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**Title: Effect of temperature on post-cure polymerization of bulk-fill composites**

**Running head: Temperature effect on post-cure**

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## **Title: Effect of temperature on post-cure polymerization of bulk-fill composites**

### **Abstract**

**Objectives:** To investigate the 24 h post-cure polymerization and the effect of temperature on the post-cure polymerization of one conventional and three bulk-fill composite materials.

**Methods:** A conventional composite GrandioSO (GR) and three bulk-fill composites: Tetric EvoCeram Bulk Fill (TECBF), Quixfil (QF) and X-tra fil (XF) were investigated. The samples were cured for 20 seconds with irradiance of 1090 mW/cm<sup>2</sup>. Composite samples were divided into two groups: the “room-temperature” group (RT, n=5) and the “body-temperature” group (BT, n=5) and they were stored in dark at 20 °C and 37 °C, respectively. Measurements of degree of conversion (DC) were made immediately after curing (0h) and 24 h post-cure (24h). To analyze the extent of post-cure DC increase, the DC values of 0h-RT/24h-RT and 0h-BT/24h-BT were compared. To analyze the difference in DC between RT and BT, the DC values of 0h-RT/0h-BT and 24h-RT/24h-BT were compared.

**Results:** DC increase 24 hours post-cure was significant for all composites and ranged between 6.3%-8.2% in RT and 12.5%-15.7% in BT. All composites demonstrated a higher DC in 24h-BT compared to 24h-RT. The difference was statistically significant for GR, TECBF and QF.

**Conclusions:** All composites demonstrated a significant post-cure effect after 24 h. Post-cure temperature increase from 20 °C to 37 °C yielded a higher post-cure DC increase.

**Clinical significance:** Due to the temperature effect on the final DC, studies performed at composite samples at room temperature may record more inferior properties than these attained in the oral cavity.

## **Introduction**

Contemporary composite materials are typically based on dimethacrylate resins which harden via free-radical polymerization. Activation by visible light is the primary mode of triggering the polymerization (1, 2). Polymerization occurs by subsequent addition of dimethacrylate monomer molecules, resulting in rapid growth and crosslinking of polymeric chains. During polymerization, free radicals are located on growing polymeric chains and represent reactive sites that must be reached by monomeric molecules for addition to take place (3). Since polymeric chains are relatively immobile due to high molecular mass, the reaction is controlled by the mobility of monomer molecules. In the initial stages of polymerization, monomer is free to flow to reactive sites and addition proceeds rapidly. However, as degree of conversion (DC) increases, it becomes increasingly difficult for monomer to reach reactive sites and therefore the rate of reaction decreases (4).

Free radical polymerization features complex kinetics, with phenomena known as autoacceleration and autodeceleration (3). Autoacceleration occurs in early stages of polymerization because limited mobility of free radicals located on growing polymeric chains inhibits termination. This results in the increase of reaction rate, despite the fact that the amount of reactants is decreasing. Autodeceleration occurs in later stages of polymerization when increasing viscosity limits the mobility of monomer molecules, thus inhibiting propagation. In other words, kinetics of polymerization reaction is largely affected by mobility of reactants during both early and late stages of reaction (5). Due to the increasing diffusivity limitations, the polymerization rate slows down significantly and reaction extends for prolonged periods of time.

Most of the polymerization reaction occurs during the first minutes after irradiation; however a significant portion of reaction may take place after curing (6). The extent of post-cure reaction depends on initial DC (7), resin composition (8), availability of free radicals (9) and the temperature of the sample (10).

Bulk-fill composites are designed for placement in layers up to 4 mm. In order to achieve properties that enable such placement, manufacturers have addressed issues of shrinkage stress and depth of cure by various composition modifications (11). These modifications may alter polymerization kinetics, especially because slower polymerization is sometimes used as a strategy to reduce shrinkage stress (12, 13).

The aims of this study were: (I) to assess the post-cure DC increase for one conventional and three bulk-fill composites, and (II) to examine the influence of temperature on the post-cure DC increase. The null-hypotheses were: (I) there is no difference between the DC values obtained immediately after curing and 24 h post-cure, and (II) there is no difference in the post-cure DC increase between the samples stored at 20 °C and 37 °C.

## **Materials and methods**

Composite materials are listed in Table 1. Three bulk-fill composites were investigated and a conventional composite GrandioSO (Voco, Cuxhaven, Germany) was used as a control.

Cylindrical samples with diameter of 3 mm and height of 6 mm were prepared using a solid stainless steel mold with polished and thus reflective surface. The outside shape of the mold was

cuboid, with dimensions of 20 x 6 x 8 mm. Uncured composite material was applied in bulk into the mold, both mold apertures were covered with a polyethylene terephthalate (PET) film and curing was performed with a LED curing unit Bluephase G2 (Ivoclar-Vivadent, Schaan, Liechtenstein) for 20 s in the wavelength range of 380-515 nm. The irradiance of Bluephase G2 in “high” mode, as stated by the manufacturer, is 1200 mW/cm<sup>2</sup> while the value of 1090 mW/cm<sup>2</sup> was measured using a single CureRite radiometer (Caulk Dentsply, Konstanz, Germany, model No. 644726). Taking into account the variability of the irradiance measurements obtained by some hand-held radiometers (14, 15), in the present study the radiometer was used for monitoring the constancy of the curing unit irradiance before the polymerization of each composite material. The variations were under 10%, and value of 1090 mW/cm<sup>2</sup> represents the arithmetic mean of ten measurements. The curing time of 20 seconds corresponded to or exceeded manufacturer’s recommendations for all tested materials. The light probe of the curing unit was positioned immediately adjacent to the mold aperture, contacting the PET film covering the sample. A fixture was used to align the center of the light probe with the center of the mold aperture. Sample curing was done at 20±1°C. During the measurement, the samples were retained inside the molds, which were used for mounting to the universal holder of the spectrometer.

For each composite, 10 samples were prepared and randomly divided into two groups: the “room-temperature” group (RT, n=5) and the “body-temperature” group (BT, n=5) which were dry stored in dark at 20 °C and 37 °C, respectively. For the storage of BT samples, the incubator Cultura (Ivoclar-Vivadent, Schaan, Liechtenstein) was used. Temperature variations were under 1°C.

DC measurements were made at the irradiated sample surface immediately after curing (0h) and 24 h post-cure (24h). DC was determined by means of FT-Raman spectroscopy, using a Spectrum GX spectrometer (PerkinElmer, Waltham, USA). The excitation was an NdYAG laser at 1064 nm wavelength, with laser power of 800 mW and resolution of 4  $\text{cm}^{-1}$ . The exposed sample surface was about 0.5 mm in diameter. For each spectrum, 100 scans were recorded. Spectra of the uncured composites (n=5) were recorded in the same manner. The spectra were processed with the Kinetics add-on for Matlab (Mathworks, Natick, Massachusetts, USA).

DC calculation was performed by comparing the relative change of the band at 1640  $\text{cm}^{-1}$ , representing the aliphatic C=C stretching mode to the aromatic C=C band at 1610  $\text{cm}^{-1}$ , before and after the polymerization. Integrated intensities of aliphatic C=C and aromatic C=C bands were used for DC calculation by the following equation:  $DC = 1 - R_{\text{polymerized}}/R_{\text{unpolymerized}}$ , where  $R = (\text{aliphatic C=C band area}) / (\text{aromatic C=C band area})$  (16).

To analyze the extent of post-cure DC increase, DC values immediately after curing and 24 h post-cure were compared within RT and BT groups for each composite, denoted as 0h-RT/24h-RT and 0h-BT/24h-BT, respectively. They were compared by dependent samples t-test. To analyze the difference in DC between RT and BT, the DC values of 0h-RT/0h-BT and 24h-RT/24h-BT were compared for each composite by independent samples t-test. Statistical analysis was performed in SPSS 20 (IBM, Armonk, NY, USA). P-values lower than 0.05 were considered statistically significant.

## Results

Mean DC values for RT and BT are presented in Table 2.

DC values immediately after curing amounted to 58.0%-65.8%, while the 24 h post-cure DC values were in the range of 65.6%-72.6% and 73.8%-78.3% for RT and BT, respectively.

All composites demonstrated a significant post-cure DC increase which ranged between 6.3%-8.2% in RT and 12.5%-15.7% in BT. Since there was no significant difference between 0h-RT and 0h-BT for each individual composite, equal initial DC for both groups was assumed. Thus, the impact of temperature on post-cure behavior was evaluated by comparing 24h-RT/24h-BT. All composites demonstrated higher DC in 24h-BT compared to 24h-RT. The differences in the DC values between 24h-BT and 24h-RT were 5.2% (GR), 11.1% (TECBF), 5.7% (QF) and 3.4% (XF). These differences were statistically significant for GR, TECBF and QF, while for XF only a trend of increase was observed.

