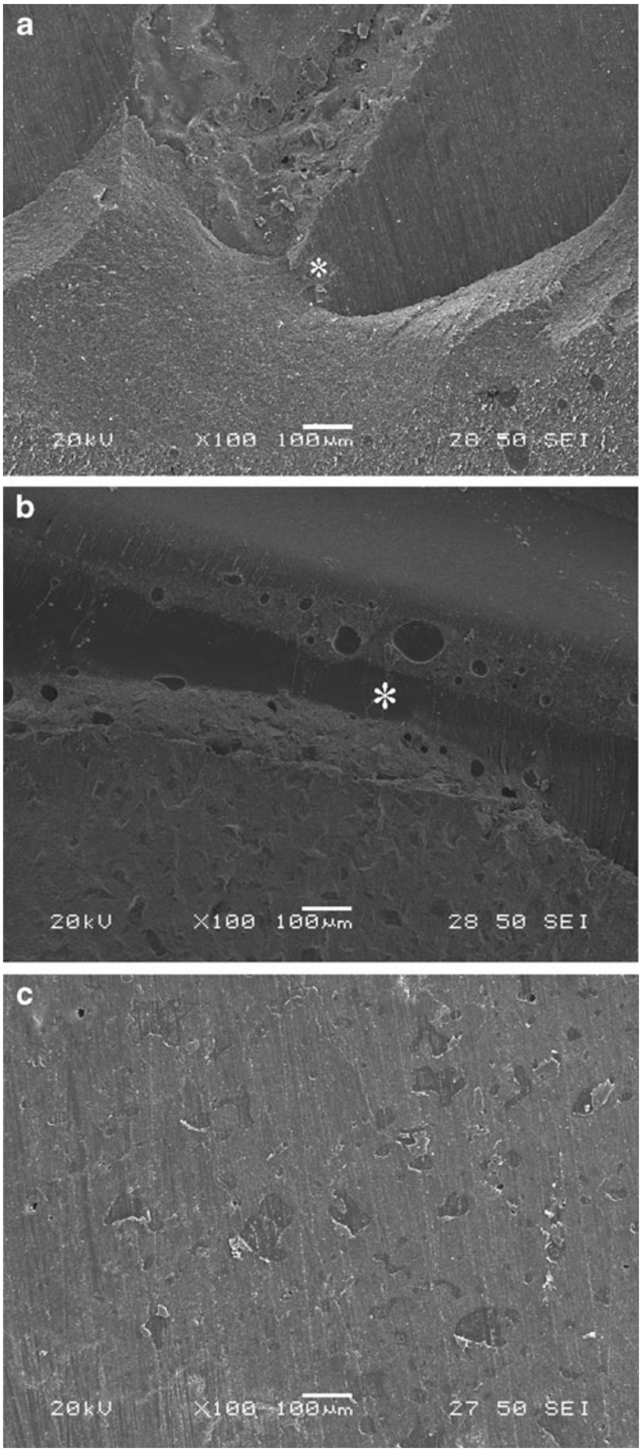
Ivoclar-Vivadent) with a length and a width of 4 mm and a height of 2 mm were prepared in a glass mold and light-cured 20 s (Bluephase, Ivoclar-Vivadent) through a mylar strip. After 24 h of storage in deionized water at 37°C, half of the specimens were subjected to an ozone application of 60 s (HealOzone, KaVo), whereas, the other specimens were left untreated. In order to reproduce the same experimental conditions applied for the shear bond strength specimens, half of the ozone-treated and half of the untreated composite specimens were tested for the micromechanical properties immediately, whereas, the other halves where prior subjected to the same thermocycling regimen, used for the shear specimens. The elastic modulus (E) and the Vicker's hardness (VH) of the composite specimens were tested in the resulting four experimental groups (n=5) with a micro hardness indenter (Fischerscope H100C; Fischer, Sindelfingen, Germany). The test procedure was carried out force controlled. A load application time of 50 s was set and subdivided as follows: the force increased at a constant speed from 0.4 to 500 mN in 20 s, the maximal force of 500 mN was kept constant for 5 s, then the force decreased at a constant speed from 500 to 0.4 mN in 20 s, and the minimal force of 0.4 mN was kept constant for 5 s. The load and the penetration depth of the indenter (Vicker's pyramid, diamond right pyramid with an angle α=136° between the opposite faces at the vertex) were continuously measured during the load–unload cycle. Six indentations were performed on the top composite surface of each specimen.

