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A study into the application of gaseous ozone combined with different adhesive procedures

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Chapter 1

General Introduction

1.1. New concepts in caries management

The classical surgical approach to caries management , firstly introduced by G.V. Black, has been the cornerstone of 20th century dentistry. According to the principle of "extension for prevention", the treatment of caries affected teeth required a peculiar cavity design, which was not limited to the sole excavation of carious tissue but included also a self-cleansing outline form, a resistance form, a retention form and a convenience form (Osborne & Summitt 1998). This traditional restorative technique implied a substantial loss of tooth structure but for many years it represented the only treatment option due to the limited choice of restorative materials and to the lack of understanding of the development and progression of the caries disease (Mount 2003).

In the last decades considerable research into the field of dental materials has been conducted, leading to the introduction of a wide amount of new adhesive restorative materials, which allowed for a reduced cavity extension, since the concepts of "retention form" and " resistance form" did not need to be strictly applied to these new materials. In addition, more attention has been focused toward the mechanisms of initiation and progression of the caries disease. Caries has been recognized as a bacterial disease, which cannot be initiated or continue in the absence of the bacterial etiological agents (Balakrishnan *et al.* 2000).

As a result, a new philosophy of caries management was recently developed, which is known as "Minimal Intervention Dentistry (MID)". According to this novel approach, besides a more conservative cavity design (Mount 2003; Noack *et al.* 2004; Tyas *et al.* 2000), the control of the bacterial

infection with antimicrobial agents represents a reliable method for improving caries lesions and avoiding caries progression (Noack *et al.* 2004; Tyas *et al.* 2000). In other words, besides the conventional "surgical" approach to caries management, a renewed interest is growing toward a "biological" approach to caries, which is recognized and treated as an infectious disease (Tyas *et al.* 2000).

1.2. Choice of disinfectans in restorative dentistry

The excavation of a carious lesion does not allow for the complete removal of cariogenic microorganisms (Kidd *et al.* 1996). These bacteria could be responsible for caries recurrence and restoration failure (Mejàre *et al.* 1979). Therefore, the application of antimicrobial agents prior to restorative procedures has been widely investigated (Al-Omari *et al.* 2006; Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006).

Several methods have been proposed for obtaining disinfection of tooth hard tissues and subsequently inhibiting caries progression or reoccurrence (Chandler & Heling 1995; Ersin *et al.* 2009; Esteves *et al.* 2010; da Silva *et al.* 2010; Pinto *et al.* 2010; Hosoya *et al.* 2010; Taniguchi *et al.* 2009; Vaidyanathan *et al.* 2009; Li *et al.* 2009; van As 2004; Walsh 2003; Zaura *et al.* 2007; Polydorou *et al.* 2006; Müller *et al.* 2007; Wicht *et al.* 2004). The suppression of caries-related microorganisms could be achieved with the antibacterial potential of some cavity liners (Chandler & Heling 1995) or through the application of disinfectans solutions such as chlorhexidine (Ersin *et al.* 2009; Wicht *et al.* 2004) or sodium hypochlorite (Taniguchi *et al.* 2009; Zaura *et al.* 2007). Dental bonding agents present an antibacterial activity (Pinto *et al.* 2010; Esteves *et al.* 2010; Vaidyanathan *et al.* 2009; Polydorou *et al.* 2006), which could also be enhanced by the addition of fluoride or antimicrobial

monomers to the chemical formulation of the adhesive (da Silva *et al.* 2010; Pinto *et al.* 2010; Hosoya *et al.* 2010; Vaidyanathan *et al.* 2009; Li *et al.* 2009; Polydorou *et al.* 2006). The use of antibiotics has been also suggested (Wicht *et al.* 2004). More recently, the application of laser devices for cavity preparation has been also introduced in the dental practice (van As 2004; Walsh 2003) and the antimicrobial activity of laser appliances has been investigated (Müller *et al.* 2007).

1.3. The antimicrobial potential of gasiform ozone against oral pathogens

Ozone is a strong oxidant whose high antibacterial potential has been well known for over a century. Therefore, ozone has found applications in the treatment of sewage and disinfection of drinking-water, as well as in the food preservation and equipment sterilization (Sheldon & Brown 1986; Wallhäußer 1995; Restaino *et al.* 1995; Filippi 1995). In the last years, an ozone-generating device (HealOzone, KaVo, Biberach, Germany; Fig. 1) has been developed as an alternative non-invasive treatment for primary caries. Ozone is delivered through a handpiece, which is equipped with a silicon cup. The cup is applied directly to the tooth and is able to tightly seal the application site. This ozone generator operates basically by passing dried air through an electric field. This way, oxygen molecules (O_2) are split into two oxygen atoms (O^-) by an electric discharge. As oxygen atoms are very unstable, they attach immediately to other oxygen molecules, forming ozone ($O^- + O_2 \rightarrow O_3$), which is produced at a concentration of 2,100 ppm with a changeover of 300 times per second. The high molecular instability of ozone accounts for its remarkable oxidation potential. Bacterial cell membranes undergo an oxidation reaction when coming in contact with ozone. Moreover, bacterial glycoproteins, glycolipids and other amino acids are attacked and the enzymatic control system is firstly inhibited

and subsequently completely blocked. Bacteria are killed due to the dysfunction of the cell wall permeability or due to cell lysing (Brockmann & Botzenhart 2001). Besides this direct mechanism of bacteria killing, the ozone-induced oxidation might also result in a reduction of carbohydrates and acids within the treated dental site, thus determining an indirect cariostatic effect (Claxson *et al.* 2002; Mills *et al.* 2001; Silwood *et al.* 2002; Silwood *et al.* 1999).

Several studies investigated the effect of ozone on the oral microbiota (Baysan *et al.* 2000; Nagayoshi *et al.* 2004a; Nagayoshi *et al.* 2004b; Baysan & Lynch 2004; Hems *et al.* 2005; Estrela *et al.* 2006; Estrela *et al.* 2007; Polydorou *et al.* 2006; Müller *et al.* 2007; Baysan & Beighton 2007; Huth *et al.* 2009; Johansson *et al.* 2009). Gasiform ozone has been reported to be effective against isolated strains of caries-associated microorganisms (Polydorou *et al.* 2006; Johansson *et al.* 2009), whereas Müller *et al.* found a minimal effect of ozone on the viability of different bacterial species organized in a cariogenic biofilm (Müller *et al.* 2007). However, ozone showed effectiveness against the microflora associated with primary root caries lesions (Baysan *et al.* 2000; Baysan & Lynch 2004). Moreover, it has been reported that an ozone application on non-carious dentin prevented the formation of a biofilm by *Streptococcus mutans* and *Lactobacillus acidophilus* on the tooth substrate over a four-week period (Knight *et al.* 2008).

The antimicrobial potential of gasiform ozone has been reported to be dose-, strain- and time-dependant (Huth *et al.* 2009). The application of ozone for 40s failed to significantly reduce the number of viable bacteria associated with non-cavitated occlusal carious lesions (Baysan & Beighton 2007). An application time of 30s was not enough in order to eliminate the microflora associated with open occlusal carious lesions (Hauser-Gerspach *et al.* 2009). On the contrary, more sustained exposures to gasiform ozone showed an effective antimicrobial activity (Huth *et al.* 2009; Johansson *et al.* 2009). Nevertheless, a

40s ozone application has been reported to be suff icient to kill different concentrations of *Streptococcus mutans* (Castillo *et al.* 2008).

Fig. 1: The gaseous ozone generating device for oral application.

1.4. Clinical applications of ozone gas

The clinical performance of gasiform ozone has been evaluated in several studies in the last years. Ozone has been applied for the treatment of coronal (Huth *et al.* 2005; Knežević *et al.* 2007; Dähnhardt *et al.* 2006) and root (Holmes 2003; Baysan & Lynch 2004; Baysan & Lynch 2007) caries. Moreover, the effect of ozone application on dentin hypersensitivity (Azarpazhooh *et al.* 2009) and on white spots development in orthodontic patients (Kronenberg *et al.* 2009) has also been investigated.

Ozone treatment of primary fissure caries in adult patients has been reported to be a non invasive procedure to promote caries reversal and tooth remineralization (Knežević *et al.* 2007). The application of ozone in pediatric dentistry showed encouraging results. A prospective controlled clinical study reported that ozone treatment could reverse open carious lesions in anxious children over a 8-months period, improving the compliance of the patients (Dähnhardt *et al.* 2006). Huth *et al.* investigated the effect of ozone treatment on non-cavitated fissure carious lesions in children and observed that ozone could reverse or reduce caries progression in patients with high caries risk (Huth *et al.* 2005). According to the results of different clinical investigations, the application of ozone for the management of primary root caries lesions might be a valid alternative to conventional "drilling and filling" (Holmes 2003; Baysan & Lynch 2004; Baysan & Lynch 2007).

The assessment of the clinical effectiveness of gasiform ozone on dentin hypersensitivity presented some difficulties, due to the large placebo effect recorded within the study population, which hampered the detection of significant differences between the ozone-treated and the placebo-treated groups. However, a reduction of the pain sensation after ozone application was reported (Azarpazhooh *et al.* 2009).

Ozone treatment was tested for the prevention of white spots formation in orthodontic patients during multibracket appliance therapy and the preventive effect of ozone was inferior to that of the combination Cervitec/Fluor Protector (Ivoclar-Vivadent, Schaan, Liechtenstein).

Although the preliminary clinical results suggested that ozone treatment could be considered as an alternative to conventional procedures for the management of dental caries, there is consensus among the authors of several literature reviews on this topic that more attempts should be made in order to improve the number and the quality of the clinical trials aiming to assess ozone performance (Azarpazhooh & Limeback 2008; Rickard *et al.* 2004; Stübinger *et al.* 2006; Nogales *et al.* 2008).

1.5. Interaction of disinfectants and oxidants with dental hard tissues and dental materials

An important aspect to be considered when choosing a disinfectant should be its interaction with the tooth substrate. Several studies reported that the application of some disinfectants commonly used in the dental practice could affect the hardness and other mechanical properties of dentin (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004). A similar behaviour has been reported for substances with an oxidative potential, such as bleaching agents, which determined mostly an alteration of the mechanical properties dental hard tissues (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006). The high reactivity of the ozone molecules and the substrate's dehydration possibly determined by the gas flow could be responsible of an alteration of the properties of dental hard tissues. A laboratory study investigated the effect of

ozone treatment on Knoop microhardness, contact angle and acid resistance of human enamel and showed that these properties were not impaired by ozone application (Celiberti *et al.* 2006). This thesis contains a study aimed at assessing the effect of gasiform ozone on dentin's micromechanical properties.

Another issue related to the application of disinfectants or oxidative agents to tooth structure is the effect that these substances exert on the adhesion to dental hard tissues. Many laboratory investigations described an inhibitory action of chlorhexidine and sodium hypochlorite on the bond strength to dentin (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006). Nevertheless, recent studies reported a favourable action of chlorhexidine on the stability of dentin bonds (Breschi *et al.* 2009; Breschi *et al.* 2010). Moreover, the oxidative activity of bleaching agents has been reported to be detrimental for the adhesion to dentin (Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005) and for the polymerization of dental adhesives (Breschi *et al.* 2007; Cadenaro *et al.* 2006). Gasiform ozone showed no negative effect on the bond strength of different dental adhesives to enamel and dentin (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005). Moreover, the penetration and the microleakage of a dental sealant have been reported to be unaltered by an ozone application (Celiberti *et al.* 2006). This thesis presents a CLSM investigation assessing the impact of ozone on the dentin infiltration of different bonding systems. Another purpose of this project was the evaluation of the micromechanical properties of different adhesives bonded to ozone-treated dentin. Two studies were conducted in order to address the latter issue. A preliminary study evaluated the micromechanical properties of differently formulated adhesives comparing them to those of glass-ionomer cements, in order to define approximately an interval of values in which these properties could vary. A further study focused on the effect exerted by ozone on the micromechanical properties of selected bonding systems. Moreover, another

study was performed in order to evaluate the bond strength to ground enamel of different types of pit and fissure sealants after ozone appl ication.

Secondary caries has been reported to be one of the most common reasons of restoration's failure (York & Arthur 1993; Hickel & Manhart 2001; Hickel *et al.* 2000; Hickel *et al.* 2007a; Hickel *et al.* 2007b; Manhart *et al.* 2004). A minimal invasive treatment has been suggested, which foresees the repair rather than the replacement of failed restorations (Tyas *et al.* 2000; Mjör & Gordan 2002). If a restorations failed due to a secondary caries and a repair procedure is chosen, the additional disinfection of the repair site would be desirable. An unfavourable effect of hydrogen peroxide on composite repair procedures due to its oxidative potential has been described (Papacchini *et al.* 2007). In the last part of this thesis are presented two studies aimed a t evaluating the effect of ozone on restoration's repair. One study investigated the influence of ozone treatment on the repair strength of a conventional resin composite, whereas in the other study the effect of gasiform ozone on the repair of a silorane-based and of an ormocer-based composite was assessed.

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Chapter 2

Application of gasiform ozone prior to bonding to den tin

2.1. Concerns about the use of disinfectans and oxida nts prior to bonding procedures

A major attempt when treating caries affected teeth is to eliminate cariogenic bacteria from the carious lesion prior to restoring the tooth, in order to avoid secondary caries (Mejàre *et al.* 1979) and the subsequent restoration's failure (York & Arthur 1993; Hickel *et al.* 2000; Hickel & Manhart 2001; Hickel *et al.* 2007a; Hickel *et al.* 2007b; Manhart *et al.* 2004). As the simple excavation of caries does not determine the complet e elimination of bacteria from the affected tooth substrate, the use of cavity disinfectans has been suggested (Al-Omari *et al.* 2006; Pappas *et al.* 2005).

An unfavourable effect on the bond strength to dentin has been reported when common oral disinfectans, such as chlorhexidine or sodim hypochlorite, were used for cavity disinfection (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006). However, the contribution of chlorhexidine to the durability of the a dhesion to dentin has been recently reported (Breschi *et al.* 2009; Breschi *et al.* 2010). Moreover, an alteration of dentin's mechanical properties has been proved to occur after the application of several types of disinfectans (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004).

Due to its antimicrobial potential (Baysan *et al.* 2000; Baysan & Beighton 2007; Baysan & Lynch 2004; Polydorou *et al.* 2006; Huth *et al.* 2007; Estrela *et al.* 2007; Müller *et al.* 2007; Hems *et al.* 2005), which is enabled by its strong oxidative potential, gaseous ozone could also be used for dentin's

disinfection (Schmidlin *et al.* 2005). Nevertheless, an inhibitory effect on bonding procedures (Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005; Cadenaro *et al.* 2006) as well as an impairment of dentin's mechanical properties (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006) have been observed after the application of other oxidants, such as bleaching agents. The information about the impact of ozone on human dentin and on bonding to dentin is extremely limited.

In this chapter one study is presented, which assessed the effect of gasiform ozone on dentin's micromechanical properties. A further study aimed at evaluating the infiltration of selected bonding systems into ozone-treated dentin. In the last two paragraphs two studies are presented, which focused, firstly, on a preliminary assessment of the micromechanical properties of differently formulated adhesive systems compared to those of glass-ionomer cements and, secondly, on the investigation of the effect of ozone on the micromechanical properties of selected bonding agents.

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2.2. Influence of gasiform ozone on the micro mechanical properties of dentin

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Introduction

Gasiform ozone has been introduced in the dental practice as an alternative and minimally invasive treatment for occlusal (Huth *et al.* 2005; Dähnhardt *et al.* 2006) and root caries (Baysan & Lynch 2007; Holmes 2003). More recently the use of ozone for root canal (Estrela *et al.* 2007; Huth *et al.* 2009) and post-space disinfection (Bitter *et al.* 2008) has been also investigated. Ozone has been also reported not to affect the bond strength to dental hard tissues (Bitter *et al.* 2008; Schmidlin *et al.* 2005) and not to impair the micromechanical properties of dental adhesives bonded to dentin (Magni *et al.* 2008).

Several studies reported that the application of disinfectants (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) or oxidants, such as bleaching agents (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006), exerts mostly an alteration of the hardness and other mechanical properties of dental hard tissues. However, low concentrations of hydrogen peroxide have been reported not to negatively affect dentin microhardness (White *et al.* 2007).

Due to the high reactivity of the ozone molecules and to the substrate's dehydration possibly determined by the gas flow, an alteration of the properties of dental hard tissues might be induced by an ozone application. The impact of ozone treatment on some enamel's physical properties has been previously assessed and no impairment of the investigated properties has been found (Celiberti *et al.* 2006).

No information is currently available on the interaction between ozone and dentin. Thus, the aim of this study was to investigate the effect of ozone gas on the elastic modulus and Vicker's hardness of dentin. The tested null hypothesis is that the application of gasiform ozone affects dentin's properties.

Materials and Methods

Specimens preparation

Twelve sound human third molars extracted due to orthodontic reasons were collected. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were kept in 37°C saline solution (0.9% sodium chloride in water) for no longer than one month before being used in the experiment.

Each tooth was subjected to two cuts in its middle portion, parallel to its long axis, in a mesio-distal direction with a low-speed diamond saw under abundant water cooling (Isomet, Buehler, Lake Bluff, IL, USA), in order to obtain a 1 mm-thick slab, which included both coronal and root dentin. The buccal surfaces of the obtained slabs were polished with silicon carbide paper discs (1,000, 1,200 and 2,500 grit) and 1 μm-polycrystalline diamonds particles (DP-Spray, P; Struers) under water rinsing. Each slab was further divided into two halves (mesial and distal) with a longitudinal cut through its centre (Isomet). The specimens were then kept at room temperature in deionised water.

The mesial half of each specimen was subjected to a 60s ozone application (2,100 ppm equal to 4.2 g/m^3; HealOzone, KaVo, Biberach, Germany), whereas the distal half remained untreated and served as control. The specimens were immediately processed for the measurement of the micromechanical properties.

Measurement of the micromechanical properties

The elastic modulus (E) and the Vicker's hardness (VH) of the dentin specimens were measured with an automatic microhardness indenter (Fischerscope H100C, Fischer, Sindelfingen, Germany). Fifteen indentations were perf ormed in dentin for each specimen, resulting in 180 indentations for each experimental group. The indentation procedure was carried out force controlled. A load application time of 50s was set and subdivided as follows: the force increased at a constant speed from 0.4 mN to 30 mN in 20s, the maximal force of 30 mN was kept constant for 5s, then the force decreased at a constant speed from 30 mN to 0.4 mN in 20s and the minimal force of 0.4 mN was kept constant for 5s. 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.

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 database supplied by the manufacturer, a conversion factor between Universal Hardness and Vicker's hardness (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 substrate (E).

Statistical analysis

The normal distribution of the E and VH data was verified with the Kolmogorov-Smirnov test ($p>0.05$) and a preliminary regression analysis was performed in order to verify that the measured properties were not affected by the tooth within each experimental group ($p>0.05$). In order to compare the E and VH means of the ozone-treated and control groups the t-test for dependent samples was applied and the level of significance was set at $p<0.05$.

Results

According to outcome of the statistical analysis no significant differences in E and VH of the dentin specimens were detected between the ozone-treated and the control group ($p>0.05$). Table 1 reports means and SD of the measured properties in the experimental groups.

Table 1: Means (SD) of the elastic modulus (E) and the Vicker's hardness (VH) of dentin in the experimental groups.

Group	**E (GPa)**	**VH (N/mm^2)**
Ozone	18.4(3.7)	71.7(19.8)
Control	18.1(2.5)	75.1(17.2)

No significant differences of the micromechanical properties were detected between the ozone-treated and the control group (t-test for dependent samples, $p>0.05$).

Discussion

The application of gasiform ozone does not affect the elastic modulus and the Vicker's hardness of dentin. Thus, the tested null hypothesis was rejected.

A detrimental effect on the mechanical properties and on the microhardness of dentin has been reported to occur after the application of some common disinfectants (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) or bleaching agents (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006). Ozone gas has been introduced in the dental practice due to its antimicrobial potential (Azarpazhooh *et al.* 2008; Baysan *et al.* 2000; Bezirtzoglou *et al.* 2008; Polydorou *et al.* 2006), which is enabled by the high oxidative effect of this gas. The effect of ozone on some enamel's physical properties has been previously investigated (Celiberti *et al.* 2006). The study of Celiberti *et al.* (Celiberti *et al.* 2006) reported that ozone did not affect the enamel's hardness compared to the application of dry air for the same duration of the ozone treatment. On the contrary, a reduction of the enamel microhardness was detected when both ozone-treated and air-treated specimens were compared with an untreated baseline group. The authors speculated that this difference was probably due to the dehydration of the substrate occurred during the exposure to ozone or dry air (Celiberti *et al.* 2006).

The application of ozone on both coronal (Schmidlin *et al.* 2005; Magni *et al.* 2008) and root (Bitter *et al.* 2008) dentin prior to adhesive procedures has been also proposed. Due to its higher moisture and organic content compared to that of enamel, dentin could be more sensitive to the highly reactive ozone molecule and to dehydration. If an excessive substrate's dehydration occurs, the exposure of dentin to gasiform ozone might cause a reduction of dentin

wettability (Rosales *et al.* 1999) and could also interfere with bonding procedures (Manso *et al.* 2008).

In order to obtain a simulation of clinical conditions, in the present study the specimens of the control group were not subjected to a dry air flow. In fact, an unmotivated sustained drying of dentin is not indicated clinically. This way a direct comparison of ozone-treated dentin with an unaltered dentin substrate was allowed. As previously observed for enamel's properties (Celiberti *et al.* 2006), the results of the present investigation showed that the modulus of elasticity and the Vicker's hardness of human dentin were also not impaired by a 60s ozone gas application.

Previous studies reported that ozone does not impair bonding proce dures to coronal (Schmidlin *et al.* 2005; Magni *et al.* 2008) and root (Bitter *et al.* 2008) dentin, but no clear information was provided on the interaction between ozone and dentin. The results of this study suggested that ozone does not modify the dentinal substrate. It might be speculated that the absence of a substantial alteration of dentin due to ozone treatment could account for the observed lack of any negative effect of ozone on bonding to dentin. These observations differentiate ozone from those disinfectants (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) and oxidants (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006), which have been reported to alter dentin and also to impair the bonding to dentin (Ozturk *et al.* 2004; Pappas *et al.* 2005; Santos *et al.* 2006; Shinohara *et al.* 2005; Shinohara *et al.* 2004; Spyrides *et al.* 2000; Vieira *et al.* 2003; Cadenaro *et al.* 2006; Gürgan *et al.* 1999). Since common disinfectants or oxidants, such as bleaching agents, used in the dental practice are available mostly as solutions or gels, it could be hypothesized that also the type of formulation could be a critical factor affecting the interaction of these substances with the substrate. A liquid or gel formulation might be responsible

for a sustained persistence of the substance on dental hard tissues, which could act as reservoirs, whereas a gaseous formulation, such that of ozone, might have a milder interaction with the substrate. Nevertheless, more studies should be necessary to better clarify the mechanism of interaction between gasiform ozone and dentin.

The same indentation procedure for the measurement of elastic modulus and Vicker's hardness used in this study has been previously applied to assess the micromechanical properties of enamel and coronal dentin (Magni *et al.* 2010). The E and VH values reported for dentin in the present investigation are slightly higher than those previously reported (Magni *et al.* 2010), probably because the measurement of these properties were also performed in deep coronal and root dentin.

Conclusions

Within the limits of this *in vitro* study, it might be concluded that the application of gasiform ozone does not affect the elastic modulus and the Vicker's hardness of dentin. Thus, the application of ozone on dentin could be performed by the dental clinician without impairing the micromechanical properties of the substrate.

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2.3. Bonding to ozone-treated dentin: a CLSM investigation

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Introduction

The mechanism of bonding to dentin of dental adhesives is based on the resin infiltration into the collagen fibers exposed by dentin demineralization, with the subsequent formation of an hybrid layer (Nakabayashi *et al.* 1982; Nakabayashi *et al.* 1991), and into dentinal tubules and their lateral branches, which result filled by resin tags (Ferrari & Davidson 1996). Whether the extension and quality of hybrid layer and resins tags exert an influence on the bond strength to dentin has been questioned by some authors (Lohbauer *et al.* 2008; de Oliveira *et al* 2009).

The application on dentin of substances with an oxidative potential, such as bleaching agents, has been reported to negatively affect the polymerization (Cadenaro *et al.* 2006) as well as the bond strength (Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005) of dental adhesives. The oxidative process may influence the quality of the collagen fibers network, thus destabilizing or denaturating the dentin organic matrix (Perdigão *et al.* 2004). As dentin may act as an oxygen reservoir, the residual presence of oxygen inside dentinal tubules and within the collagen network (Nikaido *et al.* 1999) could interfere with adhesive resin impregnation (Torneck *et al.* 1990).

Gasiform ozone has been introduced in the dental practice due to its antimicrobial action against cariogenic bacteria (Baysan *et al.* 2000; Baysan & Lynch 2004; Baysan & Beighton 2007; Polydorou *et al.* 2006), which is enabled by the high oxidative potential of ozone molecules. Clinical studies assessed the effect of ozone for the treatment of occlusal (Dähnhardt *et al.* 2006; Huth *et al.* 2005; Knežević *et al.* 2007) and root caries (Baysan & Lynch 2007; Holmes

2003) and, more recently, the application of ozone on dental hard tissues prior to adhesive restorations has also been proposed (Schmidlin *et al.* 2005; Magni *et al.* 2008; Cadenaro *et al.* 2009).

Several methods have been used for characterizing the interface between bonding agents and tooth substrates, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM) (Van Meerbeek *et al.* 2000). A CLSM technique has been recently used for investigating the resin tags formation after bonding to root canal dentin (Malyk *et al.* 2010).

To date, no studies investigated the penetration of dental adhesives into ozone-treated dentin. Thus, the aim of this laboratory investigation was to assess the effect of ozone treatment on the quantity and quality of resin tags by three dental adhesives bonded to coronal dentin. The first tested null hypothesis is that ozone does not alter the adhesive penetration into dentin. Additionally, the second null hypothesis is that there is no difference in the quantity and quality of resin tags formed by the tested adhesives.

Materials and methods

Bonding procedure

Fifteen sound human third molars extracted due to orthodontic reasons were collected. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were kept in 37°C saline solution (0.9% sodium chloride in water) for no longer than one month before being used in the experiment.

The occlusal enamel was removed through grinding with silicon carbide paper discs (600 grit) under water rinsing, until a flat dentin surface was exposed. The same silicon carbide paper discs were used in order to create a clinically relevant smear layer on dentin. Each tooth was then cut in its middle portion, parallel to its long axis, in a mesio-distal direction with a low-speed

diamond saw under abundant water cooling (Isomet, Buehler, Lake Bluff, IL, USA), thus obtaining a buccal and a lingual half.

The buccal halves were subjected to a 60s ozone application (2,100 ppm equal to 4.2 g/m^3; HealOzone, KaVo, Biberach, Germany), whereas the lingual halves remained untreated and served as control.

The teeth were then divided into three experimental groups (n=5), according to the used adhesive system:

Group 1: Excite (Ivoclar-Vivadent, Schaan, Liechtenstein);

Group 2: Syntac/Heliobond (Ivoclar-Vivadent);

Group 3: Silorane Adhesive System (3M ESPE, Seefeld, Germany).

A complete list of batch numbers, chemical compositions and modes of application of the materials used in the study is reported in Table 1.

For the total-etch adhesive systems, dentin was etched with 37% phosphoric acid (Total Etch, Ivoclar-Vivadent) for 15s prior to adhesive application. The adhesives were light-cured (Elipar Freelight 2, 3M ESPE) with an output light of 1,241 mW/cm², measured by means of a calibrated fiber optic spectrally resolving radiometer (S2000, Ocean Optics, Dunedin, FL, USA) equipped with an integrating sphere. The same light-curing unit was used throughout the study.

The fluorescent dyes 0.1% Rhodamine B isothiacyanate (RITC) (Merck, Darmstadt, Germany) or Nile blue (NB) (Merck) were mixed into the components of the adhesive systems, to highlight the resin tags under CLSM, as reported in Table 1.

A 2 mm high composite build-up (Tetric EvoCeram, Ivoclar-Vivadent) was then created on the bonded surface, by adapting a Mylar stripe to the specimens and using it as a matrix.

The specimens were then stored 24h at 37°C in deionised water, prior to be processed for CLSM observation.

Two sections, with a thickness of approximately 300 µm, were obtained from each bonded half, by cutting in a mesio-distal direction with a microtome saw (Leica SP 1600, Leica, Nussloch, Germany).

CLSM observation

Three CLSM images were taken from each bonded section, respectively, at a distance of 500 µm, 1,500 µm and 2,500 µm from the mesial limit of the enamel-dentin junction (LSM-510 Meta microscope, Carl Zeiss, Jena, Germany; water-immersion objective, Achroplan·63×/0.95W). In each image the adhesive penetration was measured along a 100 µm long adhesive/dentin interface. The visualized layer was located 5 µm below the specimen's surface and was 15 µm thick (15 images, distance between images 1 µm). The image analysis and 3D reconstruction were carried out using LSM Image Browser 4.6 (Carl Zeiss). The slices were scanned with 'multitracking' mode, which allowed for the simultaneous visualization of labeled adhesives and dentinal tubules (Fig. 1). To observe the RITC labeled materials, an excitation laser beam with a wavelength of 488 nm (beamsplitter: HFT 405/488) was used, thus allowing emitted fluorescent light to pass through and hit the detector (filter: BP 530–600) (Fig. 1B). To visualize the NB labeled materials an excitation laser beam with a wavelength of 633 nm with an appropriate filter were used (beamsplitter: HFT 405/488/543/633; filter: LP 650) (Fig. 1A). To identify the dentinal tubules, which were not infiltrated by adhesive labeled with fluorescent dye a third channel was added. The reflection mode (excitation laser beam wavelength 633 nm) was used with a special beam splitter (NT 80/20) and a long-pass filter with a detection window for the reflected light (LP 420). In reflection, all dentinal tubules give a strong and clear signal because of a change in the optical properties between the dentine and the immersion medium (deionised water) (Fig. 1C).

Counting of dentinal tubules was performed on images taken on an area 15 μm×100 μm. The tubules density was indicated as tubules/mm^2. The percentages of adhesive-infiltrated tubules for the investigated bonding systems were calculated. Resin tags lengths were also measured.

Additionally, to determine the quality of adhesive penetration into dentinal tubules, completeness, continuity and evenness of resin tags were evaluated with a four-step scoring system previously described by Malyk *et al.* (Malyk *et al.* 2010), as reported below:

Completeness: homogeneity of adhesive penetration into dentinal tubules:
0=90–100% of the dentinal tubules were homogenously filled with adhesive;
1=50–90% of the dentinal tubules were homogenously filled with adhesive;
2=10–50% of the dentinal tubules were homogenously filled with adhesive;
3=0–10% of the dentinal tubules were homogenously filled with adhesive.

Continuity: uninterruptedly of the resin tags:
0=90–100% of resin tags were not interrupted;
1=50–90% of resin tags were not interrupted;
2=10–50% of resin tags were not interrupted;
3=0–10% of resin tags were not interrupted.

Evenness: equability of the resin tags length:
0=90–100% of resin tags were of equal length;
1=50–90% of resin tags were of equal length;
2=10–50% of resin tags were of equal length;
3=0–10% of resin tags were of equal length.

Statistical analysis

The normality of data distribution and the homogeneity of variances for the percentages of adhesive-infiltrated tubules were verified with the Kolmogorov-Smirnov test and the Levene's test. The percentages of adhesive-infiltrated tubules were compared between the ozone-treated and the control specimens for each adhesive with the t-test for paired samples. Moreover, the percentages of adhesive-infiltrated tubules were compared among the tested adhesives with the One-Way ANOVA, and the Tukey's test was applied for post-hoc comparisons.

Being the measured resin tags lengths not normally distributed in the experimental groups (Kolmogorov-Smirnov test), the mean tags lengths of the tested adhesives were compared with the Kruskal-Wallis Analysis of Variance followed by the Mann-Whitney test with Bonferroni's correction for post-hoc comparisons. Additionally, the Wilcoxon signed ranks test was applied for the comparison of the tags lengths data between ozone-treated and control specimens within each adhesive.

The Kruskal-Wallis Analysis of Variance was applied for comparing the scores of completeness, evenness and continuity of resin tags among the tested adhesives and the Mann-Whitney test with Bonferroni's correction was used for pairwise multiple comparisons. The Wilcoxon signed ranks test was applied for comparing the completeness, evenness and continuity of resin tags between the ozone-treated and control group within each adhesive.

The statistical analysis was handled with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA). In all the analyses the level of significance was set at $p<0.05$.

Fig. 1 Representative CLSM image of a Syntac/Heliobond specimen in the control group (magnification 63×). The Syntac Primer is labeled with the fluorescent dye Nile blue (**A**); Syntac Adhesive and Heliobond are labeled with 0.1% Rhodamine B isothiacyanate (**B**); the dentinal tubules are visible in the reflection mode (**C**); combination of the fluorescent and reflection modes (**D**).

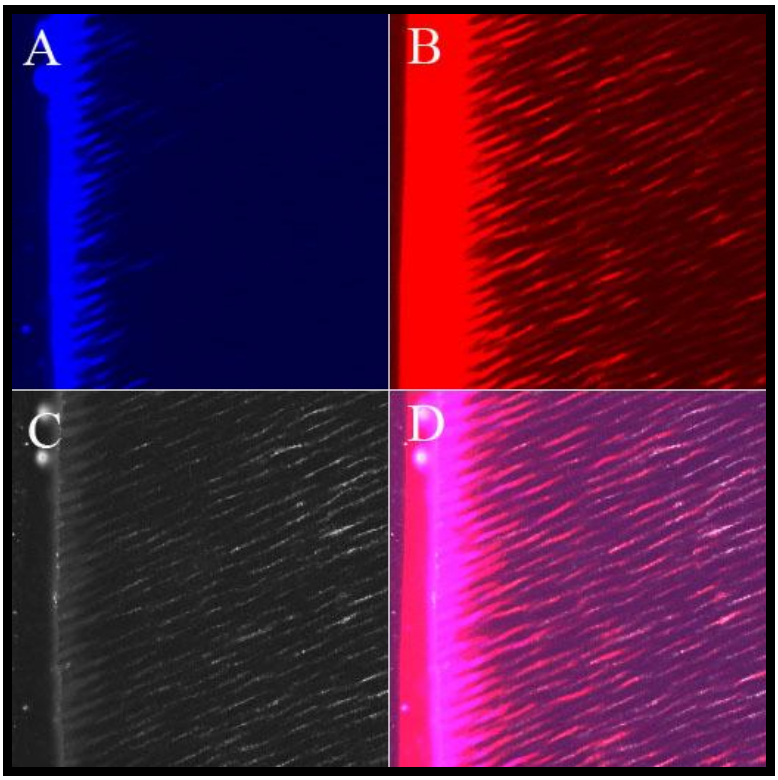

Table 1: Chemical compositions, batch numbers and modes of use of the materials used in the study.

Material	Composition	Mode of use
Total Etch* (Ivoclar-Vivadent) Batch # K14609	37% phopsphoric acid gel	Application on dentin; activation with a microbrush for 15s; rinsing with abundant deionized water; gently air drying
Excite[1] (Ivoclar-Vivadent) Batch # J18718	HEMA (2-hydroxyethyl methacrylate) Dimethacrylates Phosphonic acid acrylate Highly dispersed silicon dioxide Initiators and stabilizers Alcohol	Application on dentin surface with a microbrush for 10s; Gently air drying for 3s; Lightcuring 20s.
Syntac/Heliobond (Ivoclar-Vivadent) Syntac Primer[2]: Batch # K08247 Syntac Adhesive[1]: Batch # K02656 Heliobond[1]: Batch # K01560	Syntac Primer: Polyethylene glycol dimethacrylate, Maleic acid, Acetone, Water; Syntac Adhesive: Polyethylene glycol dimethacrylate, Glutaraldehyde, Water Heliobond: Bis-GMA (Bisphenol-A-glycidyldimethacrylate), Triethylene glycol dimethacrylate	Application of Syntac Primer on dentin surface with a microbrush for 15s; Air drying; Application of Syntac Adhesive with a microbrush; Leave undisturbed 10s; Air drying; Application of Heliobond with a microbrush; Gently air blowing; Lightcuring 20s.
Silorane Adhesive System (3M ESPE) Self-etch Primer[2]: Batch # 292319 Bond[1]: Batch # 292274	Self-etch Primer: Phosphorylated methacrylates, Vitrebond™ copolymer, Bis-GMA (Bisphenol-A-glycidyldimethacrylate), HEMA (2-hydroxyethyl methacrylate), Water, Ethanol, Silane-treated silica filler, Initiators, Stabilizers Bond: Hydrophobic dimethacrylate, Phosphorylated methacrylates, TEGDMA (Triethylene glycol dimethacrylate), Silane-treated silica filler, Initiators, Stabilizers	Application of Self-etch Primer on dentin surface for 15s; Air drying; Lightcuring 10s; Application of Bond; Air drying; Lightcuring 20s.
Tetric EvoCeram (Ivoclar-Vivadent) Batch # L16680	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 20s.

*Total Etch was used in combination with the investigated total-etch adhesives.

[1]Labeled with Rhodamine B isothiacyanate.

[2]Labeled with Nile blue.

Results

The statistics showed that the percentage of adhesive-infiltrated tubules was not affected by the ozone treatment for all tested adhesives (t-test for paired samples, $p>0.05$; Table 2). The One-way ANOVA detected a significant difference in the amount of infiltrated tubules among the tested adhesives ($p<0.05$). The combination Syntac/Heliobond showed a significantly higher percentage of infiltrated tubules compared to Excite and Silorane Adhesive System (Table 2). The amount of adhesive-infiltrated tubules of Silorane Adhesive System was significantly lower as for Syntac/Heliobond and Excite (Table 2).

The Wilcoxon test showed that ozone did not affect the completeness, evenness and continuity of resin tags for all tested adhesives (Table 3). The Kruskal-Wallis Analysis of Variance detected significant differences in the completeness of resin tags among the tested adhesives ($p<0.05$). Silorane Adhesive System has shown a significantly worse completeness mean score than Syntac/Heliobond and Excite, which were comparable (Table 3). Statistically significant differences among the adhesives were also detected in the evenness of resin tags ($p<0.05$). Syntac/Heliobond had a significantly higher evenness mean score than Excite ($p<0.001$) but was comparable with Silorane Adhesive System. The evenness scores of Excite and Silorane Adhesive System were also comparable (Table 3). The continuity scores were significantly different among the tested adhesives ($p<0.05$). Syntac/Heliobond achieved the best score for the continuity of resin tags that was significantly different from those of Excite and Silorane Adhesive System, which were comparable (Table 3).

Table 4 reports the means and standard deviations of the measured resin tags lengths in the experimental groups. No differences were detected between the ozone and control group within each adhesive ($p>0.05$). Syntac/Heliobond

showed a significantly higher mean resin tags length than Excite and Silorane Adhesive System ($p<0.05$). The latter presented the lowest resin tags length (Table 4).

Table 2: Means (SD) of the percentages of adhesive-infiltrated tubules in the experimental groups.

Adhesive	Infiltrated tubules (%)		
	Global	Ozone	Control
Excite	80.1(11.2)B [23022(5371)]	83.7(8.3)a [23867(5204)]	79.0(11.3)a [22178(5489)]
Syntac/Heliobond	86.0(7.0)C [17602(3928)]	86.0(7.7)a [18000(3660)]	85.9(6.7)a [17161(4231)]
Silorane Adhesive System	67.1(13.6)A [19845(4731)]	64.4(10.9)a [20092(4590)]	70.0(15.7)a [19580(4952)]

The means (SD) of the density of dentin tubules (tubules/mm^2) are reported in square brackets. Different uppercase letters indicate significant differences within the column ($p<0.05$). The same lowercase letters indicate statistically comparable groups within the rows ($p>0.05$).

Table 3: Means (SD) and medians of the scores of completen ess, evenness and continuity of resin tags in the experimental groups.

Adhesive		Completeness	Evenness	Continuity
Excite	Global	0.85(0.4)A [1]	1.00(0.2)A [1]	1.05(0.5)B [1]
	Ozone	0.80(0.4)a [1]	0.97(0.2)a [1]	0.97(0.6)a [1]
	Control	0.90(0.3)a [1]	1.03(0.2)a [1]	1.13(0.4)a [1]
Syntac/Heliobond	Global	0.79(0.4)A [1]	1.28(0.6)B [1]	0.56(0.5)A [1]
	Ozone	0.80(0.4)a [1]	1.33(0.5)a [1]	0.50(0.6)a [0]
	Control	0.78(0.4)a [1]	1.22(0.6)a [1]	0.63(0.5)a [1]
Silorane Adhesive System	Global	1.07(0.4)B [1]	1.14(0.6)AB [1]	0.95(0.5)B [1]
	Ozone	1.00(0.3)a [1]	1.17(0.6)a [1]	1.03(0.5)a [1]
	Control	1.15(0.5)a [1]	1.11(0.5)a [1]	0.85(0.5)a [1]

Medians are reported in square brackets. Different uppercase letters indicate significant differences in each characteris tic among the adhesives ($p<0.05$). Identical lowercase letters indicate statistically similar subgroups within each adhesive ($p>0.05$).

Table 4: Means (SD) of the resin tags lengths (μm) in the experimental groups.

Adhesive	Resin tags length (μm)		
	Global	Ozone	Control
Excite	14.80(6.2)B	14.39(6.4)a	15.23(6.1)a
Syntac/Heliobond	22.25(7.0)C	22.38(6.9)a	22.09(7.2)a
Silorane Adhesive System	14.00(6.5)A	14.05(7.2)a	13.97(5.6)a

Different uppercase letters indicate significant differences among the adhesives ($p<0.05$). Identical lowercase letters indicate statistically similar subgroups within each adhesive ($p>0.05$).

Discussion

The statistical analysis revealed that an ozone application on dentin did not affect the penetration of the tested dental adhesives into dentin in terms of percentage of infiltrated dentinal tubules, quality of the formed resin tags and resin tags length. Thus, the first null hypothesis was accepted. On the contrary, significant differences in all investigated parameters were detected when comparing quantitatively and qualitatively the infiltration of the three tested adhesive systems. Thus, the second null hypothesis was rejected.

The interaction of adhesive systems with dentinal substrate is mediated by the infiltration of the primer into the acid conditioned dentin and by its interdiffusion within the collagen fibers network along the first portion of the inner wall of dentinal tubules and the subsequent polymerization of the primer upon evaporation of solvent (Pashley *et al.* 1993; Suh 1991; Ferrari 1994). The bonding resin diffuses into the primer-infiltrated intertubular dentin and into dentinal tubules forming resin tags and its adhesion to the first portion of tubules walls is mediated by the primer.

After the application of substances with an oxidative potential, dentin may act as an oxygen reservoir (Swift & Perdigão 1998; Shinohara *et al.* 2004), thus impairing the polymerization of the adhesive monomers and potentially altering the substrate/adhesive system micromorphologic interaction. The high oxidative effect of gaseous ozone, which enables its antimicrobial activity, might result in an unfavourable oxidation of the dentinal substrate, which could lead to a qualitative or quantitative impairment of the adhesive resin penetration into dentin. This study investigated several aspects of resin tags formation by three different adhesive systems bonded to ozone-treated dentin. In order to perform a reliable and direct comparison between ozone-treated and control group, two halves of the same tooth were used as the test and as the control specimen. Moreover, the evaluation of the dentinal tubules density confirmed

that the bonding procedures were performed to homogeneous substrates in the compared experimental groups (Table 2) and the measured tubules densities were in agreement with previously reported data (Mjör & Fejerskov 1986). The results revealed that an ozone application did not influence the quality and quantity of the formed resin tags, since all investigated parameters were not altered in the ozone-treated specimens. These findings are in agreement with those previous studies, which reported that dentinal pretreatment with gasiform ozone did not compromise bonding to dentin (Schmidlin *et al.* 2005; Magni *et al.* 2008; Cadenaro *et al.* 2009). It might be speculated that the volatility of a gaseous substance, such as ozone, did not allow for the formation of a consistent amount of oxygen by-products within the dentinal substrate, which could impair the adhesion of the primer and bonding resins to the tubular walls and subsequently the quantity and quality of the resulting resin tags.

Three different adhesives were investigated in the present study. The combination Syntac/Heliobond is a three-step total-etch adhesive system, which requires an additional step for the application of the unfilled Heliobond bonding resin. The results showed that this adhesive system achieved the highest percentage of infiltrated dentinal tubules and formed longer and more continuous resin tags compared to the other two investigated adhesives. These findings support the observation that there was a general trend of adhesive systems with separate primer and bonding agents to perform better than simplified adhesives in terms of bonding to dentin (Frankenberger & Tay 2005). Moreover, a literature review on the durability of adhesion to tooth tissues highlighted the superiority of three-step total-etch adhesive systems compared to the other commercially available bonding agents (De Munck *et al.* 2005). Nevertheless, it has also been pointed out that acetone-based adhesive systems, such as Syntac/Heliobond, could be more technique-sensitive, due to required "wet-bonding" method and this issue could increase the difficulty of applying them properly in complex cavity configurations (De Munck *et al.* 2005).

Therefore, it might also be speculated that the application of Syntac/Heliobond on a flat dentinal surface could have contributed to optimize its performance in terms of dentin infiltration in the present study. Excite is a two-step ethanol-based total-etch adhesive system, which combines the primer and the bonding resin in a single bottle. The trend of this type of simplified bonding agents to perform slightly worse than those systems with separate priming and bonding steps has been previously observed (Frankenberger & Tay 2005; De Munck *et al.* 2005). According to the results of the present investigation, Excite achieved a lower percentage of infiltrated dentinal tubules and formed shorter resin tags than Syntac/Heliobond, but performed better than Silorane Adhesive System. As far as the quality of the formed resin tags was concerned, Excite exhibited the trend to form tags with more homogeneous, though inferior, lengths compared to the combination Syntac/Heliobond. It might be speculated that the unfilled resins, on which the combination Syntac/Heliobond is based, presented better flow properties, which allowed for a deeper penetration into dentinal tubules, which was limited mostly by the patency of the tubules along their lengths. On the other side, the presence of the filler in the formulation of Excite might have contributed to a slight reduction of the flow properties of this adhesive into the tubules leading to more uniform resin tags lengths. The resin tags formed by Excite presented more irregularities along their lengths than those formed by Syntac/Heliobond and it might be speculated that the difficulty in obtaining a complete solvent removal, which was previously reported for two-step etch-and-rinse adhesive systems (De Munck *et al.* 2005), could have hampered this adhesive in infiltrating the acid conditioned dentin fully. Silorane Adhesive System is a two-step self-etch adhesive system, which was developed for being used in combination with a silorane-based composite. The percentage of infiltrated dentinal tubules and the mean resin tags length of this adhesive, as well as its completeness scores, were worse than those showed by the two investigated total-etch systems. Besides the lack of an acid etching step, the

peculiar composition of the priming resin of this adhesive system could have contributed to the obtained results. In fact, the primer contains also glass-ionomer compounds, which are not usually included in the formulations of conventional self-etch adhesive systems (Moszner *et al.* 2005) and this resin requires an additional light-curing step to ensure optimal polymerization. These particular characteristics of the primer of Silorane Adhesive System were aimed at addressing the requirement of bonding the highly hydrophobic silorane-based composite to a hydrophilic substrate, such as dentin, since they guarantee an optimal seal of dentinal tubules and prevent any contact between the restorative material and the moist substrate. On the other hand, the infiltration of this adhesive system might have been hampered by the presence of a thick priming resin layer.

The confocal laser scanning microscopy was applied in the present study for observing the dentin infiltration of the tested adhesive systems. This technique has been successfully applied for the observation of adhesive interfaces between tooth substrates and dental materials, without dehydrating the specimens with the risk of introducing artefacts (Van Meerbeek *et al.* 2000). The reflection mode was used in this study in order to highlight the dentinal tubules and it allowed for a reliable estimation of the dentinal tubules density, since the results are comparable with data reported in textbooks of oral histology (Mjör & Fejerskov 1986). As the counting of dentinal tubules seemed to be trustworthy, the evaluation of the adhesive-infiltrated tubules, which was enabled by the fluorescent dyes, should have also been performed with a good approximation. However, the correlation between the adhesive infiltration into dentin, which can be assessed with microscopy techniques, and the resultant bond strength to dentin is still controversial (Lohbauer *et al.* 2008; de Oliveira *et al* 2009) and should be further investigated.

Within the limits of this laboratory study it can be concluded that the application of gaseous ozone does not affect the infiltration of the investigated

adhesive systems into dentin. Moreover, the combination of Syntac/Heliobond achieves a higher percentage of infiltrated tubules and forms longer and more regular resin tags than Excite and Silorane Adhesive System.

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2.4a. Evaluation of the mechanical properties of dental adhesives and glass-ionomer cements

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Introduction

The interface between a resin composite restoration and the tooth is subjected to stresses potentially leading to debonding and subsequent clinical failure of the restoration. The interfacial stress occurs even before the restored tooth is subjected to functional load, due to the polymerization shrinkage of the composite (Feilzer *et al.* 1988). An adhesive or a lining/base material may act as an elastic intermediate layer between the tooth and the resin composite. These materials can resist polymerization shrinkage (Kemp-Scholte *et al.* 1990) and absorb the shock produced by occlusal loads (Van Meerbeek *et al.* 1993). Besides conventional adhesives, flowable composites (Magni *et al.* 2007) or glass-ionomer cements (GICs) (Liebenberg 2006) could also be used for this purpose. Nevertheless, the information on the interfacial stress between restoration and tooth, as well as on the materials which could relieve this stress, is mainly based on the results of laboratory studies, whereas a clinical validation is still lacking.

Due to the ability to bond directly to dental tissues, that can be enhanced by a short polyalkenoic acid pretreatment for conditioning tooth surface (De Munck *et al.* 2005), and thanks to the property of fluoride release (Nikolaos *et al.* 2007), glass-ionomer cements have found several applications in the dental practice. Besides their use as base materials (Liebenberg 2006), they have been used for core build-up procedures (Bayindir *et al.* 2004) and for luting prosthetic restorations (Farrell *et al.* 2008) and orthodontic bands (Youn *et al.* 2007). They have also been proposed for the atraumatic restorative treatment (ART)

technique (Frencken *et al.* 2006), and as dental sealants (Papacchini *et al.* 2005; Papacchini *et al.* 2006). They are suitable for class III and V cavity restorations, whereas for class I or II restorations their use is advisable only in low stress bearing areas (Tyas & Burrow 2004).

The limited use of conventional glass-ionomer cements in the posterior region could be ascribed to their poor mechanical properties, compared to those of resin composites (Mount 1999; Xie *et al.* 2000). Later, resin-modified glass-ionomer cements were launched on the market. The latter showed improved mechanical properties, comparable to those of microfilled and packable composites (Ilie & Hickel 2007), though poor wear rates.

Although the mechanical properties of conventional and resin-modified glass-ionomer cements have already been assessed and compared to those of resin composites, information regarding the mechanical behaviour of these materials in comparison with bonding agents is still lacking. In a literature review by Peumans et al. (Peumans *et al.* 2005), glass-ionomer cements have been reported to provide a better clinical retention of non-carious cervical restorations as compared with conventional adhesives (Peumans *et al.* 2005). Conversely, laboratory studies reported lower bond strengths to tooth substrates for glass-ionomer cements compared to those of composite restorations bonded with dental adhesives (Papacchini *et al.* 2005; Wu *et al.* 2007). However, recent studies showed an improvement of the clinical performance of simplified adhesives (Van Landuyt *et al.* 2008; Abdalla & Garcia-Godoy 2007). Besides the loss of retention, the marginal breakdown was also reported to be a common reason for restorations' failure (Hickel & Manhart 2001). The loss of marginal integrity could also be related to poor mechanical properties of the bonding and/or restorative materials. Thus, it could be of interest to assess the mechanical features of these materials.

Therefore, the aim of this laboratory study was to compare the mechanical properties of eight adhesives (five one-step self-etch, two two-steps

self-etch and one two-steps etch-and-rinse) and six glass-ionomer cements (two conventional and four resin-modified) and to confront them with those of enamel and dentin. The tested null hypotheses were (1) that the mechanical properties of the materials within each class of materials are comparable and (2) that the mechanical properties of GICs and adhesives are similar to each other and also to those of enamel and dentin.

Materials and methods

Specimens preparation and measurement of the mechanical properties

Batch numbers, chemical compositions and modes of use of all the materials used in this study are reported in Table 1.

Forty sound human third molars were collected for the preparation of the adhesives specimens. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were disinfected in 2.5% sodium hypochlorite for 2min and stored in distilled water at 4°C until they were used for the study. The superficial enamel was removed from the occlusal aspect of the teeth by grinding with a wet 220-grit silicon carbide paper disk. The crowns were sectioned perpendicular to the long axis in order to obtain one 1-mm thick slice from each tooth (Isomet; Buehler, Lake Bluff, IL, USA). The peripheral enamel was removed with wet 220-grit silicon carbide paper in order to obtain disk-shaped specimens consisting only of dentin. A clinically relevant smear layer was created by grinding the occlusal aspect of each dentin disk with 600-grit silicon carbide paper under water cooling. Five teeth were assigned to each tested adhesive resulting in five bonded dentin disks. For the adhesives based on the etch-and-rinse technique (Admira Bond and Solobond Plus; VOCO), dentin was previously etched with 35% phosphoric acid gel (Vococid; VOCO, Batch # 560819) for 15s, abundantly rinsed with deionised water and air dried. All the

tested adhesives were applied on the dentin disks and light-cured through a Mylar stripe according to their respective modes of use as reported in Table 1.

In order to obtain the GICs specimens, a 2 mm × 3 mm × 2 mm teflon mold with opened upper and lower surfaces was positioned on a glass plate with a polyacetate sheet interposed. The materials were introduced into the mold and the upper surface was covered with a polyacetate sheet. A second glass plate was compressed on the upper surface of the mold in order to obtain specimens with flat surfaces. The specimens of the resin-modified GICs were light-cured in a top-to-bottom direction and then left undisturbed for 15 min in the mold prior to be stored. The conventional GICs were simply left undisturbed for 15 min in the mold prior to storage. Five specimens were prepared with each tested GIC.

The same LED curing unit (Elipar Freelight 2; 3M ESPE) was used throughout the study. The spectral distributions and the irradiance of the curing unit were determined by means of a calibrated fiber optic spectrally resolving radiometer equipped with an integrating sphere (S2000; Ocean Optics, Dunedin, FL, USA). The total irradiance was obtained by the integrate calculus of the irradiance as a function of the wavelength over the entire wavelength range, divided by the effective area of the curing unit tip. The diameter of the tip was measured with a digital micrometer and the effective area was defined as the area of the tip without cladding. The total irradiance of the curing unit was 1226 mW/cm^2.

All the specimens were stored for 24 h prior to testing of mechanical properties. The GICs specimens were stored in deionised water at 37°C, whereas the adhesives were kept in an environment 100% saturated with humidity at 37°C. Vicker's hardness, modulus of elasticity, elastic indentation work and creep of the tested materials were assessed. The measurements were performed by means of a micro hardness indenter (Fischerscope H100C; Fischer, Sindelfingen, Germany). The test procedure was carried out force controlled. A load application time of 50s was set and subdivided as follows: the force

increased at a constant speed from 0.4 mN to 30 mN in 20s, the maximal force of 30 mN was kept constant for 5s, then the force decreased at a constant speed from 30 mN to 0.4 mN in 20s and the minimal force of 0.4 mN was kept constant for 5s. The load and the penetration depth of the indenter (Vicker's pyramid: diamond right pyramid with an angle $\alpha = 136°$ between the opposite faces at the vertex) were continuously measured during the load -unload cycle.

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 database supplied by the manufacturer, a conversion factor between Universal Hardness and Vicker's hardness (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).

The total mechanical work (Wtot) was measured during the indentation procedure according to the formula $W = \int F dh$ (F=load; h=indentation depth). The plastic deformation work (Wp) and the work of the elastic reverse deformation (We), which are the two components of the mechanical work, were also measured. The elastic indentation work (We/Wtot) was calculated as the percentage of Wtot represented by We.

By measuring the variation of the indentation depth occurring when the maximal force was kept constant, a relative change of the indentation depth was calculated. This value represented the creep of the material. The creep (Cr) is defined as the ratio between the change in indentation depth measured during the 5s in that the force of 30 mN was maintained constant and the indentation depth measured at the maximal force of 30 mN.

The above mentioned mechanical properties had also been measured in enamel and dentin in a preliminary study of the authors, starting from the cuspal

tip and performing measurements from enamel to dent in with a distance of 100 μm between each measurement point (Fig. 1 A -D). The resulting mean values of the mechanical properties of enamel and dentin were also compared to those of the two tested materials' classes.

Fig. 1 The graphs represent the variation of (**A**) the Vicker's hardness (VH), (**B**) elastic modulus (E), (**C**) creep (Cr) and (**D**) elastic indentation work (We/Wtot) measured in enamel and dentin as function of the distance from the cuspal tip.

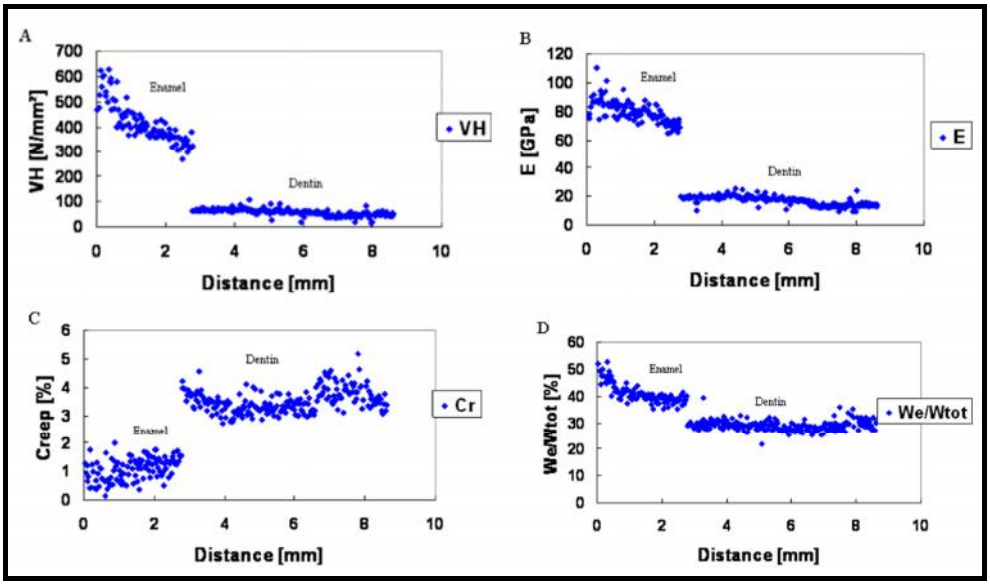

Table 1: Batch numbers, chemical compositions and modes of use of the materials used in the study.

Material	Type	Composition	Mode of use
Admira Bond (VOCO) Batch # 580606	Two-steps etch-and-rinse adhesive	Ormocers, Bis-GMA, HEMA, BHT, Acetone, Organic acids	Apply on dentin 30s. Air dry. Light-cure 20s.
Futurabond NR (VOCO) Batch # 610643	One-step self-etch adhesive	*Liquid A*: Polyfunctional adhesive monomers (Methacroyl-Phosphorus-Acid-Ester, Methacroyl-Carbon-Acid-Ester), Dimethacrylates, Functionalized SiO_2-nano-particles, Initiators *Liquid B*: Ethanol, Water, Hydrophilic adhesive monomers, Fluorides	Mix Liquid A and Liquid B 5s. Apply mixture on dentin and massage 20s. Air dry 5s. Light-cure 20s.
Solobond Plus (VOCO) Batch # 591648	Three-steps etch-and-rinse adhesive	*Primer*: Water, Acetone, Maleic acid, Acid-functionalized methacrylates, Fluorides *Adhesive*: Acetone, Dimethacrylate, Hydroxymethacrylate	Apply Primer 30s. Apply Adhesive 15s. Air dry. Light-cure 20s.
Hybrid Bond (Sun Medical) Batch # LS2	One-step self-etch adhesive	*Hybrid Base*: Methyl methacrylate, 4-methacryloxyethyiltrimellitic acid anhydride, Tris(2-hydroxyethyl)-isocyanurat-triacylate, HEMA, Acetone, Water *Hybrid Brush*: Sodium p-toluenesulfinate, Aromatic amine	Dispense few drops of Hybrid Base in the Plastic Dispensing Dish. Stir with Hybrid Brush for few seconds. Apply on dentin 20s. Air blow 5-10s. Light-cure 20s.
Clearfil SE Bond (Kuraray) Batch # 41471	Two-steps self-etch adhesive	*Primer*: HEMA, MDP, Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, Water, Accelerators, Dyes and others *Bond*: HEMA, Bis-GMA, MDP, Hydrophobic aliphatic dimethacrylate, Colloidal silica, dl-Camphorquinone, Initiators, Accelerators and others	Apply the Primer on dentin. Leave in place 20s. Air dry. Apply the Bond. Make the Bond film uniform with a gentle air blow. Light-cure 20s.
Clearfil S^3 Bond (Kuraray) Batch # 41117	One-step self-etch adhesive	2 HEMA, Ethanol, Bis-GMA, MDP, Colloidal silica, dl-Camphorquinone, Water, Initiators, Accelerators and others	Apply on dentin. Leave in place 20s. Air dry more than 5s. Light-cure 20s.
Clearfil Protect Bond (Kuraray) Batch # 41130	Two-steps self-etch adhesive	*Primer*: HEMA, MDP, MDPB, Hydrophilic aliphatic dimethacrylate, Water, Initiators, Accelerators, Dyes and others *Bond*: HEMA, sodium fluoride, Bis-GMA, MDP, Hydrophobic aliphatic dimethacrylate, Colloidal silica, dl-Camphorquinone, Initiators, Accelerators and others	Apply the Primer on dentin. Leave in place 20s. Air dry. Apply the Bond. Make the Bond film uniform with a gentle air blow. Light-cure 20s.
Experimental i Bond (Heraeus Kulzer) Batch # VP130706 Ep1	One-step self-etch adhesive	Acetone, Water, Glutaraldehyde, 4-META	Apply on dentin 30s. Air dry. Light-cure 20s.
Fuji Fil LC (Shade A3) (GC) Batch # 0512161	Resin-modified GIC	*Paste A*: Aluminosilicate glass, HEMA, Urethanedimethacrylate *Paste B*: Distilled water, Polyacrylic acid, Urethanedimethacrylate, Silicone dioxide	Mix Paste A and Paste B 15s. Dispense in the mold. Light-cure 20s.
Fuji II LC improved (Shade A3) (GC) Batch # 05101112	Resin-modified GIC	*Powder*: Aluminosilicate glass *Liquid*: Polyacrylic acid, HEMA, Proprietary ingredient, 2,2,4, Trimethyl hexamethylene dicarbonate	Mix powder with liquid no more than 20-25s. Light cure 20s.

Fuji IX (GC) Batch # 0512081	Conventional GIC	*Powder*: Polyacrylic acid, Aluminosilicate glass *Liquid*: Polyacrylic acid, Proprietary ingredient	Mix powder with liquid 15-20s. Dispense in the mold.
Photac Fil (Shade A3) (3M ESPE) Batch # 238979	Resin-modified GIC	HEMA, Polyalkenoic acid, Fluoroaluminosilicate glass	Dispense directly in the mold. Light cure 20s.
Vitremer (Shade A3) (3M ESPE)Batch # 200512272	Resin-modified GIC	Modified polyalkenoic acid, Fluoroaluminosilicate glass	Mix powder with liquid. Dispense in the mold. Light-cure 20s.
Ionofil Molar (VOCO) Batch # 580342	Conventional GIC	Polyacrylic acid, Fluoride silicate glass	Mix powder with liquid. Dispense in the mold.

Abbreviations: HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA: Bisphenol A diglycidylmethacrylate; MDPB: 12-methacryloyoxydodecylpyridinium bromide; 4-META: 4-methacryloxyethyltrimellitic anhydride; BHT: Butylated hydroxy toluene.

Statistical analysis

The normal distribution of the data and the homogeneity of variances were verified with the Kolmogorov-Smirnov test and the Levene's test respectively. The mechanical properties were then compared within each materials' class (adhesives and GICs) using the One-Way Analysis of Variance. The data of each mechanical property of all the materials belonging to the same class were pooled together, and a comparison among the average values of the mechanical properties of the two materials' types and those of enamel and dentin was performed by means of the One-Way Analysis of Variance. The Tukey HSD test was used for post-hoc multiple comparisons. In all the analyses the level of significance was set at $p<0.05$. The calculations were handled with SPSS 14.0 software for Windows (SPSS Inc.; Chicago, IL, USA).

Results

Tables 2 and 3 report the descriptive statistics of the mechanical properties of adhesives and GICs respectively. Fig. 2 reports the comparison of the mechanical properties of the materials between the two tested materials' classes and enamel and dentin.

The statistical analysis revealed significant differences in the mechanical properties among the tested adhesives (Table 2). Admira Bond and Clearfil Protect Bond showed comparable VH values ($p>0.05$), which were significantly lower as compared to those of the other tested adhesives ($p<0.05$). No significant differences in VH were detected among Futurabond NR, Solobond Plus, Clearfil SE Bond, Clearfil S^3 Bond and experimental i Bond ($p>0.05$). Hybrid Bond exhibited the highest VH value (25.6 ± 5.6 N/mm^2), which was comparable to those of Solobond Plus, Clearfil S^3 Bond and experimental i Bond ($p>0.05$).

Hybrid Bond showed the highest E (5.3 ± 1.0 GPa), though comparable to that of experimental i Bond ($p>0.05$). On the contrary, the lowest E value (3.6 ± 0.7 GPa) was shown by Clearfil Protect Bond and it was statistically similar to the E of Futurabond NR ($p>0.05$) and significantly lower to the E of all the other tested materials ($p<0.05$). No statistically significant differences in E were observed among Admira Bond, Futurabond NR, Clearfil SE Bond and Clearfil S^3 Bond ($p>0.05$). The E of Solobond Plus was significantly higher than the E of Futurabond NR ($p<0.05$) but similar to those of Admira Bond, Clearfil SE Bond, Clearfil S^3 Bond and experimental i Bond ($p>0.05$).

Concerning the creep, Solobond Plus exhibited a significantly lower value compared to the other tested materials ($p<0.05$). The Cr of Futurabond NR, Hybrid Bond and Clearfil S^3 Bond were statistically similar, as well as the Cr of Admira Bond, Clearfil SE Bond, Clearfil Protect Bond and of experimental i Bond ($p>0.05$). Moreover, Clearfil S^3 Bond showed a comparable creep to those of Clearfil SE Bond and experimental i Bond ($p>0.05$). Futurabond NR and Solobond Plus showed significantly higher values of elastic indentation work than the other tested adhesives ($p<0.05$). The We/Wtot of Hybrid Bond, Clearfil SE Bond, Clearfil S^3 Bond, Clearfil Protect Bond and experimental i Bond resulted statistically comparable ($p>0.05$). Admira Bond

presented the lowest elastic indentation work, though similar to those of Hybrid Bond, Clearfil Protect Bond and experimental i Bond.

The statistical analysis showed significant differences in the mechanical properties among the tested GICs ($p<0.05$) (Table 3). With respect of the VH, Photac Fil showed the lowest VH, which was comparable to those of Fuji Fil LC and Vitremer, but significantly lower than those of the other three tested GICs ($p<0.05$). The VH of Fuji Fil LC, Vitremer, and Ionofil Molar were statistically similar and significantly lower than those of Fuji II LC and Fuji IX ($p<0.05$), which were significantly higher in comparison to all the other tested GICs ($p<0.05$). The E of Fuji IX was significantly higher than those of the other GICs ($p<0.05$), followed by the E of Fuji II LC. Ionofil Molar, Fuji Fil LC and Photac Fil showed statistically homogeneous E values ($p>0.05$). The E of Vitremer was lower than those of all the other tested GICs, and the differences were significant when the latter was compared to Ionofil Molar, Fuji II LC and Fuji IX ($p<0.05$). Significant differences in the creep were detected only between Fuji IX and, respectively, Vitremer and Fuji Fil LC. The former presented a significantly lower creep ($p<0.05$). The We/Wtot of Fuji IX was significantly lower than that of Fuji II LC and that of Vitremer ($p<0.05$). The latter showed a We/Wtot significantly higher than the other GICs ($p<0.05$).

Finally, when the mechanical properties of the two classes of materials and those of enamel and dentin were compared, statistically significant differences in the E, creep and elastic indentation work were detected among adhesives, GICs, dentin and enamel (Fig. 2 A-C). Enamel presented the highest E value (Fig. 2A), followed by dentin, GICs and adhesives ($p<0.05$). The creep (Fig. 2B) of enamel was the lowest, followed by dentin, GICs and adhesives ($p<0.05$). Enamel showed also the highest We/Wtot (Fig. 2C), followed by adhesives, GICs and dentin ($p<0.05$). Regarding the VH (Fig. 2D), no significant difference was found between dentin and GICs ($p>0.05$) and both

showed significantly higher VH values than the adhesives and significantly lower when compared to enamel ($p<0.05$).

Fig. 2 The graphs represent the comparison among the mechanical properties of the two tested materials' classes and of enamel and dentin. Means(SD) are reported. The asterisks indicate statistically similar groups (Tukey HSD test, $p>0.05$).

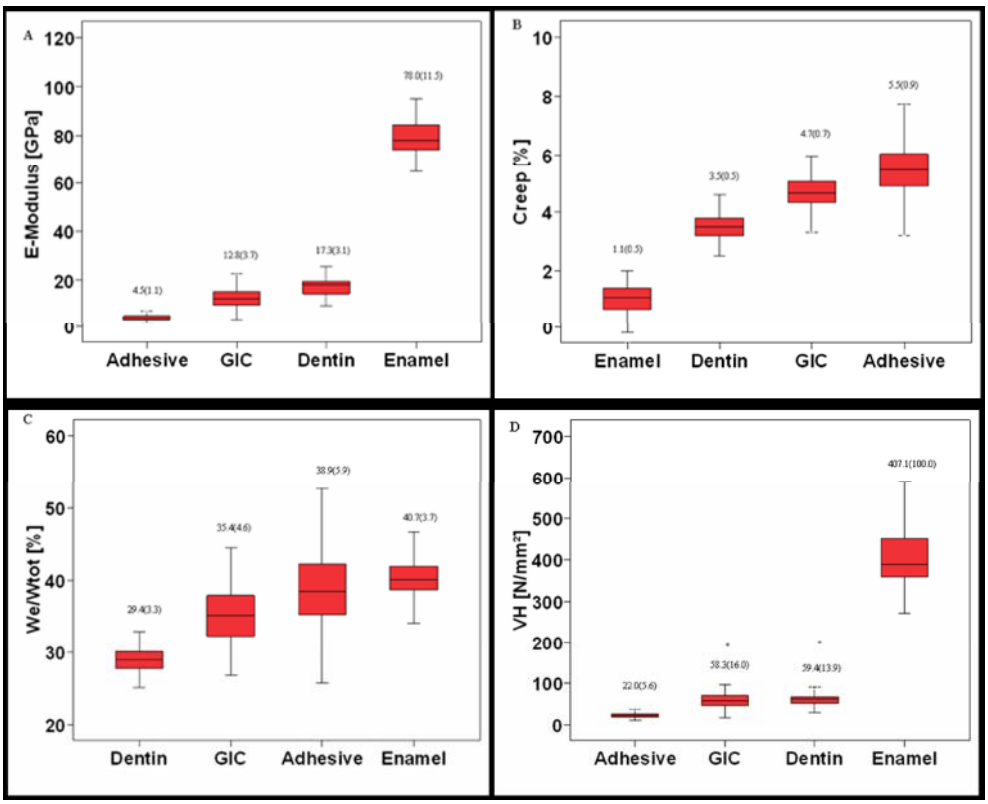

Table 2: Means (M) and standard deviations (SD) of Vicker's hardness (VH), elastic modulus (E), creep (Cr) and elastic indentation work (We/Wtot) of the tested adhesives. Different superscript letters indicate statistically significant differences (Tukey HSD test, $p<0.05$).

Adhesives	VH (N/mm^2)		E (GPa)		Cr (%)		We/Wtot (%)	
	M	SD	M	SD	M	SD	M	SD
Admira Bond	18.7^{A}	4.1	4.5^{BCD}	0.9	5.9^{D}	0.7	35.8^{A}	3.2
Futurabond NR	22.8^{B}	2.7	4.0^{AB}	1.0	5.0^{B}	0.3	43.7^{C}	5.8
Solobond Plus	24.2^{BC}	4.8	4.6^{CD}	1.0	4.5^{A}	0.5	44.0^{C}	5.4
Hybrid Bond	25.6^{C}	5.6	5.3^{E}	1.0	5.2^{B}	0.8	37.5^{AB}	5.3
Clearfil SE Bond	22.1^{B}	6.1	4.3^{BC}	1.0	5.6^{CD}	0.5	39.2^{B}	4.2
Clearfil S^3 Bond	23.9^{BC}	5.9	4.4^{BC}	1.0	5.4^{BC}	1.1	40.1^{B}	7.8
Clearfil Protect Bond	17.2^{A}	2.8	3.6^{A}	0.7	5.8^{D}	0.8	37.4^{AB}	4.8
Experimental i Bond	23.4^{BC}	5.6	5.0^{DE}	1.4	5.6^{CD}	1.0	37.4^{AB}	5.2

Table 3: Means (M) and standard deviations (SD) of Vicker's hardness (VH), elastic modulus (E), creep (Cr) and elastic indentation work (We/Wtot) of the tested GICs. Different superscript letters indicate statistically significant differences (Tukey HSD test, $p<0.05$).

GICs	VH (N/mm^2)		E (GPa)		Cr (%)		We/Wtot (%)	
	M	SD	M	SD	M	SD	M	SD
Fuji Fil LC	48.7^{AB}	13.9	11.3^{AB}	2.2	5.0^{B}	0.9	33.9^{AB}	3.6
Fuji II LC	69.2^{C}	12.6	14.7^{C}	2.2	4.6^{AB}	0.5	35.8^{B}	3.7
Fuji IX	67.9^{C}	11.3	17.2^{D}	3.9	4.4^{A}	0.5	32.4^{A}	4.5
Photac Fil	46.2^{A}	9.0	10.6^{AB}	2.0	4.8^{AB}	0.9	34.3^{AB}	4.2
Vitremer	51.4^{AB}	15.7	9.8^{A}	2.4	4.9^{B}	0.7	39.0^{C}	4.5
Ionofil Molar	57.4^{B}	15.2	12.3^{B}	2.1	4.6^{AB}	0.7	34.8^{AB}	3.3

Discussion

The tested adhesives and glass-ionomer cements differed in their mechanical properties both within each materials' class and between the two materials' types. Moreover, the mechanical properties of the two materials' classes differed from those of enamel and dentin. Thus, both null hypotheses were rejected.

Eight adhesives were tested for their mechanical properties in the present investigation. When the study was performed seven out of eight bonding systems were already on the market, whereas i Bond was still at the experimental stage, but, currently, it is also available. The adhesives differed significantly in all the tested mechanical properties, regardless of the adhesive class.