## **Discussion**

This study intended to evaluate the extent of 24 h post-cure DC increase for one conventional and three bulk-fill composites. Additionally, the impact of two temperatures (20 °C and 37 °C) on the post-cure DC increase was assessed.

Post-cure polymerization occurs due to the nature of photo-activation of composite resins. Initial rapid reaction readily causes mobility limitations; hence the rate of polymerization considerably decreases (3). Therefore, after the short irradiation interval, free radicals remain trapped within the matrix and the reaction has the potential to continue at a slow rate (9). The post-cure reaction can continue for as long as there are free radicals available (17) and reactants (i.e. pendant methacrylate groups and free monomer) are sufficiently mobile.

The most of the post-cure polymerization occurs during the first 24 h after irradiation, as assessed indirectly by microhardness (6). The amount of free radicals thereafter decreases considerably and further polymerization becomes extremely slow (9). However, it can still be observed up to 1 month after irradiation (18). Post-cure polymerization has been reported for conventional and bulk-fill composites (7, 18, 19). A study by Truffier-Boutry et al. reported no post-cure DC increase in the neat Bis-GMA/TEGDMA mixture (20), but it was documented for filled Bis-GMA/TEGDMA experimental resins (8). Contrary to the previously listed reports, one study reported no post-cure effect in a commercial Bis-GMA/TEGDMA-based composite (21). The inability to observe the post-cure effect may be due to the insufficient sensitivity of the method used. Additionally, Alshali et al. (19) reported a significant post-cure DC increase for all tested materials except for Filtek Bulk Fill, which was not the object of the present investigation.

In this study, conventional and bulk-fill composites presented a significant post-cure effect at both 20 °C and 37 °C. The first null-hypothesis was therefore rejected. The extent of the post-cure DC increase is known to depend on the resin composition and initial DC (7, 8). Generally, lower initial DC allows higher mobility which results in higher post-cure (7). Therefore, formulations containing lower ratio of diluent monomer TEGDMA that reach lower initial DC show more additional post-cure conversion (8). Also, lower initial DC due to low irradiation doses may result in higher post-cure DC increase (18). Since in the present study irradiation dose was consistent for all tested composites, the differences in post-cure DC increase are attributable to differences in composite formulations. Considering the post-cure DC increase within RT and BT, the between-material differences were relatively small, amounting to 2-3%, which is in the range of standard deviation. Thus it appears that compositional differences of the tested materials

did not significantly affect the amount of post-cure. The results also suggest that the post-cure behavior was similar for three bulk-fill composites and the conventional composite.

The time interval between the end of curing and the end of measurement was 16-17 minutes, due to sample handling (1-2 min) and Raman measurement (15 min). The portion of post-cure reaction that occurred in this interval remained undetected by our experiment. A setup with faster data collection rate may record even higher post-cure DC increase than reported here.

Positive relationship of the post-cure polymerization and temperature is explained by facilitated mobility of reactive species (10). After irradiation, reactive species are still present, but the reaction is considerably limited due to reduced mobility. If the temperature is increased, the mobility improves allowing free monomer and pendant groups to access radical sites and further react (22). From the perspective of reaction kinetics, the temperature increase may be perceived as a factor delaying autodeceleration and in turn allowing the reaction to continue at a higher rate and for a longer time (23). Temperature influence on post-cure DC has been reported previously (23-25) and a linear relationship between the post-cure temperature and DC was found (24). Our results confirm the positive influence of temperature on the post-cure effect since increasing the temperature from 20 °C to 37 °C almost doubled the post-cure DC increase (Figure 1). The effect of temperature increase was similar for the bulk-fill composites and the conventional composite. Since the temperature increase yielded a statistically significant DC increase for GR, TECBF and QF, but not for XF, the second null-hypothesis was partially rejected.

