INTRAORAL ENVIRONMENT CONDITIONS AND THEIR INFLUENCE ON MARGINAL LEAKAGE IN COMPOSITE RESIN RESTORATIONS

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ABSTRACT

Color matching in the anterior superior incisor region (ASIR) is very difficult when using a rubber dam during restorative procedures. This study measured temperature/relative humidity parameters in the ASIR and evaluated the influence of the inhalation/downtime/exhalation mouth-breathing cycle on microleakage in composite resin restorations performed in the region, using three different adhesive systems. Sixty bovine incisors were randomly assigned to six groups (n=10) according to environmental conditions (laboratory environment or intraoral conditions) and the three adhesive systems being tested (Prime & Bond NT (PB), Single Bond (SB) and Clearfil SE Bond (CL)). The composite resin restored specimens were thermocycled (800 cycles, 5-55°C), immersed in a 2% methylene blue-buffered solution and sectioned longitudinally. The dye penetration on the margin of the restoration was evaluated and non-parametric statistical analyses were performed. The temperature and humidity parameters in the ASIR showed significant differences when compared to the laboratory environment. Restorations performed in the ASIR environment showed no increases in microleakage. As it was shown that temperature/humidity in ASIR do not affect marginal sealing in direct composite resin restorations negatively, better color matching can be safely achieved without the use of a rubber dam.

Key word: dentin-bonding agents, dentin, temperature, humidity, dental leakage.

CONDIÇÕES INTRAORAIS E SUA INFLUÊNCIA NA MICROINFILTRAÇÃO DE RESTAURAÇÕES EM RESINA COMPOSTA

RESUMO

A seleção de cor na região dos incisivos superiores (RIS) é muito difícil quando se utiliza isolamento absoluto durante o procedimento restaurador. O objetivo deste trabalho foi mensurar os parâmetros de temperatura e umidade relativa na RIS e avaliar a influência do ciclo de inspiração e expiração na microinfiltração de restaurações em resina composta realizadas na RIS, utilizando três sistemas adesivos. Sessenta incisivos bovinos hígidos foram distribuídos, aleatoriamente, em seis grupos (n=10) de acordo com as condições ambientais (laboratorial ou condição intraoral) e um dos três sistemas adesivos testados (Prime & Bond NT (PB), Single Bond (SB) and Clearfil SE Bond (CL)). Os espécimes restaurados com resina composta foram termociclados (800 ciclos, 5-55°C), imersos em solução de azul de metileno a 2% e seccionados longitudinalmente. A penetração de corante ao longo da margem da restauração foi mensurada e analisada estatisticamente usando testes não paramétricos. Os parâmetros de temperatura e umidade relativa em RIS foram significativamente diferentes quando comparados aos encontrados em condições laboratoriais. As restaurações realizadas em RIS não apresentaram aumento na infiltração marginal, quando comparadas às restaurações executadas em laboratório. Como as condições de temperatura e umidade intraoral não apresentaram efeito negativo no selamento marginal de restaurações em resina composta, a não utilização do uso de isolamento absoluto pode ser considerada quando restaurações estéticas em dentes anteriores forem realizadas.

Palavras chaves: Agentes adesivos dentinários, dentina, temperatura, umidade, infiltração dental.

INTRODUCTION

The increasing demand for aesthetic dentistry and conservative restoration procedures has led to improvements in the optical characteristics of restorative dental composite resins. However, single anterior tooth restoration represents a considerable challenge for the clinician[1] due to difficulties in tooth shaping as well as color and translucency reproduction[1-3]. During restorative composite resin procedures in anterior teeth, many dentists prefer not to use a rubber dam because it can lead to tooth dehydration[4] and consequently, to a dramatic change in tooth color and opacity[3,5].

On the other hand, it has been demonstrated that when a rubber dam was not used, temperature and humidity levels were higher during bonding and restorative procedures[6]. Some researchers have studied the detrimental effects of intraoral environmental conditions on dentin bond strength[7,8], marginal leakage[9,10] and volumetric polymerization shrinkage of resin composites[11,12]. Additionally, some adhesive systems were found to be more sensitive to oral humidity[7,9]. This difference in sensitivity appears to be related to the adhesive solvent, the amount of hydrophilic monomers and the water content of the adhesive system[9,13].

Most of these adhesive system experiments were carried out *in vitro*[7-10,14] using a humidity chamber under extreme conditions of temperature and humidity (35-37°C and 90-95%RH). These environmental conditions are very similar to those present in the posterior region of the mouth, where relative humidity is maintained at a high level (78-94%RH)[6]. Additionally, humidity chambers do not reproduce the natural inhalation, down time and exhalation cycles present in the oral cavity[15]. Adhesive systems have also shown no adverse effect in their bond strength when tested under less severe temperature/humidity parameters (30°C and 80% RH)[16].

Relative humidity levels differ in accordance with tooth position in the intraoral cavity. Because the anterior superior incisors are located at the threshold between the oral cavity and the clinical environment, it can be speculated that not using a rubber dam might not cause excessively moist conditions, even in mouth-breathing patients[6].

Within this context, the aims of this study were to: 1) measure anterior superior incisors region (ASIR) temperature/humidity parameters, and 2) evaluate the influence of the inhalation/downtime/exhalation mouth-breathing cycle on microleakage in composite resin restorations performed in the ASIR, using three different solvent-based adhesive systems. The null hypothesis was that relative humidity and temperature in the ASIR oral environment do not influence microleakage around composite resin restorations when bonding procedures are performed on the enamel and dentin substrates.

MATERIALS AND METHODS

Before the beginning of the experiment, the volunteer signed the informed consent to his participation in this study. Sixty bovine incisors were stored in 0.5% chloramine T solution. After the teeth were cleaned, two cylindrical cavities, 2.0 mm in diameter and 1.5 mm in depth, were prepared on each tooth surface using diamond burs (ref. 2294, KG Sorensen, Barueri, SP, Brazil) in a water-cooled, high-speed hand-piece, and finishing was performed using a cylindrical bur (ref. 57L, KG Sorensen, Barueri, SP, Brazil) at low speed. Each bur was replaced after five cavity preparations. Cavities were prepared on the buccal surface, on the cervical third of the crown (enamel) and on the cervical third of the root (dentin). The teeth were randomly assigned to six groups (*n* = 10) according to the environmental conditions and the adhesive systems being tested (Table 1):

The materials used in this study were handled and applied according to the manufacturer's instructions. In groups I, III and V (control groups), bonding and restorative procedures were performed in a laboratory environment in order to reproduce the conditions present when using a rubber dam in a dental clinic[6]. In the experimental groups (II, IV and VI), identical bonding and restorative treatments were performed with the specimens positioned in the anterior superior incisor region (ASIR) of a volunteer's mouth, recreating exhalation/downtime/inhalation mouth-breathing cycles throughout the bonding and restoration procedures in order to simulate restorations performed without the use of a rubber dam. The temperature and humidity levels measured in each environmental condition are summarized in table 2.

The controlled temperature/ humidity conditions in the laboratory environment ranged from 20°C - 23.2°C and 35.9%RH - 45.6%RH, respectively. These values were recorded using a digital thermo-hygroscope (Minipa – MTH- 1361, Minipa Ind. Com, Ltda, São Paulo, SP, Brazil).

From the beginning of the entire bonding procedure until the light-curing of the adhesive systems in the experimental groups, each tooth was positioned

Table 1: Experimental groups (n=10), adhesive system, type of solvent and temperature /humidity environment conditions [laboratory and anterior superior incisor region (ASIR)].

Group	Adhesive	Solvent present	Temperature / Humidity	Number of specimens
I	Prime & Bond NT (PB) (Dentsply)	Acetone	Laboratory	10
II			Intraoral ASIR	10
III	Single Bond (SB) (3M- ESPE)	Water/Ethanol	Laboratory	10
IV			Intraoral ASIR	10
V	Clearfil SE Bond (CL) (Kuraray Medical Inc.)	Water/Ethanol	Laboratory	10
VI			Intraoral ASIR	10

Table 2: Distribution of groups according to adhesive systems tested, humidity and temperature environment conditions [laboratory and anterior superior incisor region (ASIR)].

Groups	Adhesive system	Extraoral humidity	Extraoral temp.	Intraoral humidity	Intraoral temp.
I	Prime & Bond NT	35.9%	23.2°C	------------	------------
II		42.6%	20.0°C	74.3%	32.0°C
III	Single Bond	38.3%	22.9°C	-------------	------------
IV		43.0%	22.6°C	73.5%	31.3°C
V	Clearfil SE Bond	43.9%	23.1°C	-------------	------------
VI		45.6%	22.9°C	72.1%	29.2°C

between the edges of the central incisors of a volunteer, who performed exhalation/down time/inhalation cyclic mouth-breathing. In an effort to create maximum humidity conditions, critical steps, such as the application of the adhesive system and solvent volatilization, were performed during the exhalation phase of the mouth-breathing cycle, when intraoral temperature and humidity were recorded at levels ranging from 30.3 - 32°C and 73.1 - 76.3%, respectively (Table 2).

After the bonding procedures were performed, the cavities were restored with hybrid composite (*Esthet X* Dentsply-Calk, Milforf, DE, USA) which was inserted in the cavity in a single increment and light-cured for 40 seconds. The teeth were stored for 24 hours at 37ºC in a 100% RH environment, after which they were finished and polished with Sof-Lex (3M ESPE Dental Products, St. Paul, MN, USA) fine and ultra-fine finishing discs. The root apices were sectioned with double-faced diamond discs (ref. 7020, KG Sorensen, Barueri, SP, Brazil) and the root canal and pulp chamber were then sealed with epoxy glue (Araldite, Brascola Ltda, São Bernardo do Campo, SP, Brazil). The entire tooth surface, except the restoration and a 1.0 mm distance measured from tooth margins, was coated with two layers of nail varnish (Colorama Maybelline, Rio de Janeiro, RJ Brazil). Next, all groups were thermocycled for 800 cycles between 5ºC and 55ºC (± 2ºC) with a 1-minute dwell time at each temperature. After thermocycling, the teeth were immersed in a 2% methylene blue-buffered solution (pH 7.0) for 2 hours, and subsequently washed and dried.

The specimens were then sectioned on the longitudinal plane – buccal-lingual – through the center of the restoration using double-faced diamond discs (ref. 7020, KG Sorensen, Barueri, SP, Brazil) at low speed under refrigeration. The dye penetration on the margin of the restoration was qualitatively evaluated using an optical stereoscopic microscope (40x magnification, Stemi 2000-c, Zeiss, Germany). Three independent examiners established the extent of microleakage, attributing the following scores:

0. No leakage;
1. Dye penetration up to one-third of the distance between the cavity margin and the axial wall;
2. Dye penetration up to half the distance between the cavity margin and the axial wall;
3. Dye penetration up to the axial wall;
4. Dye penetration beyond the axial wall.

Statistical analysis

Non-parametric statistical analyses were conducted to evaluate the effect of the substrate and adhesive system on dye penetration scores. The Mann-Whitney test was used to compare the different experimental conditions (laboratory versus intraoral – ASIR). The Wilcoxon Mann-Whitney test was performed to compare enamel and dentin substrates. Finally, the Kruskal-Wallis test was used to evaluate the differences between the three adhesive systems studied. All analyses were performed using Biostat software with a fixed significance level of 5%.

RESULTS

The humidity and temperature means, standard deviations and statistically significant differences were analyzed according to laboratory and ASIR environmental conditions, presented in table 3.1 (t-Student test; $p<0.05$).

Table 3.1: Mean and standard deviation (SD) of temperature (ºC) and humidity (%H) levels obtained in intraoral (ASIR) and laboratory environments.

		Mean	SD	
Temperature	Laboratory	27.69	0.29	A
	Intraoral (ASIR)	30.21	1.47	B
Humidity	Laboratory	50.65	2.79	A
	Intraoral (ASIR)	76.78	1.94	B

Different uppercase letters indicate statistically significant differences in vertical columns. Temperature and humidity variables are to be considered separately (Student-t test, p<0.05).

No significant difference was found between the enamel and dentin substrates (p>0.05) using Prime & Bond NT (NT) and Clearfil SE Bond (SE) adhesive systems, independently of environmental conditions. When using the Single Bond (SB) system, the dentin substrate under mouth-breathing conditions showed significantly less dye penetration (p<0.05) than the enamel under both intraoral (ASIR) and laboratory conditions, as well as less dye penetration than the dentin substrate under laboratory conditions (Table 3.2). When the adhesive systems were compared in both intraoral (ASIR) and laboratory environments, no significant difference was observed (p>0.05) for enamel margins (Table 3.3). However, when the dentin margins were evaluated, increased marginal leakage was detected when using the SE bonding system under laboratory humidity/ temperature conditions (Table 3.4).

Table 3.2: Medians and mean ranges of dye penetration scores according to environmental conditions (intraoral (ASIR) or laboratory) and adhesive systems.

		NT		SB		SE	
		Med	MR	Med	MR	Med	MR
Enamel	Laboratory	0	10.5A	0.5	11.5A	0	11.5A
	Intraoral (ASIR)	0	10.5A	0	9.5A	0	9.5A
Dentin	Laboratory	3	12.8A	3	14.05A	1	10.5A
	Intraoral (ASIR)	1	8.2A	1	6.95B	1	10.5A

Different uppercase letters indicate statistically significant differences in vertical columns. Enamel and dentin variables are to be considered separately (Mann-Whitney test, p<0.05).

Table 3.3: Medians and mean ranges (MR.) of dye penetration scores according to dental substrates (enamel or dentin) and adhesive systems.

		NT		SB		SE	
		Medians	MR	Medians	MR	Medians	MR
Laboratory	Enamel	0	1B	0.5	1.5B	0	1.2B
	Dentin	3	3.6A	3	3.8A	1	2A
Intraoral (ASIR)	Enamel	0	1.1B	0	1.3B	0	1B
	Dentin	1	2.3A	1	2.3A	1	2.1A

Different uppercase letters indicate statistically significant differences in vertical columns. Laboratory and intraoral variables are to be considered separately (Wilcoxon Mann-Whitney test, p<0.05).

Table 3.4: Medians and mean ranges (MR.) of dye penetration scores according to adhesive systems and dental substrates.

		NT		SB		SE	
		Medians	MR	Medians	MR	Medians	MR
Laboratory	Enamel	0	13B	0.5	19A	0	14.5A
	Dentin	3	12.89A	3	13.57A	1	20.04B
Intraoral (ASIR)	Enamel	0	14.65A	0	14.78A	0	16.08A
	Dentin	1	14.66A	1	15.39A	1	16.45A

Different uppercase letters indicate statistically significant differences in horizontal rows. Laboratory and intraoral variables are to be considered separately (Kruskal-Wallis test, p<0.05).

DISCUSSION

All bonding procedures for each adhesive system tested were performed under two different environmental conditions (*i.e.* intraoral (ASIR) and laboratory) because the presence of moisture could have a detrimental effect during the primer and/or resin bonding adhesive application, even after the adhesives have been light-cured. According to the literature, the effect of temperature as an isolated variable has not been proven to be a significant factor in determining dentin bond strength[12,17,18]. Therefore, this study focused on relative humidity as a determining factor in dentin bond strength.

During bonding procedures, the control of humidity in the intraoral environment is important to achieve adequate dental bond strength and marginal sealing[7-10,13-15,19]. The aim of this study was to determine if relative humidity in the ASIR (anterior superior incisor region) is a critical factor that can lead to increased microleakage in composite resin restorations that are bonded with acetone and water/ethanol solvent-based adhesive systems. This is an important consideration because rubber dam usage is recommended when performing composite resin restorations to decrease RH and improve adhesive bond strength[6]. However, it can negatively affect tooth color, appearance and translucency during composite resin restoration handling[1,3].

In spite of the detrimental effects on bond strength and microleakage demonstrated in several studies with high relative humidity[7-,10,14,15], it must be pointed out that the temperature and relative humidity parameters (35-37°C and 90-95% RH) measured in these previous studies were significantly higher than those present in the ASIR (30.21±1.47°C and 76.78±1.94% RH).

The results of this study demonstrated that microleakage in enamel and dentin substrates did not increase under the experimental environmental conditions (*i.e.* mouth-breathing). Regarding the enamel, it was expected that mouth-breathing would not be a significant factor in all the adhesive systems tested[9] due to the lower water content of enamel tissue[20] and the fact that it is less sensitive to moisture. Asmussen & Peutzfeldt (2001)[21] suggested that this is probably related to the ability of the hydrophilic bonding systems to absorb small amounts of water.

Lower dye penetration scores in dentin substrate were recorded when bonding procedures were performed under intraoral conditions for Single Bond (SB) and Prime & Bond NT (PB) adhesive systems. Using the PB system, the microleakage scores in intraoral conditions did not differ significantly from the control group, which was bonded and restored under laboratory conditions. Previous studies have demonstrated that for some total-etch adhesives, a relative humidity of about 80% did not alter performance[16,21] and may even have improved the adhesive bond strength[21]. These differences might be attributable to the agent composition, especially the solvent[22]. Jacobsen and Söderholm (1995)[23] showed that water contamination in the hybrid layer could affect bonding strength dramatically, which may be due to interference with the bonding resin polymerization process, mainly when water/HEMA primer is used. These observations have been confirmed in other studies, which demonstrated lower dentin bond strength[14,15] and an increase in dentin microleakage[9,10] under extreme temperature/relative humidity conditions. Conversely, in the present experiment, the SB adhesive system (water/ethanol solvent and HEMA) significantly reduced microleakage under experimental, mouth-breathing conditions. These humidity conditions (72-74% RH) probably did not lead to a detrimental amount of water in the resin/dentin interface, which could have compromised the degree of polymerization conversion in the bonding resin. On the other hand, this level of humidity may have provided favorable moisture conditions in terms of maintaining open diffusion channels for primer/bond resin infiltration[24] to increase the hydrophobic components in the hybrid layer[25].

The self-etching primer system (Clearfil SE Bond / CB) did not alter dentin microleakage independently of the temperature/humidity conditions tested in this study. This may have been due to the fact that changes in humidity levels were below a critical value, which would lead to alterations in its bonding performance. In addition, Miyazaki et al. (2001)[17] and Finger & Tani (2002)[13] have demonstrated that self-etching adhesive systems were not affected by a 75-80% RH level, regardless of changes in temperature. Although some authors[13,17] have pointed out that self-etching systems are less susceptible to environmental humidity conditions during application, other studies have demonstrated a lower dentin bond strength under extremely high humidity conditions (90-95% RH)[14].

Water is an essential component in self-etching systems as it generates hydrogen ions for both the smear layer and the hard dental tissue demineralization process[26]. Since self-etching adhesives perform better when applied on an air-dried smear layer, studies have suggested that they contain a sufficient amount of water for the initial ionization of the acidic components needed to etch dental tissue[27]. Excessive water levels on the dentin surface can reduce primer acidity[27] and consequently the ability to counteract the buffering capacity of the smear layer/dentin[28]. This may explain why self-etching systems had the lowest performance under extremely high humidity conditions, since environmental conditions affect dental surface moisture[14].

It is interesting to note that the primed dentin surface should be air-dried because the primer contains solvent[17] and air-drying facilitates solvent evaporation, as well as moisture removal from the dental surface during the bonding procedures.

Although the ASIR humidity/temperature conditions evaluated in this study did not affect the marginal sealing of composite resin restorations, environmental conditions should be taken into account during bonding procedures because of their potential effect on the outcome of the performance of the restoration. In spite of the importance of microleakage analysis on the marginal deterioration and longevity of the restorations, other factors such as enamel and dentin bond strength should be evaluated in further studies.

Despite encouraging results, it is worth noting that this study does not aim to take a position on whether or not a rubber dam should be used during bonding procedures, since each adhesive agent has different susceptibility to moisture conditions. Furthermore, complete control of intraoral temperature/humidity parameters is difficult to achieve. However, in accordance with patients' ever-increasing demand for excellence in aesthetic results, and the difficulty of matching natural tooth color and appearance in direct composite resin restorations, bonding and anterior restorative procedures performed without the use of a rubber dam, should be considered a viable alternative.

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