The Vicker's hardness of Admira Bond and Clearfil Protect Bond was significantly lower compared to those of the other adhesives. Admira Bond is a two-steps etch-and-rinse adhesive based on the organically-modified ceramic ("ormocer") technology, which combines an inorganic backbone based on silicon dioxide with polymerizable organic units, in order to form three-dimensional compound polymers (Hickel *et al.* 1998; Wolter *et al.* 1994). This ormocer-based adhesive was developed to be used in combination with the ormocer-based composite Admira. Previous studies reported that the mechanical properties of an ormocer-based composite were comparable to those of other restorative materials (Manhart *et al.* 2000a; Manhart *et al.* 2000b). The present investigation showed that the tested ormocer-based adhesive had lower mechanical properties than other tested adhesives. In fact, among the tested adhesives, Admira Bond presented also the lowest We/Wtot value and the highest Cr (which indicates an increase of indentation depth under maximal load). Nevertheless, the absence of filler particles in this ormocer-based adhesive could have contributed to the lower mechanical properties. However, as the restorative system Admira/Admira Bond showed a clinical performance

comparable to that of conventional bis-GMA based materials (Bottenberg *et al.* 2007), it may be speculated that the mechanical behaviour of the whole ormocer-based restorative system compensates for the slightly lower mechanical properties of the adhesive. Nevertheless, the correlation between the mechanical properties of a material and its clinical performance is still not clear. Clearfil Protect Bond is a two-steps self-etch adhesive, which has antibacterial properties due to the presence of the monomer 12-methacryloyoxydodecylpyridinium bromide (MDPB) in the primer solution (Imazato *et al.* 1998) and of sodium fluoride in the bond. Interestingly, with the exception of the Cr, Clearfil Protect Bond showed significantly lower mechanical properties than Clearfil SE Bond, which has an analogous chemical composition but does not contain MDPB. Thus, it could be hypothesized that the addition of the antimicrobial, which, on the other side, has been reported not to impair the microtensile bond strength (Peris *et al.* 2007), could have lowered the mechanical properties. Although it does not contain fillers, Hybrid Bond showed the highest E and VH among the tested adhesives. This adhesive contains an aromatic amine in the dispensing brush, which additionally activates the chemical polymerization, making this adhesive based on a dual-curing mechanism, which could be responsible for a better curing. The results of this study highlighted that the *in vitro* mechanical behaviour of dental adhesives does not necessarily reflect their clinical performance. In fact, according to Peumans et al. (Peumans *et al.* 2005), three-steps etch-and-rinse adhesives and two-steps self-etch adhesives showed the best clinical performance. On the contrary, less favourable clinical performance was observed for two-steps etch-and-rinse adhesives and one-step self-etch adhesives (Peumans *et al.* 2005), possibly due to their higher technique sensitivity. Nevertheless, in terms of mechanical properties this trend was not confirmed. However, it should be considered that the review of Peumans et al. (Peumans *et al.* 2005) did not include the results of more recent clinical studies (Van Landuyt *et al.* 2008; Abdalla & Garcia-Godoy 2007), which showed an

improved clinical performance of new simplified adhesives, like those tested in this study.

Since the mixing procedure does not affect the microhardness and the modulus of elasticity of GICs (Ilie & Hickel 2007) cements that are available both in capsules and in hand-mixing formulation, the latter was used in this investigation. It was previously reported that the hardness and the modulus of elasticity of the examined RMGICs did not decrease in the subsurface layers of the materials, where the curing light might penetrate with lower intensity (Ilie & Hickel 2007). The latter finding indicated that the chemical hardening based on the glass-ionomer reaction still plays an important role for these materials (Ilie & Hickel 2007), as well as for conventional GICs. Despite their excellent clinical performance (Peumans *et al.* 2005), resin-modified GICs did not seem to benefit from the addition of methacrylate-based monomers in their chemical composition, if compared to conventional GICs, as far as micromechanical properties are concerned. As a matter of fact, the tested resin-modified GICs did not show consistently superior values of each measured mechanical property than the conventional ones. Nevertheless, in terms of macromechanical properties, such as flexural strength, diametral tensile strength and compressive strength, the resin-modified GICs have been reported to be superior to conventional GICs (Ilie & Hickel 2007), thus suggesting that the light cured methacrylate-based polymers improve the first phase of the polymerization of these materials, making them less susceptible to the formation of cracks due to dehydration.

In order to compare adhesives and GICs in terms of mechanical properties all the measurements performed within each materials' class were pooled and statistically analyzed. This way, it was possible to evaluate to what extent each property varied within the materials' classes. Moreover, as the materials involved in a restorative system should ideally have a mechanical behaviour as similar as possible to that of the adjacent tooth structures, the

mechanical properties of enamel and dentin were used as references. The GICs had a higher mean E and VH values than adhesives, conversely the mean We/Wtot was lower. The measured E and VH values of the GICs spread within a range which was close to that of the values measured in dentin, whereas adhesive showed values more than 50% lower. When compared to enamel, GICs presented average E and VH almost seven times lower and the discrepancies were much wider for adhesives. The Cr of the adhesives was higher than those of GICs and enamel and dentin, thus suggesting that adhesives presented a lower stability under load. As far as the elastic indentation work is concerned, the adhesives and the GICs presented values, which are intermediate between those of enamel and dentin. The latter finding indicates that under load both materials' classes showed a higher plastic deformation when compared to enamel, but presented a more elastic behaviour than dentin. These differences could be significant if considering that during functional load the stresses tend to concentrate at the interfaces between structures with different mechanical behaviour, possibly contributing to the loss of integrity of the enamel margins of restorations. However, the GICs establish a chemical bond with calcium ions of hydroxyapatite (De Munck *et al.* 2005), which could contribute to stabilize the interface between these materials and the tooth structures.

It might be questioned that the direct comparison among the mechanical properties measured in thin adhesives' layers applied on dentin and in 2-mm thick GICs' specimens could have been inappropriate, since completely different types of specimens have been used for the two materials' classes. Nevertheless, under the loading conditions applied in the present study, the depth of penetration of the micro indenter was limited to few micrometres under the specimen's surface. Thus, the specimen's thickness, as well as the substrate on which the tested materials have been applied, did not play any role on the outcome of the measurements.

It is still not proved whether the mechanical properties of adhesives and GICs correlate with their clinical performance. However, since fracture and marginal defects have been reported to be principal reasons for re storations' failure (Hickel & Manhart 2001), the investigation of properties which could explain the behaviour of a material under load could be also of clinical relevance.

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2.4b. Effect of ozone gas application on the mechanical properties of de ntal adhesives bonded to dentin

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Introduction

The excavation of a carious lesion does not necessarily imply the complete elimination of pathogenic bacteria from dentin (Kidd *et al.* 1996). This issue could become important when a minimal cavity design is performed in order to avoid an excessive tooth substance loss (Mount 2003; Brostek 2004). Secondary caries has been reported to be one of the most common reasons of restoration's failure (York & Arthur 1993; Hickel *et al.* 2000; Hickel & Manhart 2001; Hickel *et al.* 2007a; Hickel *et al.* 2007b; Manhart *et al.* 2004) and may be related to the presence of residual bacteria under restorations (Mejàre *et al.* 1979). Therefore, additional methods for ensuring an optimal disinfection of the cavity should be considered.

Chlorhexidine and sodium hypochlorite solutions have been proposed for the disinfection of dentinal cavities (Al-Omari *et al.* 2006; Pappas *et al.* 2005), but their use in combination with adhesive restorations arises some concerns, due to the observed inhibition o f the bond strength to dentin (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006). Moreover, sodium hypochlorite was reported to affect the mechanical properties of dentin (Machnick *et al.* 2003; Sim *et al.* 2001; Grigoratos *et al.* 2001) and to enhance the demineralization of freshly remineralized dentinal lesions (Zaura *et al.* 2007). Thus, the investigation of other methods for cavity disinfection is advisable.

Gasiform ozone has been introduced in the den tal practice due to its antimicrobial potential against common oral pathogens (Baysan *et al.* 2000;

Baysan & Beighton 2007; Baysan & Lynch 2004; Polydorou *et al.* 2006; Huth *et al.* 2007; Estrela *et al.* 2007; Müller *et al.* 2007; Hems *et al.* 2005). Clinical studies assessed the effect of ozone for the treatment of occlusal (Dähnhardt *et al.* 2006; Huth *et al.* 2005; Knežević *et al.* 2007) and root caries (Baysan & Lynch 2007; Holmes 2003) and, more recently, the application of ozone on dental hard tissues prior to adhesive restorations has been proposed (Schmidlin *et al.* 2005; Azarpazhooh & Limeback 2008).

Oxidants, like bleaching agents, have been reported to impair the bond strength to dentin (Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005) and to influence the polymerization of dental adhesives (Cadenaro *et al.* 2006) if applied on the substrate immediately prior to bonding procedures. Thus, some concerns could arise on performing an adhesive procedure after the use of ozone.

Currently, no information is available on the effect that the application of ozone on dentin could exert on the subsequent application of different dentin adhesives. Therefore, the aim of the present study was to evaluate the effect of the ozone pre-treatment on some mechanical properties of different classes of dental adhesives, 30min and 24h after the bonding procedure. The null hypothesis tested is that the adhesive type, the ozone treatment and the time of measurement do not affect the investigated mechanical properties.

Materials and methods

Bonding procedure and ozone application

Twelve extracted sound human molars were collected for the study. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were then disinfected in 2.5% sodium hypochlorite for 2min and stored in distilled water until they were used for the study. The superficial enamel was removed from the occlusal aspect of the teeth through grinding with a wet 220 -

grit silicon carbide paper disk. The crowns were then sectioned perpendicular to the long axis in order to obtain three 0.7mm-thick slices from each tooth (Isomet, Buehler, Lake Bluff, IL, USA). The peripheral enamel was removed with a wet 220-grit silicon carbide paper disk. A clinically relevant smear layer was created by grinding the occlusal aspect of each dentin slice with 220-grit silicon carbide paper under water cooling (Koibuchi *et al.* 2001).

The teeth were divided into four experimental groups (n=3) according to the class of adhesive to be bonded: Group1: An acetone-based total-etch two-steps adhesive (Prime&Bond NT, Dentsply, Caulk, Milford, DE, USA); Group 2: An ethanol-based total-etch two-steps adhesive (Excite, Ivoclar-Vivadent, Schaan, Liechtenstein); Group 3: A self-etch two-steps adhesive (Silorane Adhesive System, 3M ESPE, Seefeld, Germany); Group 4: A total-etch three-steps adhesive (Syntac/Heliobond, Ivoclar-Vivadent). Twelve dentin slices were bonded in each group.

Prior to bonding each slice was further cut into two halves in a bucco-lingual direction (Isomet, Buehler); one half was pre-treated with ozone for 120s (2100ppm equal to 4.2g/m^3; HealOzone, KaVo, Biberach, Germany), the other half was left untreated and served as control. The adhesives were light-cured for 20s (Elipar Freelight 2, 3M ESPE, Seefeld, Germany) through a Mylar stripe with an output light of 1241mW/cm^2, measured by means of a calibrated fiber optic spectrally resolving radiometer (S2000, Ocean Optics, Dunedin, FL, USA) equipped with an integrating sphere. After curing, the specimens were left undisturbed for 30min in an environment 100% saturated with humidity at 37°C before the mechanical testing, in order to obtain a non-sticky adhesive surface. Table 1 reports compositions, batch numbers and mode of use of the tested adhesives.

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

Material	Composition	Mode of use
Total Etch* (Ivoclar-Vivadent) Batch # K14609	37% phopsphoric acid gel	Application on dentin; activation with a microbrush for 15s; rinsing with abundant deionized water; gently air drying
Prime&Bond NT (Dentsply) Batch # 0602002202	Di- and Trimethacrylate resins PENTA (dipentaerythritol penta acrylate monophosphate) Nanofillers-Amorphous Silicon Dioxide Photoinitiators Stabilizers Cetylamine hydrofluoride Acetone	Application on dentin surface with a microbrush for 20s; Removal of excess solvent with through gently air drying for 5s; Lightcuring 20s.
Excite (Ivoclar-Vivadent) Batch # J18718	HEMA (2-hydroxyethyl methacrylate) Dimethacrylates Phosphonic acid acrylate Highly dispersed silicon dioxide Initiators and stabilizers Alcohol	Application on dentin surface with a microbrush for 10s; Gently air drying for 3s; Lightcuring 20s.
Syntac/Heliobond (Ivoclar-Vivadent) Syntac Primer: Batch # K08247 Syntac Adhesive: Batch # K02656 Heliobond: Batch # K01560	Syntac Primer: Polyethylene glycol dimethacrylate, Maleic acid, Ketone, Water; Syntac Adhesive: Polyethylene glycol dimethacrylate, Glutaraldehyde, Water Heliobond: Bis-GMA (Bisphenol-A-glycidyldimethacrylate), Triethylene glycol dimethacrylate	Application of Syntac Primer on dentin surface with a microbrush for 15s; Air drying; Application of Syntac Adhesive with a microbrush; Leave undisturbed 10s; Air drying; Application of Heliobond with a microbrush; Gently air blowing; Lightcuring 20s.
Silorane Adhesive System (3M ESPE) Self-etch Primer: Batch # 292319 Bond: Batch # 292274	Self-etch Primer: Phosphorylated methacrylates, Vitrebond™ copolymer, Bis-GMA (Bisphenol-A-glycidyldimethacrylate), HEMA (2-hydroxyethyl methacrylate), Water, Ethanol, Silane-treated silica filler, Initiators, Stabilizers Bond: Hydrophobic dimethacrylate, Phosphorylated methacrylates, TEGDMA (Triethylene glycol dimethacrylate), Silane-treated silica filler, Initiators, Stabilizers	Application of Self-etch Primer on dentin surface for 15s; Air drying; Lightcuring 10s; Application of Bond; Air drying; Lightcuring 20s.

*Total Etch was used in combination with all the investigated total-etch adhesives.

Measurement of the mechanical properties of the tested adhesives

The Vicker's hardness, the modulus of elasticity, the elastic work and the creep of each tested adhesive were assessed. The measurements were performed with an automatic micro hardness indenter (Fischerscope H100C, Fischer, Sindelfingen, Germany) 30min and 24h after the bonding procedure. Between the two measurements the specimens were stored in an environment 100% saturated with water at 37°C. Fifteen indentations were performed for each tested specimen at each testing time. The test procedure was carried out force controlled. The test load increased and decreased at a constant speed between 0.4mN and 30mN. The force application time can be non-standardly varied: The force increased from 0.4mN to 30mN in 20s, the maximal force of 30mN was kept constant for 5s, then the force decreased from 30mN to 0.4mN in 20s and the minimal force of 0.4mN was kept constant for 5s. The load and the penetration depth of the indenter (Vicker's pyramid: diamond right pyramid with an angle $\alpha = 136°$ between the opposite faces at the vertex) were continuously measured during the load-unload-hysteresis

The universal hardness (UH) is defined as the test force divided by the apparent area of the indentation and is determined only during the increasing phase of the force. The measured values of universal hardness were stored in a database supplied by the manufacturer, which allowed to calculate a conversion factor between universal hardness and Vicker's hardness (VH), so that the results were expressed in Vicker's hardness units.

The indentation modulus (Y_{UH}) is calculated from the slope of the tangent of the indentation depth-curve at maximal force and is comparable to the modulus of elasticity of the material (E).

The mechanical work (W_{tot}) measured during the indentation procedure is only partly consumed as plastic deformation work. During the removal of the force the remaining part was set free as work of the elastic reverse deformation (W_e). According to the definition of the mechanical work as $W = \int F dh$ (F=load;

h=indentation depth) and considering the force variation during load and discharge, the total mechanical work and its components were calculated.

By measuring the variation of the indentation depth occurring when the maximal force was kept constant, a relative change of the indentation depth was calculated. This value represented the creep of the material. The creep (Cr) is indeed defined as the ratio between the change in the indentation depth measured during the 5s in that the force of 30mN was maintained constant and the indentation depth measured at the maximal force of 30mN.

Statistical analysis

Preliminary linear regression analysis showed that neither the tooth, nor the slice of origin had a significant influence on the four investigated parameters for the tested adhesives. Therefore the data were pooled together and statistically analyzed. The normality of data distribution and the homogeneity of group variances were verified for the investigated mechanical properties with the Kolmogorov-Smirnov test and the Levene's test. The data were then analyzed with a Multivariate Analysis of Variance with elastic modulus (E), elastic work (We/Wtot), Vicker's hardness (VH) and creep (Cr) as the dependent variables and adhesive, dentin treatment and time of testing as independent factors. The Tukey's HSD test was used for post hoc comparisons. The partial Eta-squared statistic (η_p^2) was reported in order to estimate the actual proportion of variation of the dependent variables attributable to each independent factor. In all the tests, the level of significance was set at $p<0.05$ and calculations were handled by the SPSS 12.0 software for Windows (SPSS Inc.; Chicago, IL, USA).

Results

The Multivariate Analysis of Variance revealed that the adhesive and the time of testing were significant factors affecting all the investigated parameters

($p<0.001$). The dentin treatment was a significant factor affecting the mechanical properties ($p<0.001$) except the We/Wtot ($p=0.075$). The interactions between the factors were also significant ($p<0.001$) with exception of adhesive×treatment ($p=0.56$), treatment×time ($p=0.76$) and adhesive×treatment×time ($p=0.72$) for the elastic modulus. The partial Eta - squared statistic (Table 2) showed that the adhesive and the time contributed effectively to the variations observed for all the tested mechanical properties ($\eta_p^2 \geq 0.06$). On the contrary, the dentin treatment, though significant, exerted only a minimal effect on the investigated parameters ($\eta_p^2 \leq 0.01$).

Table 3 reports means and standard deviations of the measured mechanical properties. All the tested materials tended to improve the mechanical properties between the 30min and the 24h measurements regardless of the dentin treatment, in most of the cases the differences resulted statistically significant. An exception is represented by the Silorane System Adhesive, which showed statistically significant differences between the 30min and 24h measurements only for the creep, which was significantly improved after 24h, while the other properties resulted comparable in both ozone treated and control groups (Table 3).

The ozone treatment tended not to significantly affect the mechanical properties of the tested adhesives irrespective of the time of measurement. Small, but significant differences were detected at the 30 min measurement in the creep and the elastic work of Excite, which resulted respectively reduced and increased in the ozone group, and in the Vicker's hardness of the Silorane System Adhesive, which was lower after the ozone treatment. Differences between the ozone and control group were detected also in the elastic work, creep and Vicker's hardness of the Syntac/Heliobond system at the 24h measurement (Table 3). Ozone did not determine variations of the tested properties exceeding the 10% compared to the control values.

Table 2: Partial Eta-squared statistic, showing the strength of association between the factors and the tested mechanical properties.

Factor	Dependent Variable	Partial Eta-squared (η_p^2)
Adhesive	E*	0.337
	We/Wtot#	0.547
	Cr**	0.429
	VH##	0.144
Treatment	E*	0.009
	We/Wtot#	0.000
	Cr**	0.007
	VH##	0.010
Time	E*	0.176
	We/Wtot#	0.060
	Cr**	0.358
	VH##	0.356

*Elastic modulus
#Elastic work
**Creep
##Vicker's hardness

Table 3: Means (SD) of the mechanical properties of the investigated adhesives ($p<0.05$)

Material	Treatment	Time	E*(N/mm^2)	We/Wtot$^{\#}$(%)	Cr**(%)	VH$^{\#\#}$(N/mm^2)
Prime&Bond NT	ozone	30min	4.04(1.04)^{ef}	38.50(4.01)^{de}	5.39(0.63)^{f}	15.69(3.69)^{c}
		24h	4.73(1.12)^{g}	40.11(4.97)^{ef}	5.00(0.56)^{cd}	19.86(3.70)^{ef}
	control	30min	4.17(1.25)^{f}	38.89(3.69)^{e}	5.29(0.55)^{ef}	16.07(3.37)^{c}
		24h	4.81(0.85)^{g}	40.57(4.81)^{fg}	4.93(0.42)^{c}	20.43(2.94)^{fg}
Excite	ozone	30min	3.53(0.74)^{cd}	33.97(4.78)^{b}	6.43(0.60)^{i}	13.01(3.16)^{ab}
		24h	4.58(0.73)^{g}	36.98(3.08)^{cd}	5.78(0.41)^{g}	20.13(3.35)^{fg}
	control	30min	3.75(1.09)^{de}	31.92(3.15)^{a}	6.65(0.77)^{j}	12.10(2.38)^{a}
		24h	4.77(0.71)^{g}	36.18(2.70)^{c}	5.68(0.43)^{g}	20.10(2.91)^{fg}
Syntac/Heliobond	ozone	30min	2.68(0.39)^{a}	41.82(4.43)^{g}	6.08(0.41)^{h}	13.72(2.46)^{b}
		24h	3.53(0.45)^{cd}	44.05(3.72)^{h}	4.84(0.53)^{bc}	18.56(3.00)^{d}
	control	30min	2.73(0.45)^{a}	41.10(5.44)^{fg}	6.08(0.68)^{h}	13.65(3.17)^{b}
		24h	3.68(0.35)^{d}	46.34(4.80)^{i}	4.46(0.41)^{a}	21.23(3.29)^{g}
Silorane System Adhesive	ozone	30min	3.06(0.52)^{b}	47.32(3.10)^{i}	5.25(0.36)^{ef}	18.63(3.38)^{de}
		24h	3.35(0.69)^{bc}	46.23(4.34)^{i}	4.85(0.29)^{bc}	19.56(3.37)^{def}
	control	30min	3.28(0.41)^{bc}	47.36(3.94)^{i}	5.15(0.39)^{de}	20.07(2.70)^{fg}
		24h	3.48(0.59)^{cd}	47.06(3.34)^{i}	4.70(0.36)^{b}	20.60(3.45)^{fg}

Different lowercase letters indicate statistically signi ficant differences.
*Elastic modulus
$^{\#}$Elastic work
**Creep
$^{\#\#}$Vicker's hardness

Discussion

The results of the present study indicated that the adhesive type, the dentin treatment and the time of measurement affected the mechanical properties of the four tested adhesives to different extents. The type of adhesive and the time of testing showed the highest influence on the investigated properties, while the effect of the dentin treatment was very low. Due to these findings, the tested null hypothesis was rejected.

The antimicrobial effect of ozone on oral microbiota has been investigated in both *in vitro* (Baysan *et al.* 2000; Baysan & Beighton 2007; Estrela *et al.* 2007; Müller *et al.* 2007) and *in vivo* (Baysan & Lynch 2004) studies. According to a study of Polydorou *et al.* (Polydorou *et al.* 2006), the application of ozone with a concentration of 2100 ppm for 80s on an *in vitro*-infected dentinal cavity model has been reported to be successful in reducing the number of microorganism, thus confirming the potential of this treatment to disinfect carious cavities (Noack *et al.* 2004). This could be of particular interest in those cases of minimal cavity extension (Mount 2003), when the limited dentin removal could lead to a considerable number of residual bacteria within the cavity walls. However, the effect of ozone application on dental hard tissues prior to restoration has been poorly investigated. Ozone has been reported not to negatively influence the microleakage and penetration of a sealant (Celiberti *et al.* 2006) and not to impair the shear bond strength of dental adhesives to bovine enamel and dentin (Schmidlin *et al.* 2005). But the implications of applying ozone on dentin prior to bonding procedures on the subsequent characteristics of the adhesive layer have not been clarified yet.

In the present study, the effect of a dentin pretreatment with ozone on different mechanical properties of four different types of dental adhesives was investigated. According to the partial Eta-squared statistics, the dentin treatment exerted only a minor influence on all the investigated properties. The partial Eta-squared is defined as the proportion of total variation of the dependent variable attributable to a factor, excluding other factors from the total nonerror variation. It represents an index of strength of association between an experimental factor and the dependent variable and ranges normally between 0 and 1 (Pierce *et al.* 2004). 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 investigated properties, thus avoiding a misinterpretation of the results of the Multivariate Analysis of Variance.

The Vicker's hardness has been previously used as indirect indicator of the extent of polymerization (Cadenaro *et al.* 2005; Ferracane 1985; Rueggeberg & Craig 1988) and together with the other parameters tested in this study reflects the elastic behaviour of the material during the indentation procedure. Recently it has been reported that the extent of polymerization of dental adhesives was significantly reduced if they were bonded to dentin disks immediate after whitening with 35% hydrogen peroxide, regardless of the type of adhesive class (Cadenaro *et al.* 2006). Although ozone, being an oxidant agent, could be expected to exert a similar effect, the results of the present study showed that this treatment did not affect the mechanical properties of the four types of adhesives in the same way.

Prime&Bond NT and Excite are two-steps, total-etch adhesives, which differ in their chemical composition. Prime & Bond NT is an acetone-based adhesive and Excite, on the contrary, contains ethanol as solvent. None of the mechanical properties of Prime&Bond NT were significantly affected by ozone, irrepective of the time of testing and the weak effect of ozone on the elastic work and on the creep of Excite observed at the 30min measurement was reversed over a 24h period. These results suggest that regardless of the chemical composition and of the type of solvent, the ozone treatment did not impair the mechanical properties of this class of adhesives.

The Silorane System Adhesive has been recently introduced to the market and was still at the experimental stage when this study was performed. The choice to include it as representant of a two-steps self-etching system was done taking into account that the use of the siloranes is specifically indicated for restorations of the posterior teeth, which are susceptible by carious lesion, that could benefit of ozone application prior to restoration. When the 30 minutes results are considered, the Silorane System Adhesive exhibited a low but significant reduction of the Vicker's hardness in the ozone group compared to the control. This adhesive system is based on a two-steps self-etching technique

and consists of a hydrophilic primer and a hydrophobic adhesive resin. When the primer is applied on dentin previously treated with ozone, it could be more susceptible to the action of the ozone, due to the lack of a rinsing step, which is on the contrary performed for the total-etch systems, and could theorethically contribute to the rehydration of dentin and to the elimination of residual oxygen from the substrate (Spyrides *et al.* 2000; Swift & Perdigão 1998). However, after 24h all the mechanical properties of this adhesive did not significantly differ between the ozone and the control group, indicating that the influence of the ozone treatment is reversible. An interesting finding of the present study was that the Silorane System Adhesive did not exhibit significant differences in the mechanical properties between the first and the second measurement excepting the creep, thus suggesting that the maximal degree of conversion is reached more rapidly than in other adhesives. The manufacturer did not provide information regarding the amount of initiators contained in the primer and in the bonding resin, but since both of them present an intense yellowish color, it may be speculated that they could contain a conspicuous amount of camphoroquinone, thus accounting for a fast conversion of the monomers.

