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Research Article

"RELEASE OF MONOMERS FROM DENTAL COMPOSITE MATERIALS" - AN IN VITRO STUDY

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ABSTRACT

A study was conducted to evaluate release of BisGMA and TEGDMA from flowable dental composite materials after different polymerization and storage times. Two flowable dental composite materials, Tetric N-flow (Ivoclar Vivadent, Liechtenstein) and Esthet X flow (Dentsply Caulk, International Inc,USA) were used in the study. Three groups (n=10) of each material were fabricated, one for each polymerization time of: 20 s, 30 s, and 40 s. After curing specimens were placed immediately in 2ml of absolute alcohol. The samples were stored at room temperature and the storage medium was renewed after 24 hours and was stored for 7 more days. Ethanol samples were measured using High Performance Liquid Chromatography Unit (SHIMADZU, Model SPD 20A, Shimadzu Corporation, Kyoto, Japan) to know the amount of monomers released. The results were statistically analyzed by using Fisher's test and Student-t test. TEGDMA and BisGMA were detected in the samples. Regardless of polymerization time, the material or storage time, a higher amount of BisGMA was released compared to TEGDMA. No significant difference was found between samples polymerized for 20 s, 30 s and 40 s concerning the elution of monomers. The elution of TEGDMA from Esthet X-Flow and Tetric N-Flow was higher within 24 hours than 7 days. But the elution of BisGMA from Esthet X-Flow was higher within 24 hours than 7 days and elution of BisGMA from Tetric N-Flow was higher after 7 days compared to 24 hours. The findings of present study revealed that the effect of polymerization times 20 s, 30 s and 40 s on elution of monomers were not significant for both Esthet X-Flow and Tetric N-Flow. The release of monomers did not reduced after 24 hours and 7 days after polymerization. The 40 s that are usually used for the polymerization of resin composites seems insufficient in order to prevent higher release of monomers.

Keywords: BisGMA, TEGDMA, Polymeriztion time, High Performance Liquid Chromatography.

INTRODUCTION

Resin composites have good esthetic and less toxicologic considerations and hence they are used as an alternative to amalgam, ceramic and gold restorations[1,2]. Dental composite is nothing but a complex mixture of materials which consists of an organic polymerizable resin matrix, reinforcing inorganic filler and a silane-coupling agent which connects the inorganic fillers and the organic matrix[3]. The polymerizable resin matrix contains one or more base monomers like BisGMA and/or UDMA, a diluent co-monomers like EGDMA, DEGDMA, TEGDMA. It also contains various additives, like photoinitiators (e.g. Camphorquinone), co-initiators (e.g. Dimethyl-aminobenzoic-acid-ester), inhibitor of polymerization and photostabilizers (e.g. benzophenone).

The term degree of conversion is used for the amount of monomer getting converted to polymer during polymerization[4]. According to various studies the degree of conversion is between 43-73%[5]. The first factor which influence the degree of conversion is related to the material and the second factor is related to the clinician handling.

The composition of monomers, concentration of activator and inhibitor present, viscosity of monomers, diffusion limitation of reactive media present and size, shade, opacity of filler particles are material related factors. The factors which can be controlled by the clinician are light intensity of the curing unit, duration of light irradiation, temperature produced during polymerization and thickness of restorative material used during restoration[6].

The two main mechanisms which may influence the release of monomers from polymeric materials are unbound monomers and/or additives that are eluted by solvents after setting and degradation or erosion over time[7].

Leaching of monomers were evaluated using various solvents such as distilled water, saliva, ethanol, methanol and acetonitrile[8,9,10,11]. In several studies ethanol has been used as a solvent to simulate and accelerate aging of composite restorations and ethanol has the solubility parameter which matches that of BisGMA. A maximum softening of resin is due to close match in solubility parameter[10,12]. Ethanol can also penetrate the resin matrix and expands the space between polymer chains, and soluble substance leading to release of unreacted monomers.

Eluted monomers from composite resins were cytotoxic to fibroblasts and macrophages[13]. A study has shown that the BisGMA-based polymer frequently used as matrix material for resin composites, is highly susceptible to chemical softening[8]. BisGMA has shown strong hemolytic potency, which is due to its chemical structure with a high hydrolytic nature. In vitro studies have also shown cytotoxic effect of TEGDMA in various cell cultures. TEGDMA can easily penetrate membranes and subsequently may react with intracellular molecules. In addition, TEGDMA has been identified as an important resinous sensitizer in patients and professionals[14].

Hence there is a need for the study to assess the release of monomers of composite materials

MATERIAL AND METHODS

A two commonly used flowable composite materials Esthet X-Flow (Dentsply Caulk, International Inc, USA) and Tetric N-Flow (Ivoclar Vivadent, Liechtenstein) were used for the study. Their composition and manufacturers are given in table 1.

Table 1: Materials used, their composition and manufacturers

Material used	Composition	Manufacturer
Esthet X Flow	Silica (Amorphous) Barium boron fluoro alumino silicate glass UDMA (< 5%), BisGMA (< 20%) and TEGDMA (< 40%).	Dentsply Caulk, International Caulk, USA.
Tetric N-Flow	Dimethacrylates (< 40 % BisGMA, TEGDMA and UDMA), inorganic fillers, ytterbium trifluoride, initiators, stabilizers and pigments	Ivoclar Vivadent AG, Bendererstrasse 2, FL -9494 Schaan Fürstentum Liechtenstein

Preparation of composite samples

Samples were polymerized using composite Quartz Tungsten Halogen curing unit (QHL 75 curing light, Dentsply Caulk, Milford, CT, USA). Three different polymerization times were used which were grouped into; Group 1: 20 seconds polymerization (20 s), Group 2: 30 seconds polymerization (30 s) and Group 3: 40 seconds polymerization (40 s)

From each of the two composite materials, three groups (n=10), one for every tested polymerization time. Samples were standardized using a teflon mould of dimension 2x2x2 mm. The mould was positioned on a transparent plastic matrix strip lying on a glass plate and was filled with composite material. After inserting the material into the mould a transparent plastic matrix strip was placed on top of them to avoid oxygen-inhibited superficial layer.

After curing specimens were immediately immersed in 2 ml of absolute alcohol (Ethyl alcohol [99.9% v/v min], Hayman Limited, Eastways Park, Witham, Essex, CM83YE, England.). The samples were stored at room temperature and the storage medium was renewed after 24 hours and again stored for 7 more days. After 7 days the composite blocks were removed from the storage medium (absolute alcohol) and samples were prepared for measurements.

Evaluation of samples

The samples were measured using High Performance Liquid Chromatography (SHIMADZU, Model SPD 20A, Shimadzu Corporation, Kyoto, Japan.). A reverse phase High Performance Liquid Chromatography unit was used to detect the release of monomers. The separation of monomers took place with a CC 125/4 Nucleodur 100-5 C18ec HPLC-Column. For the analysis of extracted residual monomers a reference standards of TEGDMA (CAS No. 494356, Sigma Aldrich Chemical Co., USA) and BisGMA (CAS No. 261548, Sigma Aldrich Chemical Co., USA) were purchased. 20 µl from the solution was injected into HPLC system and standard chromatograms were obtained for both the monomers individually.

Results were tabulated under each group and were statistically analyzed using Fisher's test and Student-t test.

RESULTS AND DISCUSSION

The mean values and the standard deviations of TEGDMA and BisGMA monomers released from Esthet X-Flow and Tetric N-Flow, for different polymerization times and different storage times were calculated. Testing the release of TEGDMA from Esthet X-Flow and Tetric N-Flow based on different polymerization time groups, the statistical results have shown no significant effect. Fisher's test showed no significant difference in release of TEGDMA between 20s, 30s and 40s polymerization times for Esthet X-Flow at $p = 0.085$ for 24 hours and $p = 0.697$ for 7 days and test for Tetric N-Flow showed no significant difference at $p = 0.794$ for 24 hours and $p = 0.836$ for 7 days. On comparing release of TEGDMA from Esthet X-Flow and Tetric N-Flow, 24 hours has shown higher release than 7 days. Student t- test showed statistical significant difference for Esthet X-Flow at $p < 0.001$ for 20 s, $p < 0.001$ for 30 s, and $p < 0.001$ for 40s and for Tetric N-Flow test showed statistical difference at $p < 0.001$ for 20 s, $p = 0.003$ for 30 s and $p < 0.001$ for 40 s. On comparing release of TEGDMA between composite materials, Esthet X-Flow has shown higher release than Tetric N-Flow. Student t-test showed highly significant difference at $p < 0.001$ for 20 s, $p < 0.001$ for 30 s, and $p < 0.001$ for 40 s for 24 hours storage and for 7 days storage test showed statistical difference at $p < 0.001$ for 20 s, p = 0,003 for 30 s and $p < 0.001$ for 40s.

There was no statistical significant effect in release of BisGMA for different polymerization time groups for both Esthet X-Flow and Tetric N-Flow. Fisher's test showed no significant difference between 20s, 30s and 40s polymerization times for Esthet X-Flow at $p = 0.970$ for 24 hours and $p = 0.864$ for 7 days and for Tetric N-Flow, test showed no statistical difference at $p = 0.940$ for 24 hours and $p = 0.795$ for 7 days. On comparing release of BisGMA from Esthet X-Flow and Tetric N-Flow results have shown no significant difference for storage periods. Student-t test showed no significant difference in release of BisGMA for Esthet X-Flow at $p = 0.23$ for 20 s, $p = 0.054$ for 30 s, and $p = 0.346$ for 40 s and for Tetric N-Flow test showed no statistical difference at $p = 0.244$ for 20 s, $p = 0.199$ for 30 s, and p = 00.139 for 40 s. On comparing release of BisGMA between composite materials, Tetric N-Flow has shown higher release than Esthet X-Flow. Student-t test showed highly significant difference at $p = 0.005$ for 20 s, $p = 0.004$ for 30 s, and $p < 0.001$ for 40 s for 24 hours storage and for 7 days storage test showed statistical difference at $p < 0.001$ for 20 s, $p = 0.003$ for 30 s and $p < 0.001$ for 40s.

As new generations of resin-based dental restorative materials have been developed, they have a wide variety of applications in dentistry. They are used in restorative procedures, prosthodontics, orthodontics and preventive dentistry, making them one of the most important groups of materials in dental practice.

Many brands of composite are relatively highly viscous which makes them difficult to handle and place in some clinical situations. So, manufacturers developed lower-viscosity composite resins which are marketed "Flowable" composite resins that can quickly and easily be placed. Their percentage filler content by weight (50% to 70%) is less than that of traditional hybrid composite resins (70% to 80%).

The release of unpolymerized monomers from polymerized composite resin has been considered as a source of a wide variety of adverse biological reactions, including local and systemic toxicity, pulp reactions, allergic and estrogenic effects. In many of the studies the cytotoxicity ranking of the basic monomers has been shown like: BisGMA > UDMA > TEGDMA >>> HEMA[7,15,16,17,18].

By considering above mentioned factors we can say that clinical success of composite materials, however, depends not only on the physical and chemical properties of the materials, but also on their biological safety.

So, the present study aim was to evaluate the amount of release of BisGMA and TEGDMA monomers from flowable dental composite materials, for different polymerization times and different storage periods.

In present study Esthet X-Flow and Tetric N-Flow dental composites were selected and TEGDMA and BisGMA were selected as target compounds, as they were the main constituents.

In present study 100% ethanol was used as a solvent for immersing the samples. To simulate clinical scenario Ferracane and Condon advocate a rapid immersion of samples in the ethanol solvent. Ethanol has shown maximum ability to extract unreacted monomers. Ethanol solution has the solubility parameter that matches with BisGMA.[10, 12] Ethanol has the ability to penetrate and swell the polymer chains and which in turn can facilitate the release of residual monomers from the set composites[9,19,20].

In present study TEGDMA and BisGMA were identified by their retention times in HPLC. This is a standard method used for the determining release of monomers from Dental composites[21,22].

Standardization of conditions was one of the aims of the present study. So, different polymerization times like 20 s, 30 s and 40 s were applied in order to simulate the different polymerization conditions that might take place in dental practice. In present study there was no significant difference in release of TEGDMA and BisGMA from Esthet X-Flow and Tetric N-Flow (Table 2 and Figure 1 and Table 3 and Figure 2) based on different polymerization times.

Table 2: Mean values in Area % and standard deviations of TEGDMA and BisGMA monomers released from Esthet X-Flow based on different polymerization time and storage periods.

	Esthet X-Flow: Mean values Area % (S.D)					
	20 s		**30 s**		**40 s**	
	TEGDMA	**BisGMA**	**TEGDMA**	**BisGMA**	**TEGDMA**	**BisGMA**
24 hours	20.567 (3.162)	22.673 (2.858)	17.920 (1.678)	22.418 (2.039)	17.896 (2.949)	22.208 (5.672)
7 days	11.038 (1.486)	20.712 (3.720)	10.557 (2.104)	20.244 (2.494)	10.370 (1.728)	19.819 (4.770)

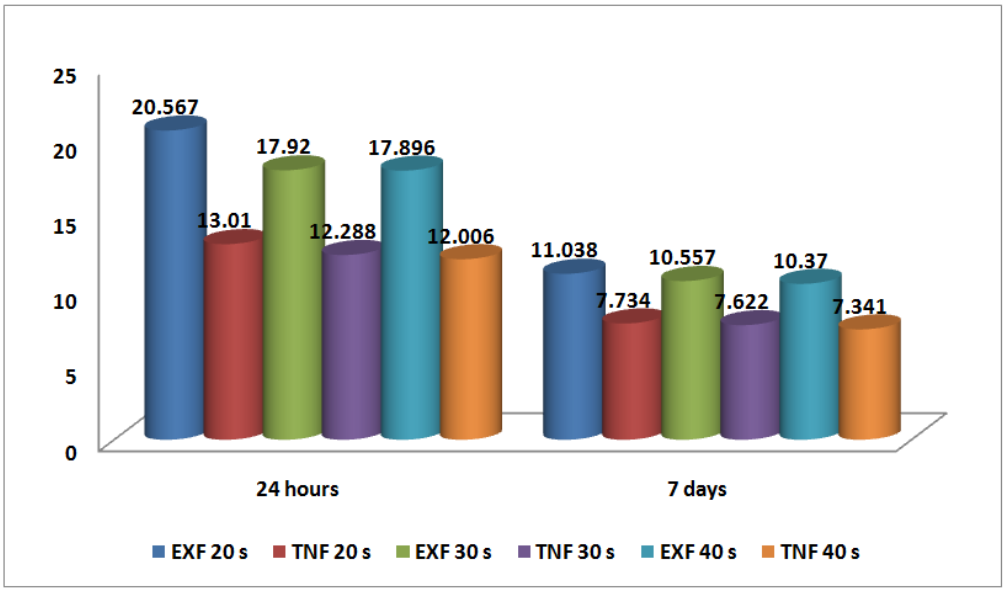

Fig. 1: The abbreviations EXF and TNF are for Esthet X-Flow and Tetric N-Flow, respectively. The amount of TEGDMA released from Esthet X-Flow was higher compared to Tetric N-Flow based on different polymerization time and storage periods.

Table 3: Mean values in Area % and standard deviations of TEGDMA and BisGMA monomers released from Tetric N-Flow based on different polymerization time and storage periods.

	Tetric N-Flow: Mean values Area % (S.D)					
	20 s		**30 s**		**40 s**	
	TEGDMA	**BisGMA**	**TEGDMA**	**BisGMA**	**TEGDMA**	**BisGMA**
24 hours	13.010(4.063)	35.882(11.171)	12.288(3.778)	34.971(11.223)	12.006(1.930)	34.418(3.950)
7 days	7.734(1.320)	41.044(6.597)	7.622(1.873)	40.667(7.418)	7.341(1.012)	38.885(8.230)

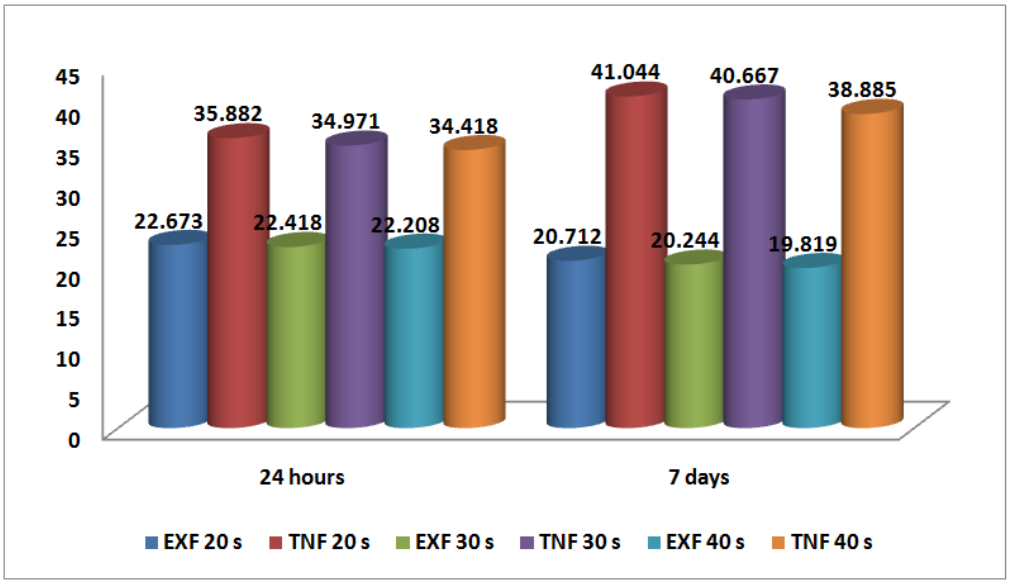

Fig. 2: The abbreviations EXF and TNF are for Esthet X-Flow and Tetric N-Flow, respectively. The amount of BisGMA released from Tetric N-Flow-Flow was higher compared to Esthet X-Flow based on different polymerization time and storage periods.

But mean difference of BisGMA was more than TEGDMA release in both Esthet X-Flow and Tetric N-Flow suggesting more release of BisGMA. This finding is an agreement with the findings of Olga Polydorou et al. and Komurcuoglu et al. who have shown more release of BisGMA than TEGDMA in their studies[23,24]. It might be due to difference in chemical properties and reactive potentials of BisGMA and TEGDMA, