

Monomer Release from Nanofilled and Microhybrid Dental Composites after Bleaching

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Abstract

Objective: The aim of this study was to assess the effect of bleaching on elution of monomers from nanofilled and microhybrid composites.

Materials and Methods: 80 samples (5mm diameter and 3mm thickness) of each composite were prepared. After curing, half of them were randomly polished. Each group was divided into 8 subgroups and immersed in water or 10%, 20% and 30% H₂O₂ for 3 or 8 hours. Eluted Bis-GMA (Bis-phenol A Glycidyl Dimethacrylate), TEGDMA (Triethyleneglycol Dimethacrylate), UDMA (Urethane Dimethacrylate) and BisEMA (Bis-phenol A ethoxylate Dimethacrylate) were quantified by high performance liquid chromatography and the results were analyzed by univariate ANOVA and t-test (P<0.05).

Results: Bleach significantly increased the overall release of monomers (P<0.001); TEGDMA was released more than Bis-GMA (P<0.001). Supreme released more TEGDMA compared to Z250 (P<0.001). Bleaching increased the release of this monomer (P<0.001). Increasing both the concentration of H₂O₂, and the immersion time, increased the release of TEGDMA (P<0.001). Polishing had no effect on release of this monomer (P=0.952). Supreme released more Bis-GMA than Z250 (P=0.000). The more concentrated H₂O₂ caused more elution of Bis-GMA (P= 0.003); while the effect of immersion time was not significant (P=0.824). Polishing increased the release of Bis-GMA (P=0.001). Neither the type of composite nor Bleaching had any effect on release of UDMA (P=0.972) and (P=0.811) respectively. Immersion duration increased the release of UDMA (P=0.002), as well as polishing (P=0.024).

Conclusion: Bleaching increased the release of monomers. Nanofilled composites released more monomer than the microfilled.

Key words: Monomer elution; Dental composites; Chromatography; Bleaching

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INTRODUCTION

Dental composite resins are used in most dental treatments as restorative or adhesive materials. Besides direct fillings, these resins are also used as cements, dentin adhesives and

luting agents for inlays, crowns, orthodontic brackets, and veneers [1, 2]. Other than UDMA and Bis-GMA, high quantities of TEGDMA as the diluents of monomer and Hema, a hydrophilic monomer, are common

components of resin composites [2-4]. Despite being considered highly stable structures, dental composites are susceptible to degradation due to the incomplete polymerization and the influence of the aqueous oral environment [5]. It has been demonstrated that unconverted monomers can be released from the resin composites into adjacent aqueous environment [6-9].

These monomers can be released into saliva and contact the mucosal tissues, and even reach the pulp via dentinal tubules [10]. HEMA and TEGDMA are the main monomers released from resin-based materials [11]. Elution of monomers from composites has consequences like reduced mechanical properties and increased biological hazards, thus it is important to study the pattern of monomer release in different clinical situations. Polydorou et al. investigated the elution of monomers from different composites and concluded that the release of monomers from a nanohybrid and a chemically-cured composite was significantly higher thanOrmocer [12]. Tooth Bleaching is another part of esthetic dentistry. Different concentrations of hydrogen peroxide have been used in bleaching procedures [13]. Studies concerning the interaction between composite restorations and bleaching materials have extensively evaluated the bond strength of adhesives to enamel and dentin after bleaching [14, 15], effect of bleaching agents on surface texture, hardness and roughness [16-19], and color and microleakage of dental composites [20].

There are few studies on the effect of bleaching agents on release of monomers from composites. Recently, Polydorou et al. studied the effect of bleaching on the elution of monomers from anOrmocer and a nanohybrid composite and reported a reduction in monomer release after bleaching [21]. Patients seeking bleaching treatment may have teeth restored with different types of composites. Interaction of bleaching materials with resin composites in the oral cavity is a serious concern because oxidative properties of active agents in bleaching materials can have side effects on resin composites [17-20]. Understanding the behavior of monomer release and monitoring the leachable components are crucial to clear the degradation process of polymer-based materials in different clinical procedures. The aim of this study was to detect and quantify the main monomers released from polished or unpolished microhybrid and nanofilled composites after different storage times in different concentrations of hydrogen peroxide, using high performance liquid chromatography (HPLC). The quantities of monomers released were the dependent variables, while the types of composite resins, polishing, H2O2 concentration and duration of immersion were the independent variables.

MATERIALS AND METHODS

Table 1 shows the components of composites used in the study. First 80 disk-shaped samples of each composite (5x3 mm) were prepared in stainless steel molds.