The Syntac system is based on a total-etch three-steps approach with the final application of the Heliobond resin, which was subjected to the indentations in the present study. Although a weak effect of ozone on the Vicker's hardness, elastic work and creep of this adhesive system was detected after 24h, the measured values, regardless of the dentin treatment, were similar to those previously reported for common dentin adhesives and measured under the same experimental conditions as this study (Trixner *et al.* 2007). Since the role of the adhesive should be that of a stress-relieving layer, able to compensate for the stress generated due to the polymerization shrinkage of an overlaying composite restoration (Kemp-Scholte & Davidson 1990; Van Meerbeek *et al.* 1993), it may be speculated that a small alteration of the Vicker's hardness, which does not

dramatically affect the whole elastic behaviour of the adhesive itself could be not relevant from a clinical point of view.

Twenty-four hours have been previously reported as an optimal time interval for the completion of the polymerization of dental resins (Tarumi *et al.* 1999; Pilo & Cardash 1992). Therefore, in the present investigation, the final measurement of the mechanical properties was performed after this period, in order to evaluate the influence of the ozone treatment on the adhesive polymerization when the reaction should be completed. A tendency of improvement over the 24 hours was detected for all the investigated mechanical properties, irrespective of the experimental group. Regardless of the adhesive type, a previous ozone application seemed not to negatively affect this trend, suggesting that the polymerization reaction was not impaired by this oxidant agent.

According to the manufacturer of HealOzone a reductanct liquid (HealOzone Reductant, Kavo, Biberach, Germany) should be applied on the hard tissues treated with ozone, in order to achieve an antioxidant action and to facilitate the remineralization. No reductant liquid was used in this study, since it has been previously reported to reduce the shear bond strength to enamel and dentin, probably due to its fluoride content (Schmidlin *et al.* 2005).

In the present investigation ozone was applied on the dentin disks for 120s. The duration of the treatment is longer than that performed in other *in vitro* (Zaura *et al.* 2007; Schmidlin *et al.* 2005; Celiberti *et al.* 2006) and *in vivo* (Baysan & Lynch 2004; Dähnhardt *et al.* 2006; Huth *et al.* 2005; Knežević *et al.* 2007; Holmes 2003) studies, as well as that indicated by the manufacturer. The attempt was to test the mechanical properties of the adhesives after a prolonged exposure to ozone, as it is very unlikely that they could be affected by a standard clinical ozone application if they resulted completely not or only moderately affected under these experimental conditions. Moreover, unlike other studies (Holmes 2003; Celiberti *et al.* 2006), no modification of the ozone delivery

system was performed in this study in order to allow for the application of air on the control specimens for the same duration of the ozone treatment. The control specimens were, on the contrary, subjected to a standard bonding procedure in order to better simulate the clinical conditions.

Since dentin may act as an oxygen reservoir (Shinohara *et al.* 2004; Swift & Perdigão 1998), a limit of this study could be related to the fact that the tested mechanical properties were measured through indentantions performed on the adhesive surface, while the portion of adhesive proximal to the adhesive/dentin interface, which would be more likely to be influenced by the oxidative action of ozone, was not directly investigated. In fact, the maximal indentation depth was not higher than 5 µm, which was surely lower than the thickness of the adhesive layer. Thus, further research should be promoted aiming to assess the degree of polymerization of the inner adhesive surface. Moreover, studies aiming to provide a better understanding of the effect of ozone on the dentin/adhesive interface should be performed.

Conclusion

Whithin the limits of the present *in vitro* study, it may be concluded that the application of ozone on dentin for 120s was not detrimental for the mechanical properties of Prime&Bond NT, Excite, Silorane System Adhesive and Syntac/Heliobond over a 24h period, determining variations below 10%. Thus, application of ozone for cavity disinfection prior to bonding procedures performed with the tested adhesives could be performed without impairing the final outcome of the restoration.

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Chapter 3

Combining the use of gasiform ozone with pits and fissures sealing procedures

3.1. Role of pits and fissures sealing in the prevention of occlusal caries

In the past decades the attention of the dental profession has been directed toward prevention of caries and, as a consequence, a global reduction of the incidence of this disease was recorded (Pinkham *et al.* 2005). On the other side, an increase of the relative incidence of occlusal caries has also been observed (Marthaler *et al.* 1996; Mejàre *et al.* 1998).

The susceptibility to caries of the occlusal surface of posterior teeth is related to the presence of pits and fissures, which provide a protected niche for plaque accumulation. In particular, deep narrow I-shaped fissures are not self-cleansing, thus more suscpeptible to caries development than shallow wide V-shaped fissures, which tend to be caries resistant (Pinkham *et al.* 2005).

The attempts at preventing pits and fissures caries date from the 1920s, when two clinical techniques were proposed. Hyatt advocated a class I prophylactic restoration, which should include all caries-free pits and fissures (Hyatt 1924). Bodecker suggested a prophylactic odontotomy, which consisted in the mechanical eradication of fissures in order to transform these plaque retentive areas in cleansable ones (Bodecker 1929). These two techniques have been used until the use of pits and fissures sealants was introduced in the dental practice.

The development of dental sealants followed the discovery that etching enamel with phosphoric acid can increase the retention of resin materials and improve the marginal integrity of restorations (Buonocore 1955). The first sealing material was introduced in the mid-1960s and was based on

cyanoacrylate. By the late 1960s, several resin materials had been tested and a viscous resin showed resistance to degradation and formed a tenacious bond with etched enamel. This resin was obtained through the reaction between bisphenol A and glycidyl methacrylate and became known as Bis-GMA (Bowen 1982). Besides traditional Bis-GMA based sealants, different materials have also been used as pit and fissure sealants (Pinkham *et al.* 2005), including conventional (Pinkham *et al.* 2005; Papacchini *et al.* 2005) and resin modified (Pinkham *et al.* 2005; Papacchini *et al.* 2005; Papacchini *et al.* 2006) glass-ionomer cements, compomers (Pinkham *et al.* 2005; Papacchini *et al.* 2006), flowable composites (Papacchini *et al.* 2005) and dental adhesives (Baca *et al.* 2007; Bonifacio *et al.* 2009).

The persistence of cariogenic microorganisms after sealants application has been reported (Oong *et al.* 2008). Therefore, an additional disinfection of enamel could be considered. Besides conventional acid etching, other treatments of the enamel surface, such as laser irradiation, have been investigated in the last years (De Munck *et al.* 2002; Chimello-Souza *et al.* 2006; Martínez-Insua *et al.* 2006; Borsatto *et al.* 2007). Laser treatment has shown a detrimental effect on the bond strength of dental sealants to enamel (De Munck *et al.* 2002; Chimello-Souza *et al.* 2006; Martínez-Insua *et al.* 2006; Borsatto *et al.* 2007). To date, limited information is available about the combination of an ozone treatment with pits and fissures sealing procedures (Celiberti *et al.* 2006; Dukić *et al.* 2007) and the effect of gasiform ozone on the bond strength to enamel of dental sealants has not been previously investigated.

In this chapter a study is presented, which aimed at assessing the impact of an ozone application on the bond strength of different pit and fissure sealing materials to ground enamel. Additionally, the effect of ozone on two enamel's micromechanical properties has also been investigated.

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3.2. Shear bond strength to ground enamel of three pit and fissure sealing materials: effect of gasiform ozone application

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Introduction

Even though the global incidence of dental caries has decreased in the last decades, caries lesions occurring on the occlusal surfaces of posterior teeth have relatively increased (Marthaler *et al.* 1996; Mejàre *et al.* 1998). Tooth surfaces with pits and fissures are particularly vulnerable to caries development (Pinkham *et al.* 2005), thus the placement of pits and fissures sealants for the prevention of caries occurrence on the occlusal surfaces of posterior teeth has become well-accepted for dental clinicians (Mejàre *et al.* 2003). The use of sealants to arrest the progression of active occlusal caries lesions has also been suggested (Welbury *et al.* 2004; Feigal & Donly 2006). However, it has recently been reported that caries fissures cannot be sealed as adequately as sound fissures (Hevinga *et al.* 2008). Moreover, viable cariogenic microorganisms can be found on sealed teeth (Weerheijm *et al.* 1992). Thus, the application of additional antibacterial agents prior to sealant placement could be considered.

Gasiform ozone has been introduced in the dental practice due to its antimicrobial activity (Baysan *et al.* 2000; Azarpazhooh & Limeback 2008; Bezirtzoglou *et al.* 2008; Polydorou *et al.* 2006) and it has been suggested for the minimally invasive treatment of occlusal (Huth *et al.* 2005; Dähnhardt *et al.* 2006) and root caries (Baysan & Lynch 2007; Holmes 2003). More recently ozone has also been proposed as a cavity disinfectant prior to bonding procedures (Magni *et al.* 2008; Schmidlin *et al.* 2005). The application of ozone in an occlusal fissure before sealing has been reported not to impair the sealant's

penetration (Celiberti *et al.* 2006). However, no previous studies investigated the effect that ozone gas exerts on the bond strength of dental sealants to enamel.

Besides traditional Bis-GMA based sealants, firstly introduced in the late 1960s, different materials have also been used as pit and fissure sealants (Pinkham *et al.* 2005), including conventional (Pinkham *et al.* 2005; Papacchini *et al.* 2005) and resin modified (Pinkham *et al.* 2005; Papacchini *et al.* 2005; Papacchini *et al.* 2006) glass-ionomer cements, compomers (Pinkham *et al.* 2005; Papacchini *et al.* 2006), flowable composites (Papacchini *et al.* 2005) and dental adhesives (Baca *et al.* 2007; Bonifacio *et al.* 2009).

The primary aim of this laboratory study was to assess the effect of an ozone gas application on the shear bond strength to ground enamel of a Bis-GMA based sealant, a flowable compomer and a flowable composite used as pit and fissures sealants. The null hypothesis tested is that gasiform ozone does not affect the bond strength to enamel of the three tested sealing materials.

The impact of ozone on the enamel's elastic modulus and Vicker's hardness was additionally evaluated.

Materials and Methods

Shear specimens preparation

Sixty sound human third molars extracted due to orthodontic reasons were collected. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were kept in 37°C saline solution (0.9% sodium chloride in water) for no longer than one month before being used in the experiment.

The roots of each tooth were cut with a low speed diamond saw under abundant water cooling (Isomet, Buehler, Lake Bluff, IL, USA). The crowns were embedded in methacrylate cylinders (Technovit 4004, Heraeus Kulzer, Wehrheim, Germany), obtained from metallic molds with a height of 20 mm and a diameter of 15 mm. The mesial aspect of each crown was left free from

the embedding resin and the surface enamel was ground with 400 grit silicon carbide paper for 20s under water cooling.

Half of the enamel surfaces were subjected to a 60s ozone application (2,100 ppm equal to 4.2 g/m^3; HealOzone, KaVo, Biberach, Germany), whereas the other half was left untreated and served as control. A custom made silicon mold presenting a cylindrical hole with a diameter of 3 mm and a height of 2 mm in its middle portion was secured to the free surface of each metacrylate cylinder with the hole centered on the ground enamel surface.

The enamel surface of each specimen was etched for 30s with 37% phosphoric acid gel (Total Etch, Ivoclar-Vivadent, Schaan, Liechtenstein), rinsed with abundant water and air-dried. Both ozone-treated and control specimens were divided into three groups (n=10) according to the type of sealing material:

Group 1: a conventional pit and fissure sealant (Helioseal F, Ivoclar-Vivadent);

Group 2: a combination adhesive/flowable composite (Excite/Tetric EvoFlow, Ivoclar-Vivadent);

Group 3: a combination adhesive/flowable compomer (Prime&Bond NT/Dyract Flow, Dentsply Caulk, Milford, DE, USA).

The materials were directly inserted in the central hole of the silicon mold until it was completely filled and they were then light-cured 20s with a LED curing unit (Bluephase, Ivoclar-Vivadent) at an output intensity of 1,200 mW/cm^2. The mold was then removed, resulting in cylindrical sealant build-ups with a diameter of 3 mm and a height of 2 mm. The geometry of the specimens and the number of specimens tested for each experimental group followed the design of a study of Barroso *et al.* (Barroso *et al.* 2005). All stages of the preparation of the specimens were performed at room temperature.

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

The specimens were stored in deionised water for 24h at 37°C and then subjected to the shear bond strength test.

Shear strength test

The bonded specimens were introduced in an universal testing machine (MCE 2000ST, quickTest, Langenfeld, Germany) and the bond strength of the sealing materials to ground enamel 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 (mm^2) and the shear strength was expressed in megapascals (MPa).

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 sealing material and enamel;

Cohesive in the sealant (CS): when the failure occurred within the sealing material;

Cohesive in enamel (CE): when the failure occurred within enamel;

Mixed (M): when a combination of two or more of the above described modes was observed.

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.

Statistical analysis of the shear strength data

The normal distribution of the shear strength data and the homogeneity of variances' distribution among the experimental groups were verified, respectively, with the Kolmogorov-Smirnov test and the Levene's test. Data were analyzed with a two-way Analysis of Variance (ANOVA) with shear bond strength as the dependent variable and substrate treatment and type of sealing material as independent factors. The Tukey's test was applied for post-hoc comparisons. In all the analyses the level of significance was set at $P<0.05$. Calculations were handled with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA).

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

Material	Composition	Mode of use
Total Etch (Ivoclar-Vivadent) Batch # L42017	37% phopsphoric acid gel	Application on enamel; activation with a microbrush for 30s; rinsing with abundant water; gently air drying
Helioseal F (Ivoclar-Vivadent) Batch # L22819	Matrix: Bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate. Filler: highly dispersed silicon dioxide and fluorosilicate glass. Additional contents: titanium dioxide, stabilizers, catalysts.	Application directly with the disposable cannula. Wait 15s. Lightcuring 20s.
Excite (Ivoclar-Vivadent) Batch # J18718	HEMA (2-hydroxyethyl methacrylate) Dimethacrylates Phosphonic acid acrylate Highly dispersed silicon dioxide Initiators and stabilizers Alcohol	Application on enamel surface with a microbrush for 10s; Gently air drying for 3s; Lightcuring 20s.
Tetric EvoFlow (Ivoclar-Vivadent) Batch # J20474	Matrix: Dimethacrylates. Filler: Barium glass, ytterbium trifluoride, highly dispersed silicon dioxide, mixed oxide and copolymer. Additional contents: Additives, catalysts, stabilizers and pigments.	Application directly with the disposable cannula. Lightcuring 20s.
Prime&Bond NT (Dentsply Caulk) Batch # 0602002202	Di- and Trimethacrylate resins PENTA (dipentaerythritol penta acrylate monophosphate) Nanofillers-Amorphous Silicon Dioxide Photoinitiators Stabilizers Cetylamine hydrofluoride Acetone	Application on enamel surface with a microbrush for 20s; Removal of excess solvent through gently air drying for 5s; Lightcuring 20s.
Dyract Flow (Dentsply Caulk) Batch # 0802000036	Strontium-alumino-fluoro-silicate glass. Highly dispersed silicon dioxide. Ammonium salt of PENTA (dipentaerythritol penta acrylate monophosphate) and N,N-dimethyl aminoethyl methacrylate. Carboxylic acid modified methacrylate macromonomers. Diethylene glycol dimethacrylate (DGDMA). Camphorquinone. Ethyl-4-dimethylaminobenzoate. 2-Hydroxymethoxybenzophenone. Butylated hydroxy toluene (BHT) and other stabilizers. Iron pigments. Titanium dioxide.	Application directly with the disposable cannula. Lightcuring 20s.

Evaluation of enamel's elastic modulus and Vicker hardness

Twelve sound human third molars extracted due to ortho dontic reasons were collected. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were kept in 37°C saline solution (0.9% sodium chloride in water) for no longer than one month before being used in the experiment.

Each tooth was subjected to two cuts in its middle portion, parallel to its long axis, in a mesio-distal direction with a low-speed diamond saw under abundant water cooling (Isomet, Buehler), in order to obtain a 1 mm-thick slab, which included both enamel and dentin. The buccal surfaces of the obtained slabs were polished with silicon carbide paper discs (1, 000, 1,200 and 2,500 grit) and 1 μm-polycrystalline diamonds particles (DP-Spray, P; Struers) under water rinsing. Each slab was further divided into two halves (mesial and distal) with a longitudinal cut through its centre (Isomet). The specimens were the n kept at room temperature in deionised water.

The mesial half of each specimen was subjected to a 60s ozone application (2,100 ppm equal to 4.2 g/m^3; HealOzone, KaVo), whereas the distal half remained untreated and served as control. The specimens were im mediately processed for the measurement of the micromechanical properties.

The enamel's elastic modulus (E) and Vicker's hardness (VH) were measured with an automatic microhardness indenter (Fischerscope H100C, Fischer, Sindelfingen, Germany). Fifteen inde ntations were performed in enamel for each specimen, resulting in 180 indentations for each experimental group. The indentation procedure was carried out force controlled. A load application time of 50s was set and subdivided as follows: the force increase d at a constant speed from 0.4 mN to 30 mN in 20s, the maximal force of 30 mN was kept constant for 5s, then the force decreased at a constant speed from 30 mN to 0.4 mN in 20s and the minimal force of 0.4 mN was kept constant for 5s. 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.

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 database supplied by the manufacturer, a conversion factor between Universal Hardness and Vicker's hardness (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 substrate (E).

Statistical analysis of the micromechanical properties data

The normal distribution of the E and VH data was verified with the Kolmogorov-Smirnov test ($P>0.05$) and a preliminary regression analysis was performed in order to verify that the measured properties were not affected by the tooth within each experimental group ($P>0.05$). In order to compare the E and VH means of the ozone-treated and control groups the t-test for dependent samples was applied and the level of significance was set at $P<0.05$. Calculations were handled with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA).

Results

Shear bond strength

The means and standard deviations of the shear bond strengths in the experimental groups are showed in Table 2.

The Two-way ANOVA has shown that the substrate treatment was not a significant factor affecting the shear strength ($P=0.975$), whereas the type of

sealing material significantly affected the bond strength ($P<0.001$). The interaction between the two factors was not significant ($P=0.964$). Regardless of the substrate treatment, the combination Prime&Bond NT/Dyract Flow achieved the highest shear strength values, which were significantly higher than those of Helioseal F, but comparable to those of the combination Excite/Tetric EvoFlo w. The enamel's pretreatment with ozone did not affect the shear bond strength.

The observed modes of failure are listed in Table 3 and representative images of failed specimens are showed in Fig. 1. In both adhesive/flowable restorative material combinations only mixed failures were recorded, whereas the conventional sealant showed a consistent amount of adhesive failures. No cohesive failures neither in the sealing material nor in enamel were observed (Table 3).

Table 2: Means(SD) of the shear bond strengths (MPa) in the experimental groups (n=10).

Type of sealant	Ozone	Control
Helioseal F	9.66(5.0)^{A}	10.13(5.1)^{A}
Excite/Tetric EvoFlow	18.12(8.4)^{AB}	17.37(8.3)^{AB}
Prime&Bond NT/Dyract Flow	21.94(11.4)^{B}	22.42(9.5)^{B}

Different superscript letters indicate statistically significant differences (P<0.05).

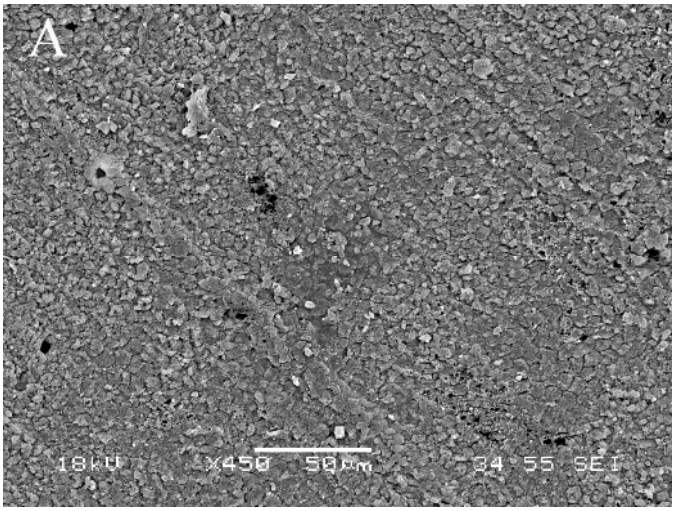
Table 3: Distribution of the failure modes within the experimental groups.

Failure mode	Helioseal F		Excite/Tetric EvoFlow		Prime&Bond NT/Dyract Flow	
	Ozone	Control	Ozone	Control	Ozone	Control
A	4	5	0	0	0	0
CS	0	0	0	0	0	0
CE	0	0	0	0	0	0
M	6	5	10	10	10	10

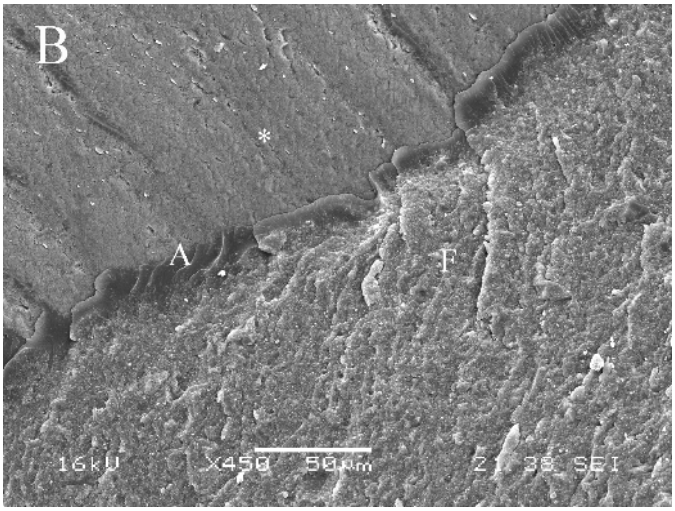
A: adhesive; CS: cohesive in the sealant; CE: cohesive in enamel; M: mixed.

Fig. 1 Representative images of failed specimens in the experimental groups (450×, bar= 50 μm).

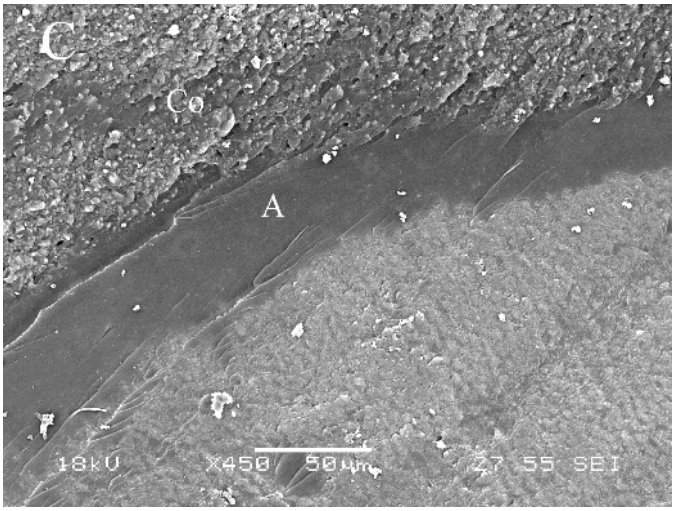
(**A**) Adhesive failure in a Helioseal F (Ivoclar-Vivadent) specimen (sealant side). No sign of cohesive fracture can be observed within the material bulk.



(**B**) Mixed failure in a Excite/Tetric EvoFlow (Ivoclar -Vivadent) specimen. The intermediate adhesive layer (A) is detectable under the flowable composite (F). The asterisk indicates the presence of a thin residual adhesive layer covering enamel's surface.



(**C**) Mixed failure in a Prime&Bond NT/Dyract Flow. The adhesive layer (A) is visible under the flowable compomer (Co).



Enamel's elastic modulus and Vicker's hardness

The results of the enamel's micromechanical properties' measurement are reported in Table 4. The mean values of E in the ozone-treated and control group were, respectively, 69.24(21.2) and GPa 70.48(22.8) GPa, whereas the measured VH were 314.13(119.8) N/mm^2 and 309.56(136.3) N/mm^2. No statistically significant differences in the measured properties were detected between the ozone and control groups ($P>0.05$).

Table 4: Means(SD) of enamel's elastic modulus (E) and Vicker's hardness (VH).

Group	**E (GPa)**	**VH (N/mm^2)**
Ozone	69.24(21.2)	314.13(119.8)
Control	70.48(22.8)	309.56(136.3)

No statistically significant differences were detected ($P>0.05$).

Discussion

The application of gasiform ozone on ground enamel did not affect the shear bond strength of the three pit and fissure sealing materials. Thus, the tested null hypothesis was accepted. Moreover, ozone did not exert any effect on enamel's micromechanical properties.

Dental sealants represent a well-established method for the prevention of caries (Mejàre *et al.* 2003). Sealants act as a physical barrier, which minimizes the action of cariogenic microorganisms on enamel (Simonsen 2002). As this barrier should be maintained intact and bonded to enamel for a lifetime, the retention of sealing materials has always been a major challenge for the dental clinician (Gwinnett & Ripa 1973; Silverstone *et al.* 1975). Therefore, several

studies investigated the bond strength of these materials to enamel (Papacchini *et al.* 2005; Papacchini *et al.* 2006; Barroso *et al.* 2005).