The dynamic nature of DC in composite materials must be taken into account while testing composite properties. Virtually every physical property of a composite is inseparably linked with DC, whose development is time- and temperature-dependent (26). With regard to the relative

influence of time and temperature, Bagis et al. reported that temperature was 12 times more influential than time on the extent of post-cure conversion (24). Our results indicate that both time and temperature are important factors for the DC development. Investigations of composites are commonly performed either at room temperature due to convenience, or at 37 °C in an attempt to simulate conditions in oral cavity. Also, curing is often performed at room temperature, followed by a post-cure period at 37 °C, as done in our study. Timing of testing is also variable, i.e. “post-cure” is being measured at different times: 40 min after curing (27), 24 h after curing (19), up to 1 month after curing (18). Since the DC changes over time and depends on the temperature, these differences in experimental conditions introduce variability that affects comparability of results reported in different studies.

The rate and extent of polymerization are known to depend on curing temperature (25, 28), as well as post-cure temperature (29). For instance, temperature increase from 22 °C to 35 °C yielded a 106% increase in the polymerization rate during the first second of curing (28). Also, higher curing temperature causes higher initial DC which in turn results in less post-cure (25). A significant impact of radiant exposure and post-cure temperature on the final conversion (determined indirectly by hardness) was reported by Quance et al. (29). These findings indicate that there are multiple factors which influence the final conversion of composite materials. Our study was limited to curing at 20 °C followed by storage at 20 °C and 37 °C, in order to compare the post-cure behavior under conditions that are most commonly used for investigating composite properties, i.e. curing at room temperature followed by storage at either room temperature or at 37 °C (6, 7, 8, 11, 18, 19, 21).

The variations in resin composition and filler content were reported to influence the DC (8, 30) and may be expected to also affect the post-cure polymerization. Bulk-fill composite QF is based

on UDMA, while TECBF and XF comprise of both Bis-GMA and UDMA base monomers. Conventional composite GR is Bis-GMA based. The filler ratio of these composites ranges from 61%-73% by volume. Since all composites showed a similar post-cure DC increase and similar influence of temperature on post-cure, it appears that neither resin nor filler composition had a significant effect on the post-cure behavior.

A significant post-cure DC increase is clinically important since it implies that composite restorations do not present their final physical properties immediately after curing, but rather develop them during the post-curing period. The post-cure DC increase may be important in this regard, since a relatively small DC increase during the late stages of polymerization largely affects the crosslinking density thus potentially improving physical properties (31). Moreover, differences in post-cure behavior at 20 °C and 37 °C suggest that studies performed at composite samples at room temperature may record more inferior properties than those attained in the oral cavity.

## **Conclusions**

- All composites demonstrated a significant post-cure effect after 24 h, both at 20 °C and 37 °C.
- Temperature increase from 20 °C to 37 °C significantly increased the extent of post-cure.
- Bulk-fill composites showed similar 24 h post-cure DC increase to the conventional composite.
- Temperature increase from 20 °C to 37 °C similarly affected the post-cure of both bulk-fill and conventional composite.

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**Figure legend:**

Figure 1 Amount of 24 h post-cure DC increase (mean values  $\pm$ SD) for samples stored at 20 °C and 37 °C.

