Time-Dependent Effect of Refrigeration on Viscosity and Conversion Kinetics of Dental Adhesive Resins

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ABSTRACT

Objectives: This study evaluated the effect of refrigeration at 4°C and post-refrigeration times (immediate, 5, 10, 15, or 20 min) on the viscosity and conversion kinetics of adhesive bonding resins.

Methods: Scotchbond Dual-Cure (3M ESPE) and Clearfil SE Bond (Kuraray) were tested. Control samples were kept at 25°C for 24 h. At each post-refrigeration time, the temperature was checked with a K-type thermocouple. Viscosity measurements as a function of temperature were performed using a cone-plate viscometer. Real-time polymerization was monitored by infrared spectroscopy. Degree of conversion (DC) was calculated for each second during polymerization, and the rate of polymerization analyzed. Data were separately submitted to two-way ANOVA and Tukey's test (P<.05).

Results: Clearfil presented faster increase in temperature after exposure to room temperature than Scotchbond. A continuous decrease in viscosity (Pa.s) was observed for both Scotchbond (0.49, 0.34, 0.30, 0.26, 0.23, 0.23) and Clearfil (0.38, 0.37, 0.34, 0.25, 0.24, 0.22). For Scotchbond, higher final DC was detected for the control (62.7%) compared with the immediate (53.3%) and 5 min (54.7%) groups. For Clearfil, the control sample (81.4%) showed higher DC than all refrigerated groups (68.8–69.5%). Clearfil always showed significantly higher DC than Scotchbond.

Conclusions: Refrigeration presented a significant time- and material-dependent effect on the viscosity and polymerization kinetics of the bonding resins. Under clinical conditions, adhesive agents should be removed from the refrigerator at least 20 min before being used. (Eur J Dent 2010;4:150-155)

Key words: Bonding resins; Degree of conversion; Polymerization kinetics; Refrigeration; Viscosity.

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INTRODUCTION

The main goal of the adhesion of restoratives materials to dental hard tissues is to obtain high bond strengths and a satisfactory, long-lasting seal.[1-3] Over the last decade, a number of investigations have reported that several factors might interfere with the bonding ability of adhesive systems to enamel or dentin, including the adhesion strategy,[4] conditioning time,[5] solvent removal method,[6,7] thickness of the adhesive layer,[8] and even the environmental humidity.[9,10]

Another factor that might affect the performance of adhesive agents is their temperature during polymerization. Manufacturers usually recommend that dental adhesive resins be stored at room temperature to prevent early evaporation of the solvent. However, they are commonly refrigerated to extend their shelf life, and dentists usually take the materials from the refrigerator and use them immediately under clinical conditions. As the temperature potentially interferes with the viscosity and the ability of the solution to penetrate into the substrate, as well with the reactivity of the monomers,[11,12] it is likely that this process may affect the efficacy of bonding agents.

Few studies have reported the influence of refrigeration on the performance of bonding agents,[13-16] and conflicting results are described in literature. Spohr et al[16] detected no significant differences in bond strength between specimens at room temperature and refrigerated specimens for etch-and-rinse systems, while Pazzinato et al[15] reported that the environmental temperature can influence the rate of spreading of the adhesive system in clinically relevant times and may influence adhesive thickness on cavity walls as well. While these studies usually concentrate on evaluating bond strengths, there is no report in the literature regarding the effect of refrigeration on the polymerization mechanism or viscosity of dental adhesive resins.

The purpose of this study was to evaluate the effect of refrigeration at 4°C and post-refrigeration times (immediate, 5, 10, 15, or 20 min) on the viscosity and conversion kinetics of bonding resins. The hypothesis tested was that refrigeration would have a time-dependent influence on both viscosity and polymerization.