Besides the conventional use of sealing materials in sound pit and fissures, the application of sealants in caries-affected teeth has also been suggested, as these materials could reduce the number of bacteria in carious lesions (Oong *et al.* 2008). Nevertheless, the persistence of cariogenic microorganisms after sealants application has also been reported (Oong *et al.* 2008). Therefore, an additional pretreatment of enamel, which could reduce the amount of bacteria without compromising the adhesion between sealing material and tooth could be desirable, especially when a suspected carious lesion could be present. Besides conventional acid etching, other treatments of the enamel surface, particularly laser irradiation, have been investigated in the last years (De Munck *et al.* 2002; Chimello-Souza *et al.* 2006; Martínez-Insua *et al.* 2006; Borsatto *et al.* 2007). Nevertheless, some concerns arose about the unfavourable effect of laser treatment on the bond strength of dental sealants to enamel (De Munck *et al.* 2002; Chimello-Souza *et al.* 2006; Martínez-Insua *et al.* 2006; Borsatto *et al.* 2007).

Recently the application of gasiform ozone prior to pit and fissure sealing has also been investigated (Celiberti *et al.* 2006; Dukić *et al.* 2007). Celiberti *et al.* (Celiberti *et al.* 2006) reported that the application of ozone did not affect the penetration and the microleakage of a dental sealant. Similar results were obtained in a study by Dukić *et al.* (Dukić *et al.* 2007), which showed that an ozone gas treatment did not compromise the use either of a conventional sealant or of a flowable composite used for pit and fissures sealing, in terms of microleakage and penetration into enamel. In the present study the shear bond strength of three types of pit and fissures sealants was investigated. The results showed that the adhesion to enamel of the tested materials was not impaired by ozone application. Thus, the results of those studies (Celiberti *et al.* 2006; Dukić *et al.* 2007), which encouraged the use of ozone prior to pit and

fissures sealing, were supported. Moreover, the findings of this study are in agreement with all the investigations, which supported the use of gasiform ozone prior to adhesive procedures (Magni *et al.* 2008; Schmidlin *et al.* 2005; Cadenaro *et al.* 2009). As the efficacy of gasiform ozone against cariogenic bacteria has been proven (Baysan *et al.* 2000; Dähnhardt *et al.* 2006; Baysan & Lynch 2007; Holmes 2003), the absence of an impairment of the bond strength of different sealing materials to enamel due to ozone application, as showed in the present investigation, might suggest the ozone treatment as an additional method for eliminating bacteria from pit and fissures prior to sealing.

Three different materials have been tested in this study. The conventional sealant showed lower shear strength values than both flowable restorative materials, regardless of the enamel pretreatment. This finding could depend on an incomplete hybridization of the etched enamel, as previously observed for another dental sealant (Tay *et al.* 2005). The failure patterns in the sealant groups also confirmed the presence of a weaker bond between this material and the tooth substrate, as a higher amount of adhesive failures were recorded. However, previous investigations reported that the performance of conventional sealants was comparable to that of flowable composites used for pit and fissure sealing, either in terms of bond strength to enamel (Papacchini *et al.* 2006) or in terms of retention *in vivo* (Amin 2008). The two adhesive/flowable restorative material combinations performed similarly as far as the bond strength to enamel as well as the modes of failure were concerned. The possibility of applying flowable composites as pit and fissures sealants has been suggested by previous studies (Papacchini *et al.* 2006; Amin 2008). Due to their low viscosity, flowable composites can penetrate and adapt into the irregularities of the occlusal surface of posterior teeth, achieving rates of retention comparable to those of conventional sealants (Amin 2008). In this study a conventional flowable composite and a flowable compomer were investigated. Compomers are composed by a glass filler and an anhydrous acid monomer matrix (Pinkham

et al. 2005). These materials have been reported to achieve higher bond strengths than conventional glass-ionomer cements (Pinkham *et al.* 2005), which, on the contrary, showed lower bond strengths to enamel than other materials used for pit and fissures sealing (Papacchini *et al.* 2005). Nevertheless, a study by Papacchini *et al.* (Papacchini *et al.* 2006) reported that a combination between a compomer-based dental sealant (Dyract Seal, Dentsply) and an adhesive (Prime&Bond NT, Dentsply) achieved lower bond strength to intact enamel than two conventional sealants. However, in that investigation enamel was pretreated with a non-rinse conditioner (NRC, Dentsply) composed by maleic and itaconic acid in water (Papacchini *et al.* 2006), whereas in the present study enamel was etched with 37% phosphoric acid. It might be speculated that etching with a stronger acid could increase the micromechanical interlocking between enamel and adhesive, thus explaining the fact that the flowable compomer performed better than the conventional sealant. Moreover, the higher mechanical properties of the flowable compomer used in this study, compared to those of the low-filled sealant, could also be a reason for the measured higher bond strength. For both flowable compomer and flowable composite only mixed failures were recorded. Both materials were used in combination with a one-bottle total-etch adhesive, which did not completely detach from the substrate, but was detectable as intermediate layer between sealing material and enamel in failed specimens (Fig. 1B and 1C), thus accounting for the mixed failure mode.

Conclusions

Within the limits of this laboratory study it can be concluded that:

1. The application of gasiform ozone does not impair the bond strength to enamel of a conventional sealant, a flowable compomer and a flowable composite used for pit and fissures sealing.

2. A flowable compomer or a flowable composite used for pit and fissures sealing could represent a reliable alternative to conventional sealants.

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Chapter 4

Exploring the application of gaseous ozone in composite repair procedures

4.1. Repair rather than replacement: a minimally invasive approach to composite restoration's failure

During the last decades the continuous development and improvement of resin composites has made these materials the main choice for all classes of restorations. Despite their ability to bond to tooth substrates and their excellent aesthetic properties, composite restorations are susceptible to failure, primarily due to secondary caries and marginal defects (Hickel & Manhart 2001; Manhart *et al.* 2004; Mjör *et al.* 2000).

The traditional approach to defective restorations consists in the removal and replacement. This operative protocol requires a cavity preparation more extended than the previous one, with the subsequent loss of healthy dental structure. More recently, a minimal invasive treatment has been proposed, which foresees the repair rather than the replacement of failed restorations (Tyas *et al.* 2000; Mjör & Gordan 2002) and increases the longevity of the original filling (Moncada *et al.* 2008).

The excavation of small carious or staining lesions at the tooth-restoration interface does not always allow for the complete removal of cariogenic microorganisms (Kidd *et al.* 1996), which could be responsible of the recurrence of caries (Mejàre *et al.* 1979), with the subsequent failure of the repair procedure. Therefore, an additional disinfection of the repair site can be desirable.

As gasiform ozone has been proven to be effective against caries-associated bacteria (Baysan *et al.* 2000), its application for disinfecting a composite repair site could be considered. Nevertheless, an unfavourable effect

on the composite repair strength has been reported for oxidative substances (Papacchini *et al.* 2007).

This chapter contains two studies, which assessed the effect of gaseous ozone on the composite repair strength. The first study evaluated the in fluence of an ozone application on the repair strength and on the micromechanical properties of a conventional methacrylate-based composite, whereas the second study investigated the impact of an ozone treatment on the repair strength of a silorane-based and an ormocer-based composite.

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4.2. Influence of ozone on the composite-to-composite bond

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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 (Hickel & Manhart 2001; Manhart *et al.* 2004; Mjör *et al.* 2000).

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 (Tyas *et al.* 2000; Mjör & Gordan 2002) and allows for an increase of the longevity of the original filling (Moncada *et al.* 2008).

The excavation of small carious or staining lesions at the tooth-restoration interface does not necessarily imply the complete removal of pathogenic bacteria (Kidd *et al.* 1996), which could be responsible of the reoccurrence of caries (Mejàre *et al.* 1979), 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 (Baysan *et al.* 2000; Azarpazhooh & Limeback 2008; Polydorou *et al.* 2006; Bezirtzoglou *et al.* 2008). Gasiform ozone has been investigated for the treatment of occlusal (Huth *et al.* 2005) and root caries

(Baysan & Lynch 2007), whereas the aqueous form, due to its biocompatibility (Huth *et al.* 2006) and to its anti-inflammatory potential (Huth *et al.* 2007), 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 (Schmidlin *et al.* 2005; Magni *et al.* 2008; Bitter *et al.* 2008; Al Shamsi *et al.* 2008), thus, it could be also 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 unfavourable for composite repair procedures (Papacchini *et al.* 2007b), 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 20s with a LED curing unit (Bluephase, Ivoclar-Vivadent) at an output intensity of 1200 mW/cm ^{2}. The same curing unit was used thorough the study.

After storage (deionised water, 24h, 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 60s 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 30s;

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 10s;

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 20s.

Repair composite cylinders (Teric 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 20s. The specimens were then stored 24h in deionised 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 deionised water, dwell time 30s, transfer time 5s; 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 an 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 (mm^2) and the shear strength was expressed in megapascals (MPa).

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;

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 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 $p<0.05$.

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 # L16680 A2: Batch # 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 20s.
ESPE Sil (3M ESPE) Batch # 313387	Silane	MPS, ethanol	Apply on the composite surface. Allow to dry 30s.
Adper Scotchbond 1 XT (3M ESPE) Batch # 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 15s. Air-thin for 5s. Light-cure for 10s.
Tetric EvoFlow (Ivoclar-Vivadent) B3: Batch # 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 20s.

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

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, 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 20s (Bluephase, Ivoclar-Vivadent) through a mylar strip. After 24h of storage in deionised water at 37°C, half of the specimens were subjected to an ozone application of 60s (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 50s was set and subdivided as follows: the force increased at a constant speed from 0.4 mN to 500 mN in 20s, the maximal force of 500 mN was kept constant for 5s, then the force decreased at a constant speed from 500 mN to 0.4 mN in 20s and the minimal force of 0.4 mN was kept constant for 5s. 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 database supplied by the manufacturer, a conversion factor between Universal Hardness and Vicker's hardness (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 (η_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 24h groups, regardless of the pretreatment (Table 2, $p<0.05$).

Mixed failures were the most represented regardless of the experimental group (Table 3 and Figs. 1A-1B). Adhesive 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 ($p>0.05$).

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	$14.12(6.05)^{ABC}$ m=2.3 σ_0=19.34	$19.24(7.74)^{BCDE}$ m=2.5 σ_0=21.61	$19.95(8.17)^{BCDE}$ m=2.2 σ_0=11.39	$20.10(7.01)^{CDE}$ m=3.1 σ_0=14.19
Silane	$10.08(4.91)^{A}$ m=2.7 σ_0=20.90	$12.60(4.11)^{AB}$ m=2.8 σ_0=22.39	$17.16(8.57)^{ABC}$ m=2.9 σ_0=16.62	$19.20(9.35)^{BCDE}$ m=3.7 σ_0=18.83
Silane+Adhesive	$14.82(6.37)^{ABC}$ m=2.8 σ_0=22.40	$16.96(5.10)^{ABC}$ m=2.8 σ_0=22.75	$18.58(8.53)^{BCD}$ m=3.0 σ_0=15.77	$19.85(7.48)^{BCDE}$ m=2.7 σ_0=21.69
Flowable composite	$20.74(5.73)^{CDE}$ m=3.1 σ_0=30.20	$18.52(2.80)^{BCD}$ m=5.5 σ_0=26.99	$26.51(6.80)^{E}$ m=4.3 σ_0=22.78	$24.93(5.46)^{DE}$ m=7.7 σ_0=19.71

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

Table 3: Failure modes distribution within the experimental groups.

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

A: adhesive failures; CC: cohesive failures within the composite; CR: cohesive failures within the repair; M: mixed failures.

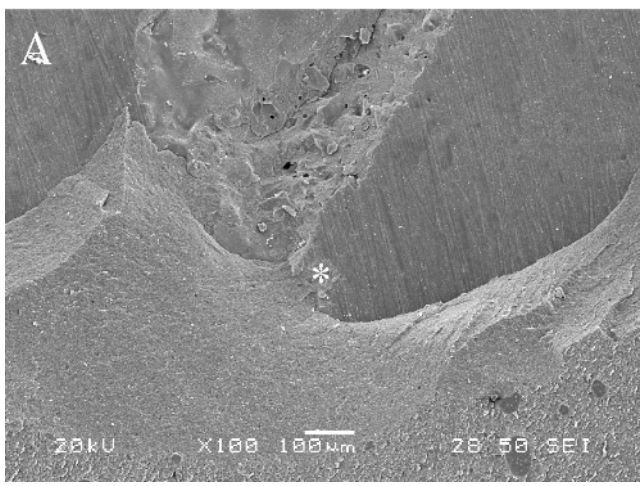
Table 4: Means (SD) of Vicker's hardness (VH) and elastic modulus (E) of the composite surface.

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

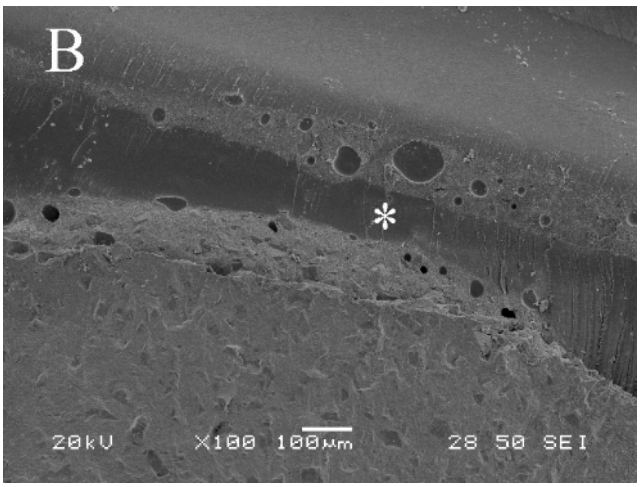
Different superscript letters indicate statistically significant differences ($p<0.05$).

Fig. 1 Representative SEM images of two mixed (A-B) and one adhesive (C) failure (100×, bar= 100 μm).

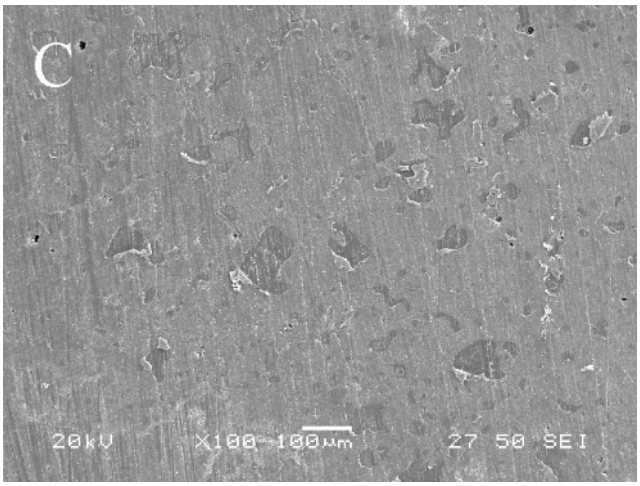
A: Mixed failure in one ozone-treated and not thermocycled specimen with the flowable composite as the intermediate layer. The asterisk in dicates remnants of the intermediate agent on the composite surface.



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.



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 (Lager *et al.* 2003) 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 (Mejàre *et al.* 1979) the combination of the lesion's excavation with the cavity's disinfection has been suggested (Noack *et al.* 2004). 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 (Ozturk & Ozer 2004; Santos *et al.* 2006), novel products have been investigated (Noack *et al.* 2004). Previous studies encouraged the application of ozone on dental hard tissues prior to adhesive procedures (Schmidlin *et al.* 2005; Magni *et al.* 2008; Bitter *et al.* 2008; Al Shamsi *et al.* 2008) 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 (Pierce *et al.* 2004). 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 moduli 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-1B) 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 (Schmidlin *et al.* 2005; Magni *et al.* 2008; Bitter *et al.* 2008; Al Shamsi *et al.* 2008). The study of Papacchini *et al.* (Papacchini *et al.* 2007b) 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 unfavourable 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 (Papacchini *et al.* 2007b). 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 (Gurgan & Yalcin Cakir 2008; Gurgan & Yalcin 2007; Lima *et al.* 2008). 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 (Calais & Söderholm 1988; Ferracane & Berge 1995). Nevertheless, the mechanical behavior of resin composites under challenging conditions is still contradictory (Ilie & Hickel 2009).

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 (Bouschlicher *et al.* 1997; Papacchini *et al.* 2008; Papacchini *et al.* 2007a). In this study an adhesive, a silane coupling agent, a 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 (Bouschlicher *et al.* 1997). 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 (Papacchini *et al.* 2007b; Bouschlicher *et al.* 1997; Brosh *et al.* 1997). 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 (Brosh *et al.* 1997). Also the poor repair potential previously observed by using solely silane couplings was confirmed by the results of this study (Papacchini *et al.* 2007c). The application of flowable composites as intermediate agents in composite repair procedures has been recently

investigated with the microtensile technique (Papacchini *et al.* 2008) and showed promising results even after aging (Papacchini *et al.* 2007c), mainly attributed to their stress-absorbing ability (Ferracane 2005) and to their superior hydrolytic stability compared to more hydrophilic intermediate agents (Papacchini *et al.* 2007c). 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 (Sideridou *et al.* 2004). 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.* (Papacchini *et al.* 2007c), which showed a significant reduction of the composite-composite repair strength after thermocycling only if a non pre-hydrolized 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 pre-hydrolized silane or other silane/adhesive blends (Papacchini *et al.* 2007c). However, a significant effect of thermocycling on the repair strength was reported when a higher number of cycles was performed (Passos *et al.* 2007).

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.

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4.3. Influence of ozone application on the repair strength of silorane -based and ormocer-based composites

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Introduction

During the last decades resin composites became the main choice for dental restorations. These restorative materials have been traditionally based on the technology of methacrylates (Peutzfeldt 1997) and have been continuously improved in order to achieve better physical properties and an optimal esthetic appearance. More recently, new restorative materials, such as ormocer -based and silorane-based composites, have also been developed as an alternative to conventional resin composites (Ilie *et al.* 2007; Guggenberger & Weinmann 2000; Weinmann *et al.* 2005; Wolter *et al.* 1994).

The ormocer-based composites have been developed on the basis of the organically-modified ceramic ("ormocer") technology, which combines an inorganic backbone consisting of silicon dioxide with polymerizable organic units, in order to form three-dimensional compound polymers (Wolter *et al.* 1994; Hickel *et al.* 1998). Previous studies reported that the laboratory (Manhart *et al.* 2000a; Manhart *et al.* 2000b; Ilie & Hickel 2009a) and clinical (Bottenberg *et al.* 2009; Mahmoud *et al.* 2008) behavior of ormocer-based composites is comparable to that of conventional restorative materials.

Siloranes are ring opening monomers obtained by merging siloxanes and oxiranes (Guggenberger & Weinmann 2000; Weinmann *et al.* 2005). They have been introduced in the dental practice in the attempt to overcome the polymerization shrinkage and the subsequent polymerization stress of methacrylate-based resin composites (Guggenberger & Weinmann 2000; Weinmann *et al.* 2005).

Despite the continuous improvement of restorative materials, dental restorations are susceptible to failure, mainly due to secondary caries and marginal defects (Hickel & Manhart 2001; Manhart *et al.* 2004; Mjör *et al.* 2000). Besides replacement, a more conservative approach for the management of failed restorations has also been proposed, which implies the repair of a defective restoration (Mjör & Gordan 2002; Tyas *et al.* 2000).

The persistence of cariogenic bacteria (Kidd *et al.* 1996) after the excavation of small carious or staining lesions at the tooth-restoration interface could determine the reoccurrence of caries (Mejàre *et al.* 1979), with the subsequent failure of the repair procedure. Therefore, additional methods for the disinfection of the repair site might be considered.

Ozone has been introduced in the dental practice due to its antimicrobial activity (Baysan *et al.* 2000; Azarpazhooh & Limeback 2008; Bezirtzoglou *et al.* 2008; Polydorou *et al.* 2006) and it has also been investigated as a cavity disinfectant prior to bonding procedures (Magni *et al.* 2008; Schmidlin *et al.* 2005). Therefore, the application of ozone for the disinfection of a repair site could also be considered. Nevertheless, some concerns arose about the application of an oxidant prior to the repair of a methacrylate-based restorative material (Papacchini *et al.* 2007a).

Ormocer-based composites also rely on radical polymerization of methacrylates, which represent the functional organic units of these compounds. Moreover, an amount of traditional methacrylates is consistently being added to all ormocer-based composites currently on the market. On the other hand, silorane-based restorative materials do not contain methacrylate in their chemical composition, even though these materials are used with a specially developed methacrylate-based adhesive.

The present study evaluated the effect of gasiform ozone on the repair strength of an ormocer-based composite and a silorane-based composite, using the corresponding adhesive of each restorative material as the intermediate

repair agent. The null hypothesis tested was that ozone application does not affect the repair strength of the tested restorative systems.

Materials and Methods

Shear specimens preparation

One hundred and sixty 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. Half of the cavities were filled with a single increment of a silorane-based restorative material (Filtek Silorane, 3M ESPE, Seefeld, Germany), whereas the other half were filled with a single increment of an ormocer-based composite (Admira, VOCO, Cuxhaven, Germany). The same LED curing unit (Bluephase, Ivoclar-Vivadent, Schaan, Liechtenstein) with an output intensity of 1,200 mW/cm^2 was used thorough the study.

After storage (one week, deionized water, 37°C) the free surfaces of the restored cavities were ground with 400-grit silicon carbide paper and the specimens of each restorative material were divided into two main experimental groups (n=40):

Group 1: The specimens were subjected to a 60s 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).

The corresponding adhesive system was then applied as the inter mediate repair agent for each restorative material in both ozone-treated and control groups: Admira Bond (VOCO) was used for the ormocer-based composite specimens, whereas the Silorane System Adhesive-Bond (3M ESPE) was applied on the silorane specimens.

Repair cylinders with a diameter of 3 mm were then built up in two 2 mm-thick increments on the surfaces of the specimens with the corresponding restorative material by means of a silicon mold. The specimens were then stored 24h 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.

After storage, half of the specimens of each group were subjected to thermocycling (Willytec, Dental Research Division, Munich, Germany) for 5,000 cycles (temperature changing from 5°C to 55°C in deionized water, dwell time 30s, transfer time 5s) 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 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 (mm^2) and the shear strength was expressed in megapascals (MPa).

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 old material surface or the repair surface;

Cohesive in the filling (CF): when the failure occurred within the original filling;

Cohesive in the repair (CR): when the failure occurred within the repair cylinder;

Mixed (M): when a combination of two or more of the above described modes was observed.

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

Material	Composition	Mode of use
Admira (VOCO) Batch # 0840046	Ormocers, Bis-GMA (Bisphenol A diglycidylmethacrylate), TEGDMA (Triethylene glycol dimethacrylate), UDMA (urethane dimethacrylate), BHT (Butylated hydroxy toluene), SiO_2, Ba-Al-B-Si glass	Application of 2 mm increments; Lightcuring each increment 40s.
Admira Bond (VOCO) Batch # 0840050	Ormocers, Bis-GMA (Bisphenol A diglycidylmethacrylate), HEMA (2-hydroxyethyl methacrylate), BHT (Butylated hydroxy toluene), Acetone, Organic acids	Apply 30s; Air drying; Lightcuring 20s.
Filtek Silorane (3M ESPE) Batch # 7AR	3,4-Epoxycyclohexylethylcyclo-polymethylsiloxane (5-15 wt %) Bis-3,4-epoxycyclohexylethyl-phenylmethylsilane (5-15 wt %) silanized Quartz Yttriumfluoride 76 wt %	Application of 2 mm increments; Lightcuring each increment 20s.
Silorane System Adhesive Bond (3M ESPE) Batch # 292274	Hydrophobic dimethacrylate, Phosphorylated methacrylates, TEGDMA (Triethylene glycol dimethacrylate), Silane-treated silica filler, Initiators, Stabilizers	Application of Bond; Air drying; Lightcuring 20s.

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 Two-Way Analysis of Variance with pretreatment and thermocycling as the main factors was used to analyze the shear bond strength data within each restorative material. The statistical analysis was handled with the SPSS 16.0 software for Windows (SPSS, Chicago, IL, USA). 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.

Results

The pretreatment, the thermocycling and their interaction did not significantly affect the repair strength of both tested materials ($p>0.05$). Table 2 reports means (SD) of the repair strengths measured in the experimental groups. Ta ble 3 reports the failure modes distribution within the groups.

Irrespective of the restorative material the ozone treatment did not significantly affect the repair strength. No significant differences in repair strength were detected between the specimens tested after 24h and those thermocycled (Table 2).

The silorane-based composite showed lower repair strengths compared to those of the ormocer-based composite (Table 2).

The mean repair strengths varied between 27.7 MPa and 29.5 MPa in the silorane-based composite groups and between 31.4 MPa and 35.6 MPa in the ormocer-based composite groups. The highest mean repair strength (35.6 MPa) was achieved by the ormocer-based composite in the control group after 24h, whereas the lowest mean bond strength (27.7 MPa) was recorded in the ozone-treated group of the silorane-based composite after thermocycling.

Mixed failures were the most represented in all experimental groups. A small amount of cohesive failures within the original filling were also recorded in all groups. No adhesive failures occurred, as well as cohesive failures in the repair (Table 3 and Figs. 1-2).