Table 1. Ingredients of composites used in the study

Composite	Manufacturer	Organic matrix	Filler	Particle size
Filtek TM Z250	3M ESPE	BisGMA/UDMA TEGDMA bisEMA	Zirconia/silica (60%)	0.01-3.5nm
Filtek TM Supreme	3M ESPE	BisGMA/UDMA TEGDMA bisEMA	Zirconia/silica (59.5%)	5-20 nm

A Mylar strip was placed on the top of the composite and the polymerization was completed with a light-curing unit (Ultralume 2, LED, Ultradent. USA), with 600 Mw/cm² output, controlled by a radiometer (Bisco, USA). The manufacturer's instructions were followed for each material.

Randomly, half of the cured composites were polished by medium and fine grit polishing disks (OptiDisk, Kerr Howe, USA).

All of the samples were randomly immersed in 2 cc of distilled water (as controls), or 10%, 20% and 30% H2O2, for 3 to 8 hours at room temperature. Table 2 summarizes the studied groups.

Glass tubes used for immersion of the samples were completely sealed by paraffin and were covered by aluminum foil to keep the samples away from light. After the immersion procedure, the tubes were refrigerated until HPLC analysis. Bis-GMA, BisEMA and UDMA with 100% purity and TEGDMA with 90% purity were used to create calibration curves. Table 3 shows the monomers used for this purpose. The released monomers were detected and quantified as microgram/liter. Effect of the type of composite, polishing, concentration of H2O2 and immersion time on release of each monomer was evaluated and analyzed using Univariate ANOVA.

Table 2. Detail of the subgroups tested

Composite Type	Nanofilled						Microhybrid															
	Polished			Unpolished			Polished			Unpolished												
Surface	Control		10% H2O2	20% H2O2	30% H2O2	Control		10% H2O2	20% H2O2	30% H2O2	Control		10% H2O2	20% H2O2	30% H2O2							
Medium	Control		10% H2O2	20% H2O2	30% H2O2	Control		10% H2O2	20% H2O2	30% H2O2	Control		10% H2O2	20% H2O2	30% H2O2							
Time(hours))	3	8	3	8	3	8	3	8	3	8	3	8	3	8	3	8	3	8	3	8	3	8

Table 3. Standard Monomers used for HPLC

Monomer	Company
Bisphenol A Glycidyl Dimethacrylate (Bis-GMA)	(Aldrich - USA)
Bisphenol A ethoxylate Dimethacrylate (Bis-EMA)	(Aldrich - USA)
Urethane Dimethacrylate (UDMA)	(Aldrich - USA)
Triethyleneglycol Dimethacrylate (TEGDMA)	(Fluka Germany)

Since the three-way and two-way interactions were significant in the subgroups, independent sample t-test was used. Bonferroni's correction method was used to counteract the problem of multiple comparisons. $P < 0.05$ was considered the limit of significance.

RESULTS

Tables 4-6 show the effect of independent variables on release of Bis-GMA, TEGDMA and UDMA, respectively. As can be observed, the interactions were significant. Table 7 shows the mean and SD of Bis-GMA released from Supreme and Z250. Table 8 depicts the mean and SD of TEGDMA released from Supreme and Z250. Bleaching significantly increased the overall release of monomers ($P < 0.001$), while TEGDMA was released more than Bis-GMA ($P < 0.001$). The effect of independent variables on release of monomers is summarized as follows:

Bis-GMA:

Supreme released more Bis-GMA than Z250 ($P = 0.000$). The more concentrated H₂O₂ caused more elution of Bis-GMA ($P = 0.003$); while the effect of immersion time was not significant ($P = 0.824$). It was observed that polishing significantly increased the release of Bis-GMA ($P = 0.001$).

TEGDMA:

TEGDMA was released significantly more in Supreme composite compared to Z250 ($P < 0.001$). Bleaching significantly increased the release of this monomer ($P < 0.001$). Increasing both the concentration of H₂O₂, and the immersion time, increased the release of TEGDMA ($P < 0.001$). But polishing had no effect on release of this monomer. ($P = 0.952$).

UDMA:

The type of composite had no effect on release of UDMA ($P = 0.972$). Bleaching was also ineffective ($P = 0.811$). Immersion duration in-

creased the release of UDMA ($P = 0.002$). Polishing increased the release of this monomer ($P = 0.024$).

BisEMA:

No evidence of BisEMA release was observed in this study

DISCUSSION

The most frequently used substances for bleaching vital teeth are 10% to 37% carbamide peroxide, and 1.5% to 38% hydrogen peroxide; the more concentrated products are used professionally in the dental office [22]. The H₂O₂ concentrations used in the present study are among the usual concentrations applied in clinical bleaching procedures. There is controversy about the impact of low concentrated 10%–16% carbamide peroxide gels or 35% hydrogen peroxide on surface microhardness of restorative composite materials [17-19]. Such wide variations in data suggest that some tooth colored restorative materials may be more susceptible to alterations caused by some bleaching agents. This is why in this study composites with the same matrix composition were used. The detection method used was HPLC; which is a valuable and popular method of analysis, not limited by the volatility or stability of the sample compound; many researchers have used this method in dentistry [23-25]. The results confirmed that TEGDMA was released significantly more than Bis-GMA. This observation was in agreement with Nathanson et al [26], who showed that smaller molecules are released faster and more than the larger ones because of their mobility. TEGDMA is added to Bis-GMA to decrease viscosity of the composition. Tabatabaee et al. showed that TEGDMA was released more than Bis-GMA from both nanofilled and flowable composites cured with either halogen or LED curing units [27]. The greater release of Bis-GMA and TEGDMA in higher concentrations of H₂O₂ was confirmed by Durner et al,

Table 4. Analysis of variance of BisGMA released in the tested media

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.398(a)	31	.658	5.159	.000
Intercept	74.377	1	74.377	583.113	.000
CONCENTR	2.758	3	.919	7.208	.000
TIME	6.383E-03	1	6.383E-03	.050	.824
COMPOSIT	1.190	1	1.190	9.329	.003
POLISH	1.496	1	1.496	11.726	.001
CONCENTR * TIME	1.270	3	.423	3.320	.025
CONCENTR * COMPOSIT	.513	3	.171	1.342	.268
TIME * COMPOSIT	.203	1	.203	1.590	.212
CONCENTR * TIME * COMPOSIT	1.074	3	.358	2.808	.046
CONCENTR * POLISH	5.997	3	1.999	15.672	.000
TIME * POLISH	.510	1	.510	4.002	.049
CONCENTR * TIME * POLISH	.163	3	5.441E-02	.427	.735
COMPOSIT * POLISH	4.634E-02	1	4.634E-02	.363	.549
CONCENTR * COMPOSIT * POLISH	1.897	3	.632	4.958	.004
TIME * COMPOSIT * POLISH	9.548E-02	1	9.548E-02	.749	.390
CONCENTR * TIME * COMPOSIT * POLISH	.675	3	.225	1.763	.162
Error	8.801	69	.128		
Total	103.910	101			
Corrected Total	29.200	100			

Table 5. Analysis of variance of TEGDMA released in the tested media

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	3.327(a)	31	.107	20.294	.000
Intercept	20.499	1	20.499	3876.261	.000
CONCENTR	.197	3	6.580E-02	12.442	.000
TIME	.584	1	.584	110.412	.000
COMPOSIT	.659	1	.659	124.704	.000
POLISH	1.906E-05	1	1.906E-05	.004	.952
CONCENTR * TIME	.283	3	9.429E-02	17.829	.000
CONCENTR * COMPOSIT	8.154E-02	3	2.718E-02	5.140	.003
TIME * COMPOSIT	5.147E-02	1	5.147E-02	9.732	.003
CONCENTR * TIME * COMPOSIT	6.027E-02	3	2.009E-02	3.799	.014
CONCENTR * POLISH	.201	3	6.711E-02	12.689	.000
TIME * POLISH	4.476E-02	1	4.476E-02	8.464	.005
CONCENTR * TIME * POLISH	.111	3	3.712E-02	7.020	.000
COMPOSIT * POLISH	4.150E-02	1	4.150E-02	7.848	.007
CONCENTR * COMPOSIT * POLISH	.144	3	4.799E-02	9.074	.000
TIME * COMPOSIT * POLISH	3.563E-03	1	3.563E-03	.674	.415
CONCENTR * TIME * COMPOSIT * POLISH	9.470E-02	3	3.157E-02	5.969	.001
Error	.365	69	5.288E-03		
Total	23.266	101			
Corrected Total	3.692	100			

who reported that hydrogen peroxide could affect the three dimensional polymer network of composites, resulting in more monomer release [28]. Effect of different concentrations of H₂O₂ showed that the greater concentration resulted in greater release of both Bis-GMA and TEGDMA.

This can be interpreted as a positive relation between the presence of H₂O₂ and possibility of monomer release.

This observation did not confirm the results obtained by Polydorou et al. who studied the effect of 38% hydrogen peroxide for 45 min and 15% carbamide peroxide for 56h, on release of monomers from two different resin composites, a nanohybrid and an ormocer.

They concluded that bleaching agents reduced the amount of the monomers released from the two composite materials [21].

It should be mentioned that in the study conducted by Polydorou et al, after exposure to bleaching substance, the composite samples were washed and transferred to ethanol as the holding medium; therefore, some of the leached monomers may have been washed away. This may justify the different results, since in our study the bleaching medium was analyzed for monomer elution after removal of the composites. Polymerization conditions and the holding medium may affect the amount and type of released monomers. Moharamzade et al. used distilled water, saline solution, artificial saliva, serum-free culture medium, and culture medium with 10% fetal calf serum to extract monomers from different composites and concluded that the type of extraction medium may have significant effect on monomer release [25].

Table 6. Analysis of variance of UDMA released in the tested media

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	98.616(a)	31	3.181	1.837	.019
Intercept	193.178	1	193.178	111.550	.000
CONCENTR	1.664	3	.555	.320	.811
TIME	18.235	1	18.235	10.530	.002
COMPOSIT	2.207E-03	1	2.207E-03	.001	.972
POLISH	9.178	1	9.178	5.300	.024
CONCENTR * TIME	23.429	3	7.810	4.510	.060
CONCENTR * COMPOSIT	5.900	3	1.967	1.136	.341
TIME * COMPOSIT	1.073	1	1.073	.619	.434
CONCENTR * TIME * COMPOSIT	1.303	3	.434	.251	.860
CONCENTR * POLISH	1.241	3	.414	.239	.869
TIME * POLISH	3.048	1	3.048	1.760	.189
CONCENTR * TIME * POLISH	13.672	3	4.557	2.632	.057
COMPOSIT * POLISH	.844	1	.844	.487	.488
CONCENTR * COMPOSIT * POLISH	.665	3	.222	.128	.943
TIME * COMPOSIT * POLISH	1.610E-02	1	1.610E-02	.009	.923
CONCENTR * TIME * COMPOSIT * POLISH	5.557	3	1.852	1.070	.368
Error	119.491	69	1.732		
Total	439.284	101			
Corrected Total	218.107	100			

The other explanation for these different results may be the difference in bleaching material, duration of immersion and type of the composites. Available information on the effect of bleaching medium on monomer elution is rare. Synergistic toxic effect of TEGDMA and H₂O₂ has been reported though the exact mechanism of this effect is not clear [29].

One possible explanation may be the softening effect of bleaching agent on composites which has been reported by Hanning et al, [30] and Basting et al [31]. However, other studies have not proven this [16, 19]. It seems that type and concentration of the bleaching material, time of immersion and the type of composite play key roles in this regard [19].

Table 7. Mean and SD of Bis GMA released in the tested media

Composite	Surface	Medium	Time	Mean	SD
Z ₂₅₀	unpolished	Control	3.00	.75676	.655376
Supreme				.75328	.652364
Z ₂₅₀	Polished			.76056	.658696
Supreme				.75961	.657874
Z ₂₅₀	unpolished	Control	8.00	.77068	.667590
Supreme				.76372	.661409
Z ₂₅₀	Polished			.75867	.657030
Supreme				.76182	.659760
Z ₂₅₀	unpolished	H ₂ O ₂ (10 %)	3.00	.00000	.000000
Supreme				.00000	.000000
Z ₂₅₀	Polished			1.13594	.002182
Supreme				1.15047	.015590
Z ₂₅₀	unpolished	H ₂ O ₂ (10 %)	8.00	.00000	.000000
Supreme				.75961	.657975
Z ₂₅₀	Polished			1.13509	.001264
Supreme				1.18082	.045475
Z ₂₅₀	unpolished	H ₂ O ₂ (20 %)	3.00	.00000	.000000
Supreme				1.16634	.001575
Z ₂₅₀	Polished			1.14637	.010403
Supreme				1.16755	.014087
Z ₂₅₀	unpolished	H ₂ O ₂ (20 %)	8.00	.77226	.668812
Supreme				1.15649	.019880
Z ₂₅₀	Polished			1.17798	.006295
Supreme				1.20520	.005353
Z ₂₅₀	unpolished	H ₂ O ₂ (30 %)	3.00	1.44932	.101309
Supreme				1.23070	.116244
Z ₂₅₀	Polished			1.15902	.032184
Supreme				1.21845	.013443
Z ₂₅₀	unpolished	H ₂ O ₂ (30 %)	8.00	1.19251	.015668
Supreme				1.22919	.055360
Z ₂₅₀	Polished			.00000	.000000
Supreme				1.24942	.003041

The higher concentration of H₂O₂ and the more immersion time in our study caused more monomer release. Since bleaching can both soften and roughen the composite surface, it is highly possible that it affects monomer release from composite restorations and thus affects the biocompatibility of the resins. The effect of polishing on monomer release was another issue studied in this research.

It is documented that polymerized composite under the Mylar sheet has a surface layer rich in low molecular weight monomers [32, 33]. During immersion in extracting medium, these smaller monomers are readily released. Removal of this layer by polishing may lead to less release of TEGDMA from polished samples. However, more research is needed in this regard.

Table 8. Mean and SD of TEGDMA released in the tested media

Composite	Surface	Medium	Time	Mean	SD
Z ₂₅₀	unpolished	Control	3.00	.47963	.026763
Supreme				.58361	.061515
Z ₂₅₀	Polished			.44729	.010293
Supreme				.48774	.010883
Z ₂₅₀	unpolished		8.00	.50918	.020948
Supreme				.71371	.141846
Z ₂₅₀	Polished			.44909	.007751
Supreme				.50351	.019043
Z ₂₅₀	unpolished	H ₂ O ₂ (10 %)	3.00	.00000	.000000
Supreme				.46103	.033444
Z ₂₅₀	Polished			.41297	.011218
Supreme				.48126	.012570
Z ₂₅₀	unpolished		8.00	.42477	.003730
Supreme				.47171	.014885
Z ₂₅₀	Polished			.44990	.010186
Supreme				.49820	.006123
Z ₂₅₀	unpolished	H ₂ O ₂ (20 %)	3.00	.43402	.028153
Supreme				.45854	.009892
Z ₂₅₀	Polished			.13372	.231610
Supreme				.47225	.018743
Z ₂₅₀	unpolished		8.00	.46054	.009333
Supreme				.54973	.015353
Z ₂₅₀	Polished			.42738	.002704
Supreme				.55649	.027321
Z ₂₅₀	unpolished	H ₂ O ₂ (30 %)	3.00	.00000	.000000
Supreme				.46636	.027863
Z ₂₅₀	Polished			.30123	.262764
Supreme				.48270	.021187
Z ₂₅₀	unpolished		8.00	.54252	.019238
Supreme				.79309	.125934
Z ₂₅₀	Polished			.55234	.022887
Supreme				.67821	.106796

On the other hand, the results showed that nanofilled composite leached more TEGDMA than microhybrid composite. Da Silva et al. have reported that nanofilled composites may present higher degradation in the oral environment than hybrid ones [34]. This happens as the result of water sorption which leads to monomer elution [34, 35]. According to the manufacturer, composites tested in this study have the same polymeric matrix; thus, the size and arrangement of filler particles can be responsible for the significant differences observed in TEGDMA and Bis-GMA release. The sorption phenomenon in resin composites is mainly dependent on the hydrophilicity of their polymeric matrices [36]. In addition, the theoretically larger total surface area of nanofiller particles allows more water to accumulate at the filler particle-polymeric matrix interfaces, thus increasing the water sorption [9]. According to Santos et al, water accumulated at the aggregated zirconium/silica cluster filler-organic matrix interface in the nanofilled composite can create paths for water diffusion towards the inside of aggregates, where microvoids are probably present, due to lack of 5-20 nm-sized primary particles being impregnated in the polymeric matrix [37]. Part of the absorbed water in composites diffuses through the network and is trapped in polymer nano-voids; therefore the total void volume in the polymer network dictates the amount of absorbed water [34].

Initially, water sorption causes polymer softening as a result of reducing the frictional forces between the polymer chains [38]. After relaxation process, the unreacted monomers trapped in the polymer network are released at a rate controlled by the polymer's swelling and relaxation capacity [25]. It is clear that the more water absorbed, the more components leach out of resin composites [39, 40]. This phenomenon possibly explains the greater release of monomers from nanofilled composite in this study.

CONCLUSION

In the present study, H₂O₂ was applied according to common bleaching procedures. Taken together, TEGDMA was the main monomer released in this study and bleaching increased its release. Potential systemic adverse effects caused by this molecule and reduced mechanical and chemical properties of composite restorations after contact with bleaching materials must be addressed in treatment planning.

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