MATERIALS AND METHODS

The bonding resins of two adhesive systems were evaluated: Scotchbond Dual-Cure (3M ESPE, St. Paul, MN, USA) and Clearfil SE Bond (Kuraray, Tokyo, Japan). The primer solutions were not tested. Materials compositions are shown in Table 1. Control samples were defined by evaluating both viscosity and polymerization kinetics after keeping the materials at 25°C for 24 h. The bonding agents were stored under refrigeration at 4°C for 24 h. After removing the materials from the refrigerator, different post-refrigeration times were tested: immediate, 5, 10, 15, and 20 min.

Temperature

The temperature of the bonding resins at each post-refrigeration time was measured using a K-type thermocouple connected to a digital thermometer (Tecpel Co. Ltd., Taipei, Taiwan). The thermocouple tip (0.2 mm diameter) was placed into the bottle for 5 s and the temperature recorded. Two measurements were performed after refrigeration and equilibration cycles and averaged. All analyses were performed under controlled temperature (25±1°C) and humidity (60±5%) conditions.

Viscosity

Viscosity measurements for the control and all post-refrigeration times of both adhesive agents were performed using a cone-plate digital viscometer (CAP2000+; Brookfield, Middleboro, MA, USA). A constant 5 μL volume of the bonding solutions was dispensed in the equipment operating under the following settings: temperature of 25°C, speed of 200 rpm, shear rate of 1 s^{-1}, and run time of 15 s. Three specimens were tested for each material/post-refrigeration time condition. The refrigeration and equilibration cycles were conducted after placing the adhesive resins back into the refrigerator for 24 h. Data were computed in Pa.s and submitted to two-way ANOVA (material vs. post-refrigeration time) followed by Tukey's test (P<.05).

Real-time infrared spectroscopy

The real-time polymerization was evaluated by Fourier transform infrared spectroscopy (Prestige21; Shimadzu, Tokyo, Japan) as previously described,[17] using an attenuated total reflectance device composed of a horizontal ZnSe crystal. A constant volume (5 μL) of bonding solution was dispensed onto the crystal and photo-activated for 20 s using a quartz-tungsten-halogen light unit (Optilux501; Demetron Kerr, Orange, CA, USA) with 600 mW.cm^{-2} irradiance. The polymerization reaction was monitored in real time for 1 min using Happ-Genzel apodization, collecting spectra in the 1680 to 1540 cm^{-1} range, with a resolution of 8 cm^{-1}. With this setup, one spectrum (one scan) every second was acquired. Three specimens were tested for each material/post-refrigeration time condition. The refrigeration and equilibration cycles were conducted after placing the adhesive

resins back into the refrigerator for 24 h.

The degree of conversion (DC) for each scan was calculated as previously described,[17] considering the intensity of C=C stretching vibration (peak height) at 1635 cm^{-1} and using, as an internal standard, symmetric ring stretching at 1608 cm^{-1} from polymerized and unpolymerized samples. DC (%) was determined by subtracting the percentage of remaining aliphatic C=C from 100%. Final DC values were submitted to two-way ANOVA (material vs. post-refrigeration time) followed by Tukey's test (P<.05). Average conversion vs. time data were plotted and Hill's 4-parameter non-linear regressions were used for curve fitting. As the coefficient of determination was greater than 0.99 for all curves, the rate of polymerization (RP) was calculated using these data-fitted plots, and the maximum rate of polymerization (Rp^{max}) was recorded.

RESULTS

The results for temperature and viscosity as a function of the post-refrigeration time are shown in Figure 1. Clearfil presented a faster increase in temperature (increase is prominent in the first 5 min) after exposure to room temperature than
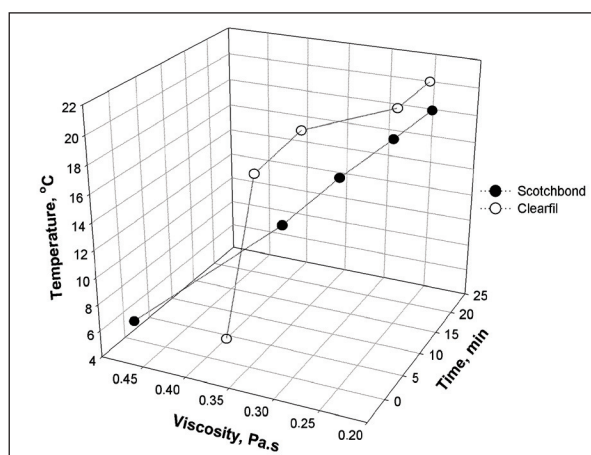