The universal hardness is defined as the test force divided by the apparent area of the indentation at maximal force. From a multiplicity of measurements stored in a

Table 3 Failure modes distribution within the experimental groups

Intermediate agent	Ozone		Control	
	24h	Thermocycling	24h	Thermocycling
Adhesive	A 0/20	A 0/20	A 0/20	A 0/20
	CC 4/20	CC 4/20	CC 3/20	CC 3/20
	CR 0/20	CR 0/20	CR 0/20	CR 0/20
	M 16/20	M 16/20	M 17/20	M 17/20
Silane	A 2/20	A 1/20	A 4/20	A 1/20
	CC 0/20	CC 1/20	CC 1/20	CC 5/20
	CR 0/20	CR 0/20	CR 0/20	CR 0/20
	M 18/20	M 18/20	M 15/20	M 14/20
Silane+Adhesive	A 0/20	A 0/20	A 1/20	A 1/20
	CC 0/20	CC 4/20	CC 0/20	CC 2/20
	CR 0/20	CR 0/20	CR 0/20	CR 0/20
	M 20/20	M 16/20	M 19/20	M 17/20
Flowable composite	A 0/20	A 0/20	A 0/20	A 0/20
	CC 0/20	CC 1/20	CC 3/20	CC 4/20
	CR 0/20	CR 0/20	CR 0/20	CR 0/20
	M 20/20	M 19/20	M 17/20	M 16/20

A adhesive failures, *CC* cohesive failures within the composite, *CR* cohesive failures within the repair, *M* mixed failures



◀**Fig. 1** Representative SEM images of two mixed (**a**, **b**) and one adhesive (**c**) failure (100×, *bar*=100 µm). **a** Mixed failure in one ozone-treated and not thermocycled specimen with the flowable composite as the intermediate layer. The *asterisk* indicates remnants of the intermediate agent on the composite surface. **b** Specimen not pretreated with ozone and not subjected to thermocycling with an intermediate adhesive layer. Part of the adhesive persisted on the composite surface (indicated by the *asterisk*). **c** Adhesive failure of an ozone-treated and silanized specimen. The failure occurred at the interface between the old composite and the repair

database supplied by the manufacturer, a conversion factor between universal hardness and VH was calculated and implemented into the software, so that the measurements were expressed in Vicker's hardness units.

The indentation modulus was calculated from the slope of the tangent of the indentation curve at maximal force and is comparable with the modulus of elasticity of the material (E).

Having checked the normality of data distribution for the measured E and VH values (Kolmogorov–Smirnov test) and the homogeneity of the groups' variances (Levene's test), the two-way ANOVA was applied in order to statistically analyze the data of each property with the pretreatment and the thermocycling as fixed factors. The Tukey's test was used for post hoc comparisons when needed. The level of significance was set at $p<0.05$.

Results

The statistical analysis detected significant differences among the experimental groups ($p<0.001$). Table 2 reports means and SD of the measured shear strengths, as well as the Weibull modulus (m) and the characteristic strength (σ_0) of each experimental group obtained through the Weibull analysis. The failure modes distribution within the experimental groups is reported in Table 3. The composite repair strength resulted affected by the pretereatment and by the intermediate agent ($p<0.001$), whereas, the thermocycling was not a significant factor. The partial eta-squared statistics showed that the intermediate agent was the main factor affecting the composite repair strength, determining more than 20% of the variability of the dependant variable, whereas, the pretreatment played a minor role ($\eta_p^2=0.1$).