Table 2: Means (SD) of the repair strengths in the experimental groups (MPa).

Group	Silorane		Ormocer-based composite	
	24h	Thermocycling	24h	Thermocycling
Ozone	28.1(13.8)	27.7(9.7)	31.5(9.3)	31.4(6.0)
Control	28.8(8.8)	29.5(11.1)	35.6(10.6)	34.2(4.5)

No significant differences were detected among the experimental groups within each restorative material ($p>0.05$).

Table 3: Distribution of the failure modes within the experimental groups.

Failure mode	Silorane				Ormocer-based composite			
	Ozone		Control		Ozone		Control	
	24h	Th.	24h	Th.	24h	Th.	24h	Th.
A	0	0	0	0	0	0	0	0
CF	5	5	6	5	4	3	4	4
CR	0	0	0	0	0	0	0	0
M	15	15	14	15	16	17	16	16

A: adhesive; CF: cohesive in the filling; CR: cohesive in the repair; M: mixed.

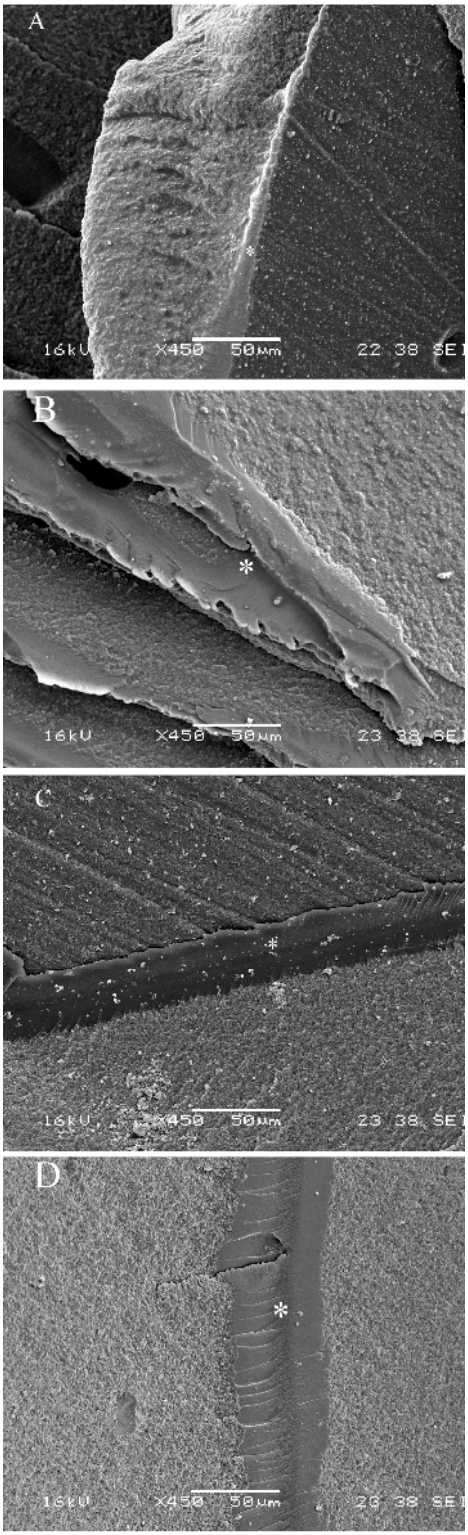

Fig. 1 Representative SEM images of ormocer-based composite shear specimens failed in a mixed mode, respectively, in the ozone-24h group (A), control-24h group (B), ozone-thermocycling group (C), control-thermocycling group (D). The asterisks indicate the remnants of the intermediate adhesive layer (450×, bar= 50 µm).

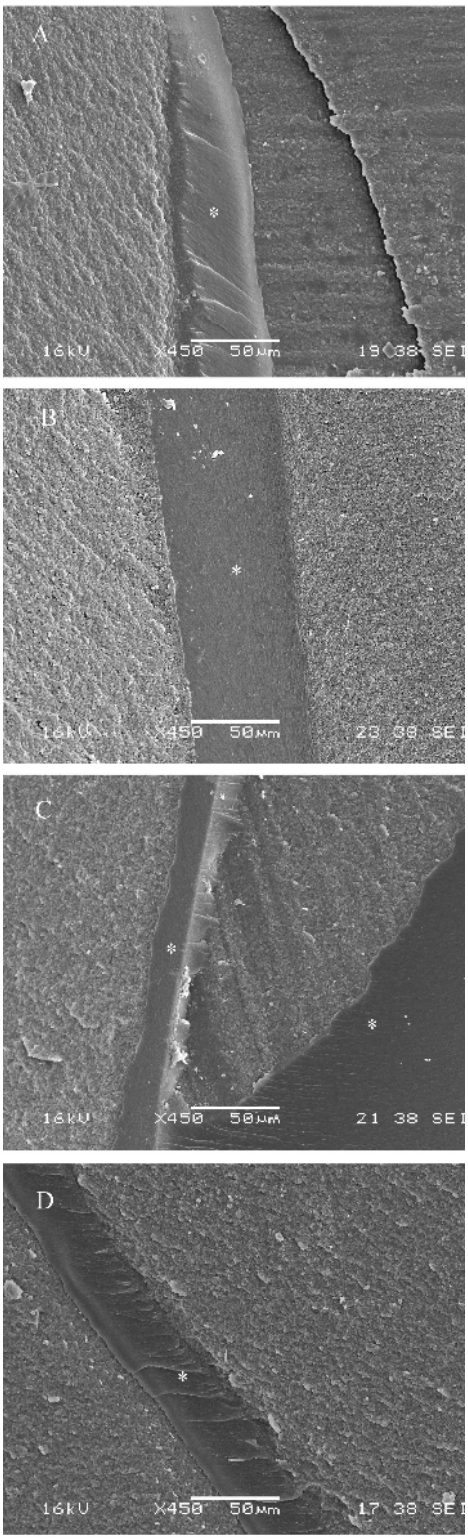

Fig. 2: Representative SEM images of silorane-based composite shear specimens failed in a mixed mode, respectively, in the ozone-24h group (A), control-24h group (B), ozone-thermocycling group (C), control-thermocycling group (D). The asterisks indicate the remnants of the intermediate adhesive layer (450×, bar= 50 µm).

Discussion

The application of gasiform ozone did not affect the repair strength of a silorane restorative material and an ormocer-based composite. Thus, the tested null hypothesis was accepted.

The occurrence of secondary caries has been reported to be the main reason for replacement of a failed restoration (Mjör *et al.* 2000). As secondary caries lesions are localized and delineated defects at the margin of a restoration, an exploratory cavity preparation at the tooth/restoration interface may help in assessing the extent of the lesion and in avoiding the replacement of the restoration. In fact, when sound tissue is exposed, the exploratory cavity may be repaired with a conventional restorative technique (Mjör & Gordan 2002). If a minimal lesion excavation approach is preferred, the additional use of a disinfectant may prevent the persistence of pathogenic bacteria (Kidd *et al.* 1996) at the repair site and, subsequently, the failure of repair procedure. Besides the antimicrobial potential, an ideal disinfectant should not interfere with the adhesion of the new restorative material to both tooth tissues and old restoration. The present study evaluated the effect of ozone on the repair strength of two innovative restorative materials.

The application of oxidants, such as bleaching agents, prior to bonding to tooth tissues (Cadenaro *et al.* 2006; Shinohara *et al.* 2005; Shinohara *et al.* 2004) and to restorative materials (Papacchini *et al.* 2007a) has been reported to be unfavorable due to the inhibitory effect of oxygen on the polymerization of methacrylate-based resins. On the contrary, several studies reported that gasiform ozone has no detrimental effect on bonding to dental hard tissues (Magni *et al.* 2008; Schmidlin *et al.* 2005; Bitter *et al.* 2008). The results of the present investigation supported these observations, indicating that the same considerations may also be extended to the application of ozone prior to repair procedures. The deleterious effect of oxidants on bonding procedures has been

commonly attributed to the formation of oxygen by-products (Papacchini *et al.* 2007a), which are retained by the substrate, that acts as an oxygen reservoir (Shinohara *et al.* 2004; Swift & Perdigão 1998). In composite repair procedures, the inhibitory effect on the repair strength was particularly evident if a methacrylate-based adhesive was applied as intermediate repair agent (Papacchini *et al.* 2007a), as in the present study. Common dental disinfectants or oxidants, such as bleaching agents, are available mostly as solutions or gels and it may be speculated that the type of formulation could also be a critical factor affecting the interaction of these substances with the substrate. A liquid or gel formulation might lead to the persistence of part of the substance on the substrate even after rinsing, whereas a gaseous formulation probably does not leave any remnants. In fact, in the present investigation the lack of any negative influence of ozone on the repair strength was observed even without rinsing the specimens after ozone application. Moreover, ozone has been reported not to impair the mechanical properties of dental adhesives (Magni *et al.* 2008). In the present study an adhesive was applied as the sole intermediate repair agent for both restorative materials. Since the intermediate agent has been reported to be a critical factor affecting the repair strength (Brosh *et al.* 1997; Papacchini *et al.* 2008; Papacchini *et al.* 2007b), it may be speculated that the absence of any alteration of the properties of the intermediate adhesive layer due to ozone application might have contributed to keep the stability of the material -adhesive-material complex unaltered. The latter finding is also confirmed by the similar failure modes distributions observed in the ozone-treated and control groups for both restorative materials (Table 3 and Figs. 1-2).

Two novel restorative materials combined with their corresponding adhesives were used in this study: a silorane-based and an ormocer-based composite. Silorane-based restorative materials have been recently introduced in dentistry as an alternative to methacrylate-based resin composites mainly due to their lower polymerization shrinkage (Ilie *et al.* 2007; Guggenberger &

Weinmann 2000; Weinmann *et al.* 2005). They showed mechanical properties comparable to those of conventional composites and a high stability (Ilie & Hickel 2009b). Their use is also encouraged by the lower susceptibility to adhere oral streptococci compared to conventional composites and the related potentially reduced occurrence of failure of silorane restorations due to recurrent caries (Buergers *et al.* 2009). A study of Tezvergil-Mutluay *et al.* (Tezvergil-Mutluay *et al.* 2008) investigated the silorane-to-silorane shear bond strength in the incremental layers bonding technique and the silorane-to-dimethacrylate strength with or without an intermediate adhesive layer, but only limited information exists on the repair potential of a silorane-based composite by using the corresponding bonding resin as intermediate layer (Moser *et al.* 2008). The repair strengths measured for the silorane-based material in the present study were slightly higher than the silorane-to-silorane strength reported by Tezvergil-Mutluay *et al.* (Tezvergil-Mutluay *et al.* 2008), suggesting that the application of the Silorane Adhesive System-Bond enhanced the coupling between the aged and the fresh silorane. Nevertheless, differences in the test methodology could also have contributed to this discrepancy. The ormocer-based composites have been reported to present a laboratory (Manhart *et al.* 2000a; Manhart *et al.* 2000b; Ilie & Hickel 2009a) and clinical (Bottenberg *et al.* 2009; Mahmoud *et al.* 2008) behavior similar to that of conventional composites. Only one previous investigation assessed the repair strength of an ormocer-based composite (Definite, Degussa AG, Hanau, Germany) with a different methodology, reporting shear bond strengths of 15.5 MPa or lower, depending on the repair procedure adopted (Hannig *et al.* 2006). In the present study the ormocer-based composite Admira (VOCO) was tested by using its corresponding adhesive (Admira Bond, VOCO) as the intermediate repair agent. Similarly to other ormocer-based composites, Admira also contains methacrylate-based monomers. The repair strengths measured in the Admira groups in the present investigation were more than two times higher than those previously reported

for Definite (Hannig *et al.* 2006). Despite its low mechanical properties (Magni *et al.* 2010), Admira Bond has been reported to form a stable bond to dentin even after fatigue loading (Abdalla *et al.* 2007). The favorable bonding potential of this intermediate adhesive layer might account for the high repair strengths achieved with the Admira/Admira Bond system. The two restorative materials used in this study were not directly compared statistically, since it was not the major aim of the investigation. However, the ormocer-based composite achieved clearly higher repair strengths than the silorane-based composite. This finding might be explained through the higher chemical compatibility between Admira and Admira Bond, which contain similar chemical components, than between Filtek Silorane and Silorane System Adhesive-Bond, the latter consisting of methacrylate-based resin monomers. However, the predominance of mixed failures and cohesive failures in the original composite in all experimental groups, rather than adhesive failures, suggests that the corresponding bonding agent achieves a stable interfacial quality for both restorative materials. Nevertheless, the dental clinician is often unable to identify the material used for the original restoration and, as a consequence, he might not use the corresponding bonding agent and the corresponding restorative material. As far as traditional methacrylate-based composites are concerned, surface treatments aimed at increasing the roughness of the composite surface to be repaired and, subsequently, at improving the mechanical interlocking between the repaired and the repairing material have been proposed, as well as the application of alternative intermediate repair agents (Brosh *et al.* 1997; Papacchini *et al.* 2008; Papacchini *et al.* 2007b). These procedures might also enhance the coupling between composites with different chemical compositions. Therefore, more studies should be performed in order to achieve a better understanding of the methods for improving the compatibility between methacrylate-based, silorane-based and ormocer-based composites.

Thermocycling has been widely used in order to simulate the stress generated by changing the environmental temperature at the interface between materials with different coefficients of thermal expansion in laboratory conditions (Sideridou *et al.* 2004). A recent study of Papacchini *et al.* (Papacchini *et al.* 2007b) investigated the hydrolytic stability of different composite repair procedures after a thermocycling regimen analogous to that used in the present investigation. According to the results of Papacchini *et al.* (Papacchini *et al.* 2007b), a significant reduction of the composite-composite repair strength after thermocycling was present only if a non pre-hydrolyzed silane mixed with an etch-and-rinse self-cure adhesive system was applied as the intermediate repair agent. In this study thermocycling did not affect the repair strength of the silorane-based and of the ormocer-based composite repaired by using their corresponding adhesives. This supports the thesis that adhesive intermediate layers achieve a stable bond also in the repair of new restorative materials. Nevertheless, a significant reduction of the composite repair strength after thermocycling was reported when a higher number of cycles were performed (Passos *et al.* 2007).

Within the limits of this laboratory study it may be concluded that the application of ozone does not affect the repair strength of the tested silorane-based and ormocer-based composites and, subsequently, the null hypothesis was accepted. Therefore, the clinician could consider the use of ozone as disinfectant after the excavation of a secondary caries lesion at the margin of a silorane-based or an ormocer-based composite restoration without impairing the repair potential of these restorative materials.

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Chapter 5

Summary, Conclusions and Future directions

5.1. Summary

The management of caries has always represented a challenge for dental clinicians. During the last century, the philosophy for treating carious lesions has changed radically. The traditional "surgical" approach, which consisted in the excavation of the carious tissue and in the realization of an extended cavity with substantial loss of tooth structure (Osborne & Summitt 1998), was progressively replaced by a "biological" approach, which allows for a more conservative cavity design and is based on the understanding of the development and progression of caries, which is recognized and treated as an infectious disease (Tyas *et al.* 2000). In light of these new concepts in caries management, a renewed interest has grown toward methods, which could prevent and control the bacterial infection. In this contest, the introduction of gaseous ozone in the dental practice was proposed.

In the initial part of this thesis (Chapter 1), an introduction of the main topic has been presented. After a brief description of the new concepts in caries management according to the principles of the "Minimal Intervention Dentistry", some considerations have been made about the use of disinfectans prior to restorative procedures.

The suppression of caries-associated microorganisms can be achieved with a wide choice of antimicrobials, which range over cavity liners (Chandler & Heling 1995), conventional disinfectans like chlorhexidine (Ersin *et al.* 2009; Wicht *et al.* 2004) or sodium hypochlorite (Taniguchi *et al.* 2009; Zaura *et al.* 2007), bonding agents (Pinto *et al.* 2010; Esteves *et al.* 2010; Polydorou *et al.* 2006; da Silva *et al.* 2010; Hosoya *et al.* 2010; Vaidyanathan *et al.* 2009; Li *et*

al. 2009), antibiotics (Wicht *et al.* 2004) and, more recently, laser irradiation (Müller *et al.* 2007).

After a concise description of the ozone-generating device, the antimicrobial potential of gasiform ozone has been considered. The effectiveness of ozone gas against isolated caries-related bacterial strains has been proven (Polydorou *et al.* 2006; Johansson *et al.* 2009), whereas its effect on associated bacterial species has not been completely clarified (Müller *et al.* 2007; Baysan *et al.* 2000; Baysan & Lynch 2004; Knight *et al.* 2008). The antibacterial effect of gaseous ozone is dose-, strain- and time-dependant (Huth *et al.* 2009).

Several clinical investigations have shown encouraging results regarding the application of gasiform ozone for the treatment of occlusal caries in adults (Knežević *et al.* 2007) and children (Dähnhardt *et al.* 2006; Huth *et al.* 2005) and for the management of root caries (Holmes 2003; Baysan & Lynch 2004; Baysan & Lynch 2007). Nevertheless, the number and quality of the clinical trials assessing the performance of gaseous ozone should be further improved prior to drawing final conclusions (Azarpazhooh & Limeback 2008; Rickard *et al.* 2004; Stübinger *et al.* 2006; Nogales *et al.* 2008).

A major issue when applying a disinfectant prior to restorative procedures is represented by its interaction with the tooth substrate and with restorative materials. A trend to alter the mechanical properties of dental hard tissues has been shown by common disinfectants (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) and also by substances with an oxidative potential (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006). Moreover, disinfectants and oxidants have been reported to negatively affect bonding procedures (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006; Spyrides *et al.* 2000; Shinohara *et al.*

2004; Shinohara *et al.* 2005; Breschi *et al.* 2007; Cadenaro *et al.* 2006) with the exception of chlorhexidine, whose role in the stabilization of adhesive interfaces has been recently reconsidered (Breschi *et al.* 2009; Breschi *et al.* 2010). As the antimicrobial effect of gasiform ozone is based on its oxidative potential, some concerns should arise about its application on dental hard tissues prior to restorative procedures. Nevertheless, the information on the interaction of ozone with tooth substrates and dental materials is extremely limited (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005; Celiberti *et al.* 2006).

In light of this issue, the application of gaseous ozone prior to bonding to dentin has been investigated (Chapter 2). The interaction of ozone with the dentinal substrate has been the object of a study presented in Paragraph 2.2, which showed that an ozone application did not alter dentin's elastic modulus and Vicker's hardness. This finding is supported by a previous study reporting that enamel's physical properties are also not impaired by gasiform ozone (Celiberti *et al.* 2006).

The effect of ozone on the infiltration of different bonding systems into dentin has been considered in Paragraph 2.3. The confocal laser scanning microscopy (CLSM) revealed that the penetration of the tested adhesives was not quantitatively and qualitatively affected by the pretreatment of dentin with gaseous ozone.

In Paragraphs 2.4a and 2.4b, two studies are presented aiming at outlining the micromechanical properties of dental adhesives bonded to dentin and at evaluating how they are influenced by an ozone application. In terms of micromechanical properties bonding agents are inferior to enamel and dentin and also to glass-ionomer cements, which can also be used as cavity liners (Paragraph 2.4a). The application on dentin of gasiform ozone prior to bonding was not detrimental for the micromechanical properties of the tested adhesive systems, suggesting that, despite its high oxidative potential, ozone does not

impair the polymerization of dental adhesives, which is indirectly expressed by their mechanical properties (Paragraph 2.4b).

The results of the studies presented in Chapter 2 suggest that the use of gaseous ozone on dentin prior to bonding procedures can be conside red without concerns about a potential impairment of the final restoration. These findings are also supported by those investigations, which reported that ozone does not negatively affect the bond strength to dentin (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005).

Chapter 3 focused on the application of gaseous ozone in combination with pit and fissures sealing procedures. An investigation on the effect of ozone on the shear bond strength of different materials used for pit and fissures sealing is presented in Paragraph 3.2. The results showed that regardless of the type of pit and fissures sealing material the enamel's disinfection with ozone does not impair the bond strength to enamel and, moreover, enamel's micromechanical properties are not affected by an ozone application. These findings confirm the previous observation that ozone does not affect the penetration of a dental sealant into enamel (Celiberti *et al.* 2006; Dukić *et al.* 2007) and does not alter enamel's physical properties (Celiberti *et al.* 2006).

In the final part of this thesis (Chapter 4), the application of gasiform ozone in composite repair procedures was considered. In Paragraph 4.2, a study investigating the effect of ozone on the repair strength of a conventional methacrylate-based composite was presented. The results suggest that ozone can be applied on the composite surface without altering it and without impairing its repair potential regardless of the intermediate repair agent and of aging conditions. The application of ozone in the re pair procedures of newly developed restorative materials has been the object of a study presented in Paragraph 4.3, which revealed that the repair site of a silorane -based and of an ormocer-based composite can be treated with gaseous ozone, without affecti ng the repair strength of these materials even after aging.

The studies presented in Chapter 4 suggest that unlike other oxidative substances, such as hydrogen peroxide (Papacchini *et al.* 2007), ozone does not represent an hazardous treatment for a composite restoration to be repaired and can be applied if an additional disinfection of the repair site is desirable.

5.2. Conclusions

On the basis of the results of the laboratory studies presented in this thesis investigating the application of gasous ozone combined with different adhesive procedures, the following conclusions can be drawn:

1. The application of gasiform ozone on dentin guarantees the respect of the micromechanical properties of the tooth substrate.
2. The infiltration into dentin of conventional bonding agents and of an adhesive system developed for bonding silorane-based composite restorations is not impaired after bonding to ozone-treated dentin.
3. As far as the micromechanical properties of dental adhesives are concerned, their elastic modulus and the Vicker's hardness vary within a range of values, which is significantly lower than that of dental hard tissues and glass-ionomer cements, whereas dental adhesives show the highest creep. Their elastic indentation work is inferior only to that of enamel.
4. If dental adhesive systems are bonded to ozone-treated dentin, their micromechanical properties are not critically impaired over a 24h period, even if a sustained ozone application is performed. Therefore, bonding to ozone-treated dentin does not negatively affect the outcome of the final restoration.

5. Enamel's elastic modulus and Vicker's hardness are not affected by an ozone application.
6. The application of gaseous ozone on occlusal pit and fissures prior to sealing procedures may represent a valid method for obtaining an additional disinfection without impairing the bond strength of the sealing material.
7. Gasiform ozone does not alter the micromechanical properties of a conventional methacrylate-based composite irrespective of the aging conditions.
8. The use of ozone in combination with composite repair procedures does not interfere with the repair potential of a methacrylate-based composite.
9. Ozone can be applied prior to repairing a silorane-based composite or an ormocer-based composite restoration without compromising the repair strengths of these innovative restorative materials.

5.3. Future directions

The modern approach to dental caries is based on recognizing and treating it as an infectious disease. Therefore, the introduction of gaseous ozone in the dental practice has been an attempt to find a painless method for arresting the progression of caries by killing its bacterial etiological agents.

This thesis findings suggest that ozone can be applied prior to several different adhesive procedures, without impairing their outcomes, suggesting that is possible to use ozone for reducing the risk of caries recurrence especially when a minimally invasive restorative treatment is performed.

The combination of gaseous ozone with restorative procedures should be also validated by clinical investigations. Moreover, the efficacy of ozone in

arresting caries progression should be evaluated when bonding is performed directly on carious dental hard tissues.

More efforts should also be made in order to identify the most appropriate time of application of gaseous ozone, in order to optimize its clinical use.

5.4. Riassunto, conclusioni e direzioni future

Il trattamento della carie ha sempre rappresentato una sfida per l'odontoiatra. Nel secolo scorso la filosofia per il trattamento delle lesioni cariose è radicalmente cambiata. Il tradizionale approccio "chirurgico", consistente nell'escavazione del tessuto cariato e nella realizzazione di un'estesa cavità con conseguente notevole perdita di struttura dentaria (Osborne & Summitt 1998), è stato progressivamente sostituito da un approccio "biologico", che consente la realizzazione di una cavità più conservativa ed è basato sulla comprensione dei processi di sviluppo e progressione della carie, che è riconosciuta e trattata come una patologia infettiva (Tyas *et al.* 2000). Alla luce di questi nuovi concetti sulla gestione della carie si è sviluppato un rinnovato interesse verso metodiche che possano prevenire e controllare l'infezione batterica. In questo contesto è stata proposta l'introduzione dell'ozono nella pratica odontoiatrica.

Nella parte iniziale di questa tesi (Capitolo 1) viene presentata un'introduzione alla tematica principale. Dopo una breve descrizione dei nuovi concetti sulla gestione della carie in accordo ai principi dell' "Odontoiatria Minimamente Invasiva", sono state fatte alcune considerazioni in merito all'impiego di disinfettanti prima di effettuare procedure restaurative.

L'eliminazione della flora microbica associata alla carie può essere ottenuta con un'ampia gamma di agenti antibatterici, che spazia dai liners cavitari (Chandler & Heling 1995), ai disinfettanti convenzionali come la

clorexidina (Ersin *et al.* 2009; Wicht *et al.* 2004) o l'ipoclorito di sodio (Taniguchi *et al.* 2009; Zaura *et al.* 2007), agli adesivi smalto-dentinali (Pinto *et al.* 2010; Esteves *et al.* 2010; Polydorou *et al.* 2006; da Silva *et al.* 2010; Hosoya *et al.* 2010; Vaidyanathan *et al.* 2009; Li *et al.* 2009), agli antibiotici (Wicht *et al.* 2004) e, più recentemente, al laser (Müller *et al.* 2007).

Dopo una concisa descrizione dell'apparecchiatura per la produzione dell'ozono gassoso, è stato preso in considerazione il suo potenziale antibatterico. L'efficacia dell'ozono nei confronti di specie batteriche cariogene isolate è stata provata (Polydorou *et al.* 2006; Johansson *et al.* 2009), mentre il suo effetto su specie batteriche associate non è stato completamente chiarito (Müller *et al.* 2007; Baysan *et al.* 2000; Baysan & Lynch 2004; Knight *et al.* 2008). L'azione antimicrobica dell'ozono gassoso dipende dalla dose, dalla specie batterica e dal tempo di applicazione (Huth *et al.* 2009).

Diversi studi clinici hanno ottenuto risultati incoraggianti riguardo all'applicazione dell'ozono gassoso per il trattamento della carie occlusale in pazienti adulti (Knežević *et al.* 2007) e bambini (Dähnhardt *et al.* 2006; Huth *et al.* 2005) e per la gestione della carie radicolare (Holmes 2003; Baysan & Lynch 2004; Baysan & Lynch 2007). Tuttavia il numero e la qualità degli studi clinici che valutano l'efficacia dell'ozono gassoso dovrebbero essere incrementati prima di poter trarre conclusioni definitive (Azarpazhooh & Limeback 2008; Rickard *et al.* 2004; Stübinger *et al.* 2006; Nogales *et al.* 2008).

Una questione fondamentale in merito all'applicazione di un disinfettante prima di procedure restaurative è rappresentata dalla sua interazione con i substrati dentari e con i materiali da restauro. I comuni disinfettanti hanno mostrato la tendenza ad alterare le proprietà meccaniche dei tessuti duri dentari (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.* 2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) e lo stesso vale anche per le sostanze con azione ossidante (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et*

al. 2002; Chng *et al.* 2005; Duschner *et al.* 2006). Inoltre è stato riportato che disinfettanti ed ossidanti influenzino negativamente le procedure adesive (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006; Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005; Breschi *et al.* 2007; Cadenaro *et al.* 2006), ad eccezione della clorexidina, il cui ruolo nella stabilizzazione delle interfacce adesive è stato recentemente riconsiderato (Breschi *et al.* 2009; Breschi *et al.* 2010). Poiché l'azione antibatterica dell'ozono è basata sul suo potenziale ossidante, dovrebbero sollevarsi alcune perplessità riguardo alla sua applicazione sui tessuti duri dentari prima di effettuare procedure restaurative. Tuttavia l'informazione sull'interazione dell'ozono con i substrati dentari e con i materiali dentali è estremamente limitata (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005; Celiberti *et al.* 2006).

Alla luce di questo fatto è stata valutata l'applicazione dell'ozono gassoso prima di procedure adesive su dentina (Capitolo 2). L'interazione dell'ozono con il substrato dentinale è stata oggetto di uno studio presentato nel Paragrafo 2.2, che ha mostrato che l'applicazione di ozono non altera il modulo di elasticità e la durezza Vicker della dentina. Questo risultato è supportato da un precedente studio che ha riportato che anche le proprietà fisiche dello smalto non sono deteriorate dall'ozono gassoso (Celiberti *et al.* 2006).

Nel Paragrafo 2.3 è stato considerato l'effetto dell'ozono sull'infiltrazione della dentina da parte di diversi sistemi adesivi. La microscopia confocale a scansione laser (CLSM) ha rivelato che la penetrazione degli adesivi esaminati non è influenzata né dal punto di vista quantitativo né da quello qualitativo dal pretrattamento della dentina con ozono gassoso.

Nei Paragrafi 2.4a e 2.4b sono presentati due studi che mirano a inquadrare le proprietà micromeccaniche di adesivi applicati su dentina e a valutare come esse vengano influenzate dall'applicazione di ozono. In termini di proprietà micromeccaniche gli adesivi risultano essere inferiori rispetto allo

smalto e alla dentina ed anche rispetto ai cementi vetroionomerici, che possono anch'essi essere utilizzati come liners cavitari (Paragrafo 2.4a). L'applicazione dell'ozono sulla dentina prima delle procedure adesive non è risultata essere dannosa per le proprietà micromeccaniche dei sistemi adesivi esaminati, indicando che, nonostante il suo elevato potenziale ossidante, l'ozono non interferisce con la polimerizzazione degli adesivi, che è indirettamente espressa attraverso le loro proprietà meccaniche (Paragrafo 2.4b).

I risultati degli studi presentati nel Capitolo 2 indicano che l'uso dell'ozono gassoso su dentina prima di effettuare procedure adesive può essere preso in considerazione senza timore per la potenziale compromissione del restauro definitivo. Questi risultati sono anche supportati da quegli studi che hanno riportato che l'ozono non influenza negativamente la forza di adesione alla dentina (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005).

Il Capitolo 3 è incentrato sull'applicazione dell'ozono in associazione con la sigillatura di solchi e fessure. Nel Paragrafo 3.2 è presentato uno studio sull'effetto dell'ozono sulla forza di adesione shear di diversi materiali utilizzati per la sigillatura di solchi e fessure. I risultati mostrano che, indipendentemente dal tipo di materiale da sigillatura, la disinfezione dello smalto con l'ozono non compromette la forza di adesione e, inoltre, le proprietà micromeccaniche dello smalto non sono influenzate dall'applicazione dell'ozono. Questi risultati confermano la precedente osservazione che l'ozono non influenza la penetrazione di un sigillante nello smalto (Celiberti *et al.* 2006; Dukić *et al.* 2007) e non altera le proprietà fisiche del substrato (Celiberti *et al.* 2006).

Nella parte finale di questa tesi (Capitolo 4) è stata considerata l'applicazione dell'ozono gassoso nelle procedure di riparazione dei compositi. Nel Paragrafo 4.2 è stato presentato uno studio che valuta l'effetto dell'ozono sulla forza di riparazione di un convenzionale composito a base di metacrilato. I risultati indicano che l'ozono può essere applicato sulla superficie del composito senza alterarla e senza comprometterne il potenziale ripartivo,

indipendentemente dall'agente riparativo intermedio utilizzato e dalle condizioni di aging applicate. L'applicazione dell'ozono nelle procedure riparative di materiali da restauro di recente introduzione è stata oggetto di uno studio presentato nel Paragrafo 4.3, che ha rivelato che il sito di riparazione di un composito a base di silorano e di un composito a base di Ormocer può essere trattato con l'ozono senza influenzare la forza di riparazione di questi materiali anche dopo aging.

Gli studi presentati nel Capitolo 4 indicano che, a differenza di altre sostanze ossidanti, come il perossido di idrogeno (Papacchini *et al.* 2007), l'applicazione di ozono non costituisce un trattamento rischioso per un restauro in composito che deve essere riparato e può quindi essere effettuata se si desidera un'ulteriore disinfezione del sito di riparazione.

Sulla base dei risultati degli studi di laboratorio presentati in questa tesi, in cui è stata valutata l'applicazione dell'ozono gassoso in associazione con diverse procedure adesive, possono essere tratte le seguenti conclusioni:

1. L'applicazione dell'ozono sulla dentina garantisce il rispetto delle proprietà micromeccaniche del substrato.
2. L'infiltrazione della dentina da parte dei sistemi adesivi convenzionali e di un sistema adesivo appositamente sviluppato per i restauri in composito a base di silorano non è compromessa se tali adesivi vengono applicati su dentina pretrattata con ozono.
3. Per quanto concerne le proprietà micromeccaniche degli adesivi, il loro modulo di elasticità e la loro durezza Vicker variano entro un range di valori che è significativamente inferiore a quello dei tessuti duri dentari e a quello dei cementi vetroionomerici, mentre gli adesivi presentano il creep più elevato. Il lavoro elastico degli adesivi è inferiore solo a quello dello smalto.

4. Se i sistemi adesivi vengono applicati su dentina pretrattata con ozono le loro proprietà micromeccaniche non subiscono modificazioni critiche nell'arco di 24 ore anche se è stata effettuata un'applicazione di ozono prolungata. Pertanto, l'adesione a dentina pretrattata con ozono non compromette il restauro finale.
5. Il modulo di elasticità e la durezza Vicker dello smalto non sono influenzati dall'applicazione di ozono.
6. L'applicazione di ozono sui solchi e sulle fessure delle superfici occlusali prima di effettuarne la sigillatura può rappresentare una valida metodica per ottenere una disinfezione aggiuntiva senza compromettere la forza di adesione del materiale da sigillatura.
7. L'ozono gassoso non altera le proprietà micromeccaniche di un convenzionale composito a base di metacrilato indipendentemente dalle condizioni di aging.
8. L'uso dell'ozono in associazione con le procedure di riparazione dei compositi non interferisce con il potenziale riparativo di un composito a base di metacrilato.
9. L'ozono può essere applicato prima di riparare un restauro di composito a base di silorano o di composito a base di Ormocer senza compromettere le forze di riparazione di questi innovativi materiali da restauro.

Il moderno approccio alla gestione della carie è basato sul suo riconoscimento e trattamento come patologia infettiva. Pertanto, l'introduzione dell'ozono gassoso nella pratica odontoiatrica ha rappresentato un tentativo di trovare una metodica indolore per arrestare la progressione della carie per mezzo dell'eliminazione dei suoi agenti eziologici batterici.

I risultati di questa tesi indicano che l'ozono può essere impiegato prima di diverse procedure adesive senza comprometterne l' esito, suggerendo che sia

possibile utilizzare l'ozono per ridurre il rischio di recidiva di carie specialmente quando si effettua un restauro minimamente invasivo.

La combinazione dell'uso dell' ozono con procedure restaurative dovrebbe essere validato anche con studi clinici. Inoltre l'efficacia dell'ozono nell'arrestare la progressione della carie dovrebbe essere valutata quando l'adesione viene effettuata direttamente su tessuti dentari cariati.

Dovrebbero essere anche svolti ulteriori studi per stabilire il tempo di applicazione dell'ozono più adeguato, al fine di ottimizzarne l'uso clinico.

5.5. Zusammenfassung, Schlussfolgerungen und künftige Richtungen

Die Behandlung der Karies stellt immer noch eine Herausforderung für den Zahnarzt. Während des letzten Jahrhunderts hat sich die Philosophie der Kariesbehandlung jedoch radikal verändert. Der traditionelle "chirurgische" Ansatz, der aus der Exkavation der Karies und der Vorbereitung einer Kavität mit einem erheblichen Verlust von Zahnsubstanz bestand (Osborne & Summitt 1998), wurde progressiv von einem "biologischen" Ansatz ersetzt, der die Vorbereitung einer konservativeren Kavität erlaubt und auf die Kenntnis der Entwicklung und der Progression der Karies als infektiöse Krankheit gestützt wird (Tyas *et al.* 2000). Aufgrund dieser neuen Konzepte in der Kariesbehandlung ist ein erneutes Interesse an Methoden, die die bakterielle Infektion verhindern und kontrollieren können, geweckt. Deswegen wurde die Anwendung des Ozons in der Zahnmedizin empfohlen.

Im ersten Teil dieser Doktorarbeit (Kapitel 1) wird eine Einführung zum Hauptthema Ozon präsentiert. Nach einer kurzen Beschreibung der neuen Konzepte in der Kariesbehandlung gemäß den Grundprinzipien der "minimalinvasiven Zahnheilkunde" wurden einige Betrachtungen über die Anwendung von Desinfektionsmitteln vor Zahnrestaurationen dargestellt.

Die Elimination von kariogenen Bakterien kann mit einer großen Zahl von antibakteriell-wirkenden Substanzen erreicht werden, die Kavitätsliners (Chandler & Heling 1995), konventionelle Desinfektionsmittel wie Chlorhexidin (Ersin *et al.* 2009; Wicht *et al.* 2004) oder Natriumhypochlorit (Taniguchi *et al.* 2009; Zaura *et al.* 2007), Adhäsiven (Pinto *et al.* 2010; Esteves *et al.* 2010; Polydorou *et al.* 2006; da Silva *et al.* 2010; Hosoya *et al.* 2010; Vaidyanathan *et al.* 2009; Li *et al.* 2009), Antibiotika (Wicht *et al.* 2004) und neulich auch Laser (Müller *et al.* 2007) umfasst.

Nach einer kurzen Beschreibung des Ozon-Gerätes wurde das antibakterielle Potenzial des Ozons berücksichtigt. Die Wirksamkeit des Ozons gegen isolierte kariogene bakterielle Arten wurde bereits bewiesen (Polydorou *et al.* 2006; Johansson *et al.* 2009). Sein Effekt auf assoziierte bakterielle Arten konnte jedoch noch nicht völlig festgestellt werden (Müller *et al.* 2007; Baysan *et al.* 2000; Baysan & Lynch 2004; Knight *et al.* 2008). Die antibakterielle Wirkung des Ozons hängt von der Dosis, von der Art der Bakterien und von der Einwirkungszeit ab (Huth *et al.* 2009).

Einige klinische Untersuchungen haben versprechende Ergebnisse über die Anwendung des Ozons in der Behandlung der Okklusalkaries in Erwachsenen (Knežević *et al.* 2007) und Kindern (Dähnhardt *et al.* 2006; Huth *et al.* 2005) sowie für die Behandlung der Wurzelkaries (Holmes 2003; Baysan & Lynch 2004; Baysan & Lynch 2007) gezeigt. Trotzdem, sollten die Anzahl und die Qualität der klinischen Untersuchungen, die die Wirksamkeit des Ozons erforschen, weiter verbessert werden, um endgültige Schlussfolgerungen ziehen zu können (Azarpazhooh & Limeback 2008; Rickard *et al.* 2004; Stübinger *et al.* 2006; Nogales *et al.* 2008).

Ein wichtiger Aspekt in der Anwendung eines Desinfektionsmittels vor Zahnrestaurationen ist seine Interaktion mit der Zahnsubstanz und den Restaurationsmaterialien. Konventionelle Desinfektionsmittel (Sim *et al.* 2001; Grigoratos *et al.* 2001; Machnick *et al.* 2003; De-Deus *et al.* 2006; Sayin *et al.*

2007; Oliveira *et al.* 2007; Slutzky-Goldberg *et al.* 2004) und auch Substanzen mit einem oxidativen Potenzial (Faraoni-Romano *et al.* 2008; Al-Salehi *et al.* 2007; Attin *et al.* 2005; Chng *et al.* 2002; Chng *et al.* 2005; Duschner *et al.* 2006) haben die Tendenz gezeigt, die mechanischen Eigenschaften von Schmelz und Dentin zu verändern. Außerdem, haben Desinfektionsmittel und Oxidationsmittel einen negativen Einfluss auf die Adhäsion der Restaurationsmaterialien mit der Zahnsubstanz (Pappas *et al.* 2005; Vieira & da Silva 2003; Gürgan *et al.* 1999; Ozturk & Ozer 2004; Santos *et al.* 2006; Spyrides *et al.* 2000; Shinohara *et al.* 2004; Shinohara *et al.* 2005; Breschi *et al.* 2007; Cadenaro *et al.* 2006). Chlorhexidin, dessen wichtige Rolle in der Stabilisierung von adhäsiven Schnittstellen neulich festgestellt wurde (Breschi *et al.* 2009; Breschi *et al.* 2010), stellt jedoch eine Ausnahme dar. Da der antibakterielle Effekt des Ozons sich auf sein oxidatives Potenzial stützt, sollte seine Anwendung auf die Zahnsubstanz vor einer Restauration in Frage gestellt werden. Dennoch, ist die Information über die Interaktion des Ozons mit der Zahnsubstanz und den Restaurationsmaterialien immer noch sehr mangelhaft (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005; Celiberti *et al.* 2006).

Daher wurde die Anwendung des Ozons vor der Adhäsion mit dem Dentin erforscht (Kapitel 2). Die Interaktion des Ozons mit dem Dentin war das Ziel einer Studie gewesen, dargestellt im Paragraph 2.2, die zeigte, dass Ozon den Elastizitätsmodul und die Vickershärte des Dentins nicht veränderte. Dieses Ergebnis wird von einer vorherigen Studie, die gezeigt hat, dass Ozon die physikalischen Eigenschaften des Schmelzes nicht beschädigt, unterstützt (Celiberti *et al.* 2006).

Der Einfluss des Ozons auf die Dentininfiltration verschiedener Adhäsiven wurde im Paragraph 2.3 berücksichtigt. Die konfokale Laserrastermikroskopie (CLSM) zeigte, dass die Infiltration der getesteten Adhäsiven von der Ozonbehandlung des Dentins sowohl quantitativ als auch qualitativ nicht beeinflusst wurde.

In Paragraphen 2.4a und 2.4b werden zwei Studien präsentiert, die als Ziel hatten, die mikromechanischen Eigenschaften von auf Dentin geklebten Adhäsiven zu erforschen und den Einfluss des Ozons auf diese Eigenschaften festzustellen. Hinsichtlich der mikromechanischen Eigenschaften schnitten die Adhäsiven schlechter als Schmelz, Dentin aber auch Glasionomer-Zemente ab. Die letzteren können auch als Kavitätsliners verwendet werden (Paragraph 2.4a). Die Ozonbehandlung des Dentins vor der Adhäsion erwies sich als nicht schädlich für die mikromechanischen Eigenschaften der getesteten Adhäsiven. Dieses Ergebnis zeigt, dass Ozon, trotz seines großen oxidativen Potenzials, die Polymerisation der Adhäsiven, indirekt charakterisiert durch ihre mikromechanischen Eigenschaften, nicht beschädigt (Paragraph 2.4b).

Die Ergebnisse der Studien vom Kapitel 2 zeigen, dass eine Ozonbehandlung des Dentins vor der Adhäsion angewendet werden kann, ohne eine potentielle Verschlechterung der endgültigen Restauration im Kauf nehmen zu müssen. Diese Ergebnisse werden auch von den Studien unterstützt, die zeigten, dass Ozon die Verbundfestigkeit mit dem Dentin nicht negativ beeinflusst (Cadenaro *et al.* 2009; Schmidlin *et al.* 2005).

Im Kapitel 3 wird die Kombination zwischen der Ozonbehandlung und der Versiegelung von Gruben und Fissuren dargestellt. Eine Studie über den Einfluss des Ozons auf die Scherfestigkeit verschiedener Materialien für die Versiegelung von Gruben und Fissuren wird im Paragraph 3.2 präsentiert. Die Ergebnisse zeigten, dass die Desinfektion des Schmelzes mit Ozon, ungeachtet der Art des Versiegelungsmaterials, die Verbundfestigkeit mit Schmelz nicht verschlechtert. Außerdem, werden die mikromechanischen Eigenschaften des Schmelzes von einer Ozonbehandlung nicht beschädigt. Diese Ergebnisse bestätigen die vorherige Beachtung, dass Ozon die Infiltration eines Versieglers in Schmelz nicht beeinflusst (Celiberti *et al.* 2006; Dukić *et al.* 2007) und die physikalischen Eigenschaften des Schmelzes nicht verändert (Celiberti *et al.* 2006).

Im letzten Teil dieser Doktorarbeit (Kapitel 4) wird die Anwendung des Ozons während einer Komposit-Reparatur in Betracht gezogen. Im Paragraph 4.2 wurde eine Studie über den Effekt des Ozons auf die Reparatur-Verbundfestigkeit eines konventionellen Methacrylat-Komposit präsentiert. Die Ergebnisse zeigen, dass Ozon auf der Oberfläche des Komposits angewendet werden kann, ohne diese zu verändern und ohne ihr Reparatur-Potenzial, ungeachtet des Reparatursmittels bzw. der Alterungsbedingungen, zu verschlechtern. Die Anwendung von Ozon während der Reparatur von neulich entwickelten Restaurationsmaterialien war das Thema einer Studie dargestellt im Paragraph 4.3 gewesen. Diese zeigte, dass die Reparatur-Stelle eines Siloran-Komposits bzw. eines Ormocer-Komposits mit Ozon durchaus behandelt werden kann, ohne die Reparatur-Verbundfestigkeit dieser Materialien, auch nach dem Thermowechselbad, negativ zu beeinflussen.

Die Studien des Kapitels 4 zeigen, dass die Anwendung von Ozon, im Gegensatz zu anderen oxidativen Substanzen wie Wasserstoffperoxide (Papacchini *et al.* 2007), kein Risiko für die Reparatur von Komposit-Restaurationen darstellt, und dass Ozon verwendet werden kann, wenn eine zusätzliche Desinfektion der Reparatur-Stelle erwünscht wird.

Angesichts der Ergebnisse der Labor-Studien, die in dieser Doktorarbeit präsentiert wurden, und die die Kombination zwischen der Ozonbehandlung und verschiedenen adhäsiven Restaurationen erforschten, können folgende Schlussfolgerungen gezogen werden:

1. Die Ozonbehandlung des Dentins garantiert unveränderte mikromechanische Eigenschaften der Zahnsubstanz.
2. Die Dentininfiltration sowohl konventioneller als auch speziell für Siloran-Komposite entwickelter Adhäsiven wird von der Ozonbehandlung des Dentins nicht verschlechtert.

3. Hinsichtlich der mikromechanischen Eigenschaften der Adhäsiven variieren ihr Elastizitätsmodul und ihre Vickershärte in einem Wertebereich, der signifikant geringer als jener des Schmelzes bzw. des Dentins und der Glasionomer-Zemente ist. Dagegen zeigen die Adhäsiven das höchste Kriechen. Die elastische Arbeit der Adhäsiven ist geringer nur als jene des Schmelzes.
4. Wenn Adhäsive mit Ozon-behandeltem Dentin geklebt werden, werden ihre mikromechanischen Eigenschaften innerhalb von 24 Stunden nicht kritisch verschlechtert, auch wenn eine verlängerte Ozonbehandlung verwendet wird. Daher hat die Adhäsion zum Ozon-behandelten Dentin keinen negativen Einfluss auf die endgültige Restauration.
5. Der Elastizitätsmodul und die Vickershärte des Schmelzes werden von einer Ozonbehandlung nicht beeinflusst.
6. Die Ozonbehandlung der okklusalen Gruben und Fissuren vor der Versiegelung kann eine zuverlässige Methode darstellen, um eine zusätzliche Desinfektion zu erreichen, ohne die Verbundfestigkeit des Versiegelung-Materials zu verschlechtern.
7. Ozon verändert nicht die mikromechanischen Eigenschaften eines konventionellen Methacrylat-Komposits ungeachtet der Alterungsbedingungen.
8. Die Kombination zwischen Ozonbehandlung und Komposit-Reparatur hat keinen Einfluss auf das Reparatur-Potenzial eines Methacrylat-Komposits.
9. Ozon kann vor der Reparatur einer Restauration aus Siloran-Komposit bzw. Ormocer-Komposit verwendet werden, ohne die Reparatur-Verbundfestigkeiten dieser innovativen Restaurationsmaterialien zu gefährden.

Die modernen Ansichten zu Kariesentstehung und Kariestherapie stützen sich auf ihre Anerkennung und ihre Behandlung als infektiöse Krankheit. Deswegen ist die Einführung des Ozons in die Zahnheilkunde ein Versuch gewesen, eine schmerzlose Methode zu finden, um die Progression der Karies durch die Elimination ihrer bakteriellen ätiologischen Agenten zu blockieren.

Die Ergebnisse dieser Doktorarbeit zeigen, dass eine Ozonbehandlung vor verschiedenen adhäsiven Restaurationen verwendet werden kann, ohne ihre Effizienz zu verschlechtern. Dies zeigt, dass die Anwendung des Ozons möglich ist, um das Risiko einer Kariesrezidive zu senken, insbesondere wenn eine minimalinvasive Restauration bevorzugt wird.

Die Kombination zwischen Ozonbehandlung und Restaurationen sollte auch in klinischen Untersuchungen erforscht werden. Außerdem, sollte die Wirksamkeit des Ozons, die Progression der Karies zu blockieren, festgestellt werden, wenn eine direkte Adhäsion mit kariöser Zahnsubstanz verwendet wird.

Weiterhin sollten auch mehrere Studien durchgeführt werden, um die optimale Anwendungszeit des Ozons festzustellen, damit seine klinische Anwendung optimiert wird.

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Curriculum Vitae

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Research activity

2006: Master of Science in Biotechnologies: Section of Dental Biomaterials, University of Siena, Italy.
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Professional positions

2005: Teacher of Restorative Dentistry, Course for Dental Assistants, ENAIP (National Institution for the Professional Education of the Italian Association of Catholic Workers) Tuscany, Pisa, Italy.

2007-2010: Teaching assistant in Basic Principles of Dentistry, University of Siena, Italy.
2009: Internship at the Department of Fixed Prosthodontics and Dental Materials of the University of Siena, Italy.

Membership in Dental Societies

2003-2005: Member of SIOI (Italian Society of Pediatric Dentistry)
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Magni E, Francini F, Ilie N, Hickel R, Ferrari M. Effetto dell'applicazione di ozono su modulo di elasticità e durezza vicker della dentina. Congresso Nazionale dei Docenti di Discipline Odontostomatologiche e Chirurgia Maxillo Facciale-Rome, April 22-23-24 2009. Mention of honour in the Endodontics Session. Poster presentation.

Coniglio I, Magni E, Cantoro A, Ferrari M. Valutazione dello spessore del cemento intorno a perni circolari e ovali in canali ovali. Congresso Nazionale dei Docenti di Discipline Odontostomatologiche e Chirurgia Maxillo Facciale - Rome, April 22-23-24 2009. Poster presentation.

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