Figure 1. Results for temperature and viscosity as a function of the post-refrigeration time. The 3D scatter plot shows that a continuous decrease in viscosity and increase in temperature with increased post-refrigeration time was observed for both bonding resins.

Scotchbond. The results for the viscosity analysis are also shown in Table 2. The factors 'material' and 'post-refrigeration time' were both significant (P<.001), and so was their interaction (P<.001). In general, all post-refrigeration times presented significant differences in viscosity compared to each other. A continuous decrease in viscosity with increased post-refrigeration time was observed for both bonding resins. For Scotchbond, the decrease was more evident up to 10 min after refrigeration, while for Clearfil the decrease was more evident after 10 min. Comparing the control and 20 min samples, slight differences in viscosity were observed for both systems. At the immediate post-refrigeration time, Scotchbond was significantly more viscous than Clearfil (P<.001), while Clearfil was significantly more viscous for the times 5 and 10 min (P<.001). Similar viscosities for these two materials were detected after 15 min at room temperature (P≥.45).

The results for final DC values are shown in Table 2. The factors 'material' (P<.001) and 'post-refrigeration time' (P=.018) were both significant, whereas the interaction between the two factors was not significant (P=.223). The profiles of polymerization kinetics are shown in Figures 2 and 3. For Scotchbond, a significantly higher final DC value was detected for the control compared with the immediate and 5 min post-refrigeration groups (P<.001). On the other hand, similar DC was observed for the times 10, 15, and 20 min in comparison with the control sample (P≥.558). For Clearfil, the control sample showed significantly higher final DC than all post-refrigeration groups (P≤.003), which were similar among them (P≥.858). Comparing the different materials, Clearfil always showed significantly higher DC than Scotchbond (P≤.012), regardless of the post-refrigeration time.

Polymerization rate profiles are shown in Figure 4. For Scotchbond, although the control group showed the highest Rp^{max} value, similar R$_p$

Table 1. Dental adhesive resins used in the study.

Material	Manufacturer	Constituents*
Scotchbond Dual-Cure	3M ESPE	Bis-GMA, TEGDMA
Clearfil SE Bond	Kuraray	Bis-GMA, MDP, HEMA, colloidal silica

*As provided by the manufacturers. The primer solutions were not tested.

Bis-GMA: bisphenol-A glycidyl dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; MDP: methacryloxydecyl dihydrogen phosphate; HEMA: hydroxyethyl methacrylate.

profiles were detected for the 10, 15, and 20 min post-refrigeration times; the immediate and 5 min times showed lower R_p^{max} compared with all other groups. In contrast, for Clearfil, all post-refrigeration times showed lower R_p^{max} values compared with the control group. Comparing the bonding resins, the R_p was slightly slower and higher R_p^{max} values were usually detected for Clearfil.

DISCUSSION

The fundamental principle of bonding to enamel and dentin relies on a micromechanical interlocking in which the inorganic phase of the demineralized substrate is exchanged by the adhesive resin. The bonding resin should be able to fully penetrate into the etched substrate and polymerize in loco, and therefore it must present proper fluidity to permit its infiltration. The results of this study demonstrated that the refrigeration of the bonding resins dramatically increased their viscosity, and similar values to those of the non-refrigerated sample were detected only 20 min after removing the materials from refrigeration. Thus, the hypothesis tested for viscosity was confirmed.