As far as the pretreatment was concerned, no significant differences were observed between the corresponding ozone and control groups when the same intermediate agent and the same aging conditions were applied (Table 2, $p>0.05$).

Among the tested intermediate agents, the flowable composite exhibited a trend to achieve higher bond strengths, even though the differences were not always significant. Also, the highest Weibull's parameters were recorded in the flowable-treated groups. On the contrary, the silane coupling agent showed the lowest bond strengths in all the experimental groups, which were significantly worse than those achieved with the flowable composite in the 24 h groups, regardless of the pretreatment (Table 2, $p<0.05$).

Mixed failures were the most represented regardless of the experimental group (Table 3 and Fig. 1a, b). Adhesive

Table 4 Means (SD) of Vicker's hardness (VH) and elastic modulus (E) of the composite surface

Group	VH (N/mm^2)		E (GPa)	
	Thermocycling	No thermocycling	Thermocycling	No thermocycling
Ozone	70.1(12.2)a	66.5(10.3)a	9.7(2.2)a	10.8(1.2)b
Control	63.7(10.5)a	65.8(8.7)a	9.9(1.4)a	10.2(1.7)b

Different letters indicate statistically significant differences ($p < 0.05$)

failures were recorded only in the silane-treated groups (Fig. 1c).

Table 4 reports the means and SD of the micromechanical properties of the composite surface under the tested experimental conditions. According to the statistical analysis, the ozone treatment was not a significant factor affecting the composite's properties ($p > 0.05$) and the thermocycling determined a significant reduction only of the E ($p < 0.05$). The interaction between the factors was not significant ($p > 0.05$).

Discussion

The application of ozone gas and the aging conditions performed in the study did not significantly affect the composite repair strength, whereas, the intermediate agent was a critical factor influencing the bond strength. Thus, the first null hypothesis was rejected. Moreover, the tested composite's micromechanical properties were not impaired by an ozone application. Therefore, the second null hypothesis was accepted.

The repair of a failed restoration represents a valid alternative to its replacement in order to avoid an excessive sacrifice of dental structure. When a secondary caries lesion or a stained restoration's margin are removed prior to restoration's repair, some concerns arise whether some cariogenic bacteria persist after the elimination of the affected tooth tissue [22] at the repair site, especially if a minimal excavation is preferred. To overcome the risk of caries recurrence due to the presence of residual infection beneath a restoration [8], the combination of the lesion's excavation with the cavity's disinfection has been suggested [23]. Besides its antimicrobial activity, a major requirement of a cavity disinfectant should be the absence of any detrimental effect on the adhesive procedures needed to perform as well as to repair a restoration. Since some common disinfectants such as chlorhexidine or sodium hypochlorite showed a negative effect on adhesion [24, 25], novel products have been investigated [23]. Previous studies encouraged the application of ozone on dental hard tissues prior to adhesive procedures [17–20] and the present investigation focused on the possibility to extend the use of ozone also in cases of restorations' repairs.