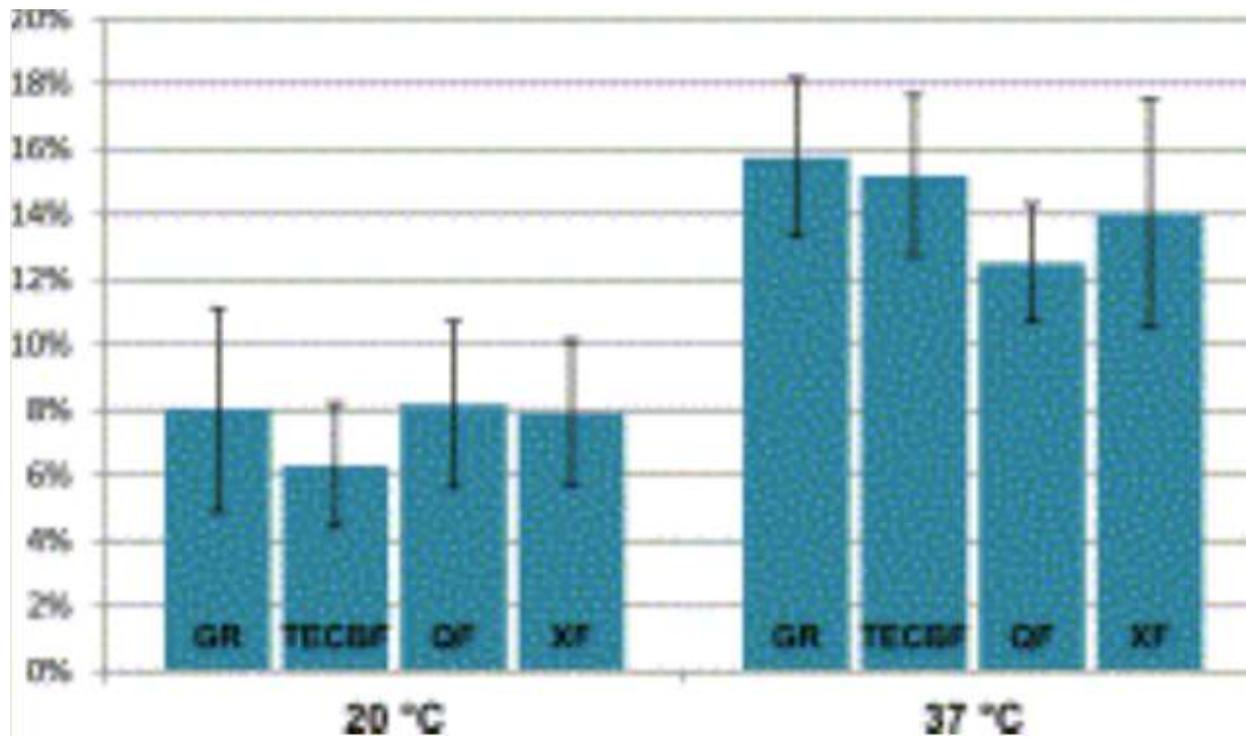


Table 1 Manufacturers' information about the composite materials used

Type	Composite material / code (Manufacturer)	Shade / LOT (EXP)	Composition	Filler amount wt% / vol%
Conventional	<b>GrandioSO / GR</b> (Voco, Cuxhaven, Germany)	A2 / 1222126 (2014/11)	inorganic fillers in a methacrylate matrix (Bis-GMA, TEGDMA)	89/73
Bulk-fill	<b>Tetric EvoCeram Bulk Fill / TECBF</b> (Ivoclar Vivadent, Schaan, Liechtenstein )	IVA / P82299 (2015/12)	Dimethacrylates: Bis-GMA, Bis-EMA, UDMA Barium glass, ytterbium trifluoride, mixed oxide and prepolymer; additives, catalysts, stabilizers, pigments	81/61
	<b>Quixfil / QF</b> (Dentsply, York, Pennsylvania, USA)	universal / 121200233 (2014/05)	UDMA, TEGDMA, di- and trimethacrylate resins, carboxylic acid modified dimethacrylate resin, butylated hydroxy toluene (BHT), UV stabilizer, camphorquinone, ethyl-4-dimethylaminobenzoate, silanated strontium aluminum sodium fluoride phosphate silicate glass	86/66
	<b>X-tra fil / XF</b> (Voco, Cuxhaven, Germany)	U / 1311472 (2015/03)	inorganic filler in a methacrylate matrix (Bis-GMA, UDMA, TEGDMA)	86/70

Bis-GMA: Bisphenol-A-glycidylmethacrylate; TEGDMA: Triethylene glycol dimethacrylate; Bis-EMA: Ethoxylated bisphenol-A-dimethacrylate; UDMA: Urethane dimethacrylate

Table 2 Mean DC values immediately after curing (0h) and 24 h post-cure (24h), n=5

Composite material	RT				BT			
	0 h		24 h		0 h		24 h	
	Mean (%)	SD						
<b>GR</b>	60.5	2.3	68.6 *	3.8	58.0	2.8	73.8 * +	2.0
<b>TECBF</b>	59.3	1.2	65.6 *	2.4	61.6	2.9	76.7 * +	1.9
<b>QF</b>	64.4	3.4	72.6 *	1.2	65.8	2.1	78.3 * +	1.4
<b>XF</b>	63.6	1.4	71.5 *	2.8	60.9	2.6	75.0 *	4.2

RT – room temperature group, 20°C

BT – body temperature group, 37°C

For a given composite, statistically significant differences within groups (RT and BT) are marked with an asterisk (\*). For a given composite and time, statistically significant differences between RT and BT are marked with the plus sign (+).