Despite the similarity in viscosity of the control samples of each bonding resin, Scotchbond showed a higher increase in viscosity due to refrigeration than Clearfil, which also showed faster increase in temperature after refrigeration. One possible explanation for this is related to the role of the components in the formulation. Both adhesives present Bis-GMA, a very viscous, high molecular weight monomer. However, while the diluent monomer for Scotchbond is the dimethacrylate TEGDMA, Clearfil contains the monomethacrylate HEMA as the main diluent, which has lower viscosity. Distinct ratios between Bis-GMA and diluent is one of the factors that might be responsible for the differences in viscosity. Another factor is the presence of fillers, which increases the viscosity of Clearfil. The fact that both materials presented similar viscosities at room temperature suggests that Scotchbond has a higher amount of Bis-GMA, which could explain the increased viscosity during refrigeration compared with Clearfil.

Another observation was that the decrease in viscosity over the course of time was dependent on the material tested. Although Clearfil showed
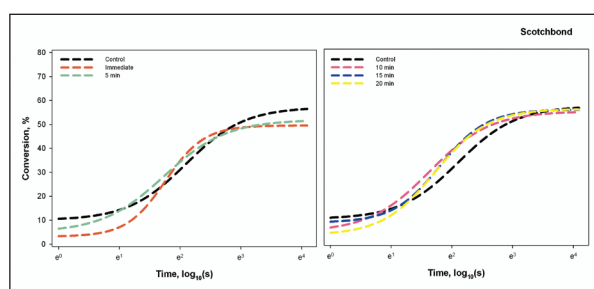
Figure 2. Profiles of polymerization kinetics for Scotchbond. Significantly higher final DC was detected for the control in comparison with the immediate and 5 min post-refrigeration groups, while similar DC was observed for the times 10, 15, and 20 min compared with the control sample.
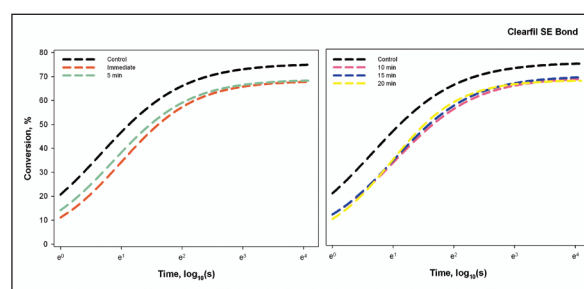
Figure 3. Profiles of polymerization kinetics for Clearfil. The control sample showed significantly higher final DC than all post-refrigeration groups.

Table 2. Means (standard deviations) for viscosity and degree of conversion.

Group	Viscosity (Pa.s)		Degree of conversion (%)	
	Scotchbond	Clearfil	Scotchbond	Clearfil
Immediate	0.487 (.006) A,a	0.376 (.007) B,a	53.3 (3.1) B,c	68.8 (5.0) A,b
5 min	0.336 (.005) B,b	0.367 (.003) A,a	54.7 (2.4) B,bc	68.9 (4.8) A,b
10 min	0.295 (.006) B,c	0.338 (.006) A,b	57.6 (2.6) B,ab	69.1 (6.0) A,b
15 min	0.257 (.004) A,d	0.254 (.005) A,c	59.4 (3.4) B,a	69.2 (3.6) A,b
20 min	0.234 (.003) A,e	0.238 (.006) A,d	59.5 (4.1) B,a	69.5 (3.2) A,b
Control	0.226 (.003) A,e	0.224 (.004) A,e	62.7 (3.3) B,a	81.4 (6.7) A,a

Means followed by distinct letters in the same line, and small letters in the same column, are significantly different at P<.05.

a faster increase in temperature after exposure to room temperature, the viscosity of Clearfil decreased more slowly than Scotchbond. This might be a result of the greater hydrogen bonding potential of HEMA compared with TEGDMA, slowing down the viscosity recovery after refrigeration. The presence of fillers in Clearfil may also explain this result, since the differences in the thermal conductivity of the glass particles compared with the resin matrix may have influenced heat transfer through the material, maintaining the viscosity of the system at higher levels for a longer period of time. As no fillers are present in Scotchbond, the system increases its fluidity faster than Clearfil.

Regarding the polymerization process, refrigeration significantly decreased the DC measured immediately and after 5 min for both adhesives. After 10 min, Scotchbond presented a similar conversion to the control sample. Therefore, the hypothesis for conversion was also confirmed, as refrigeration presented a time-dependent influence on the DC. Nonetheless, the impact of refrigeration on DC was also material-dependent; Clearfil showed significantly lower DC for all refrigerated samples, even after a 20 min post-refrigeration time. This might be linked to the aforementioned longer time required for Clearfil to increase fluidity, which may interfere with the mobility of the monomers in the system.[12] Restricted mobility may decrease the polymerization rate and the conversion of double bonds; all refrigerated groups showed lower R_p^{max} than the control sample for Clearfil. In corroboration, Lohbauer et al,[18] testing resin composites, observed lower monomer conversion for specimens at the temperature 10 ± 2^oC compared with 23 ± 2^oC, and
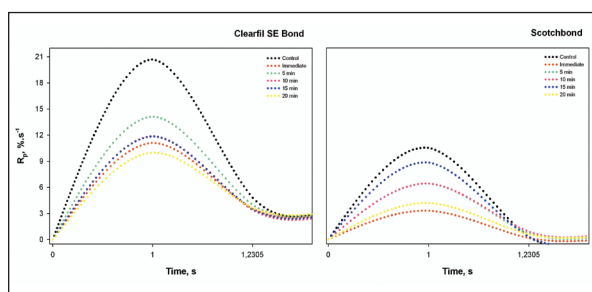
Figure 4. Profiles of polymerization rate. For Scotchbond, although the control group showed the highest R_p^{max} value, similar R_p profiles were detected for the 10, 15, and 20 min post-refrigeration times; the immediate and 5 min times showed lower R_p^{max} compared with all other groups. In contrast, for Clearfil, all post-refrigeration times showed lower Rpmax in comparison with the control group. Comparing the bonding resins, the R_p was slightly slower, and higher R_p^{max} values were usually detected for Clearfil.

also a significant linear correlation between % DC and increase in temperature.

Despite the increased effect of refrigeration on Clearfil, this bonding resin presented significantly higher DC than Scotchbond, regardless of the post-refrigeration time. This result is most probably related to the type of diluent used in each system. The dimethacrylate TEGDMA potentially renders the polymer network more densely cross-linked[11,19] but also reduces the limiting DC for Scotchbond. For Clearfil, the presence of the monomethacrylate HEMA reduces the amount of cross-linking reactions but increases the final conversion. It is difficult to forecast whether the differences in conversion observed here might predict a distinct clinical performance for the bonding resins. Although higher DC is usually linked with improved mechanical properties,[20] the cross-link density also strongly affects the properties of the polymer,[19] especially by reducing the access of water into the network, which is responsible for degrading the bonding layer over the course of time.[4]

The present outcomes showed a time- and material-dependent effect of refrigeration on both the viscosity and polymerization of bonding resins. Therefore, it seems adequate to indicate that adhesive systems should be removed from refrigerator and exposed to room temperature for at least 20 min before they are used. In addition, it can be suggested that the post-refrigeration period should be longer for materials containing fillers. According to Lohbauer et al,[18] the recommendation for resin restoratives is similar; the authors indicated that composites should be used at room or physiological temperatures. However, the effect of refrigeration is likely to be even greater for composites; thus, earlier removal from the refrigerator is advisable. The present results do not take into account other effects that might affect the performance of refrigerated materials in a clinical situation, such as differences in temperature and humidity in the oral environment. Further investigations are necessary to define the time required for proper performance of adhesive systems after refrigeration.

CONCLUSIONS

Refrigeration at 4^oC presented a significant time- and material-dependent effect on the viscosity and polymerization kinetics of the two den-

tal adhesive resins tested. Under clinical conditions, adhesive agents should be removed from refrigerator at least 20 min before they are used.

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