The ANOVA showed that the composite repair strength was not significantly different between ozone-treated and control groups irrespective of the aging conditions and of the intermediate agent (Table 2). The partial eta-squared represents an index of strength of association between an experimental factor and the dependent variable and ranges normally between 0 and 1 [26]. This parameter was included in the statistical analysis of the present study in order to assess the actual effect that the ozone treatment exerted on the composite repair strength and its outcome confirmed that the ozone application played a minor role in determining variations of the dependant variable. Also, the Weibull analysis did not reveal remarkable differences in Weibull modulus and characteristic strength after ozone application, suggesting that the chance of failure was not impaired by the pretreatment (Table 2). This finding is also supported when the distribution of failure's modes is considered (Table 3): mixed failures were the most observed in all experimental groups (Fig. 1a, b) and the additional use of ozone did not change the failures' pattern, suggesting that this disinfectant had no detrimental effect on the final composite-intermediate agent-repair system. These results are in agreement with those studies, which observed no detrimental action of ozone on the adhesion to dental hard tissues [17–20]. The study of Papacchini et al. [21] showed a detrimental effect of hydrogen peroxide on the composite repair strength particularly when an adhesive was used as the intermediate agent. The authors explained their results by considering the unfavorable interaction of residual hydrogen peroxide and oxygen by-products on the composite surface to be repaired and the oxygen coming from atmospheric air, which could impair the polymerization of the intermediate agent in the repair procedure [21]. It might be speculated that the oxidative effect of ozone on the composite surface did not lead to the formation of a critical amount of oxygen by-products. The absence of any effect of ozone on the composite's micromechanical properties immediately and after thermocycling (Table 4) also supports the thesis that an eventual chemical modification of the composite's surface through ozone application is unlikely to occur. On the contrary, common bleaching agents such as hydrogen or carbamide peroxide have been reported to affect some physical properties of composite resins [27–29]. The reduction of the elastic modulus observed after thermocycling is in agreement with the results of those studies, which reported a detrimental effect of artificial aging on some mechanical properties of dental composites [30, 31]. Nevertheless, the mechanical behavior of resin composites under challenging conditions is still contradictory [32].

According to the statistics, the intermediate agent was the major factor affecting the composite repair strength. Different agents have been proposed for composite repair procedures [33–35]. In this study, an adhesive, a silane coupling agent, flowable composite, and the combination between adhesive and silane have been investigated. The application of an adhesive layer has been proposed in order to mediate the composite-to-composite bond, since the sole use of procedures aimed to increase the roughness of the composite surface to be repaired is still controversial [33]. An intermediate low-viscosity resin layer should improve the surface wetting and the chemical bonding in composite repair procedures and the previous application of a silane has been also investigated [21, 33, 36]. In the present study, the additional application of a silane did not significantly improve the composite-to-composite bond strength compared to the use of the adhesive alone, and this finding is in accordance with previous results [36]. Also, the poor repair potential previously observed by using solely silane couplings was confirmed by the results of this study [37]. The application of flowable composites as intermediate agents in composite repair procedures has been recently investigated with the microtensile technique [34] and showed promising results even after aging [37], mainly attributed to their stress-absorbing ability [38] and to their superior hydrolytic stability compared to more hydrophilic intermediate agents [37]. A trend to achieve higher composite repair strengths by using an intermediate flowable composite layer was confirmed in the present study by using the shear bond strength test and was also supported by the higher Weibull's parameters recorded.

Thermocycling has been widely used in laboratory studies in order to simulate the stress generated by changing the environmental temperature at the interface between materials with different coefficients of thermal expansion [39]. In this investigation, the composite repair strength was not affected by the aging conditions, regardless of the composite surface pretreatment and of the intermediate repair agent. Similar results were obtained in a study of Papacchini et al. [37], which showed a significant reduction of the composite–composite repair strength after thermocycling only if a nonprehydrolized silane coupling agent mixed with an etch-and-rinse self-cure adhesive system was applied as the intermediate repair agent. On the contrary, no significant effect on the repair potential was reported by using an unfilled resin, a flowable composite, a prehydrolized silane, or other silane/adhesive blends [37]. However, a significant effect of thermocycling on the repair strength was reported when a higher number of cycles was performed [40].

Within the limits of the present laboratory study, it might be concluded that an application of ozone gas prior to repair procedures of a nanohybrid composite does not significantly impair the achieved composite-to-composite bond strength or the mechanical properties of the composite surface to be repaired. Therefore, the dental clinician could take the use of ozone into consideration if an additional disinfection of the repair site is desirable.

Conflict of interest The authors declare that they have no conflict of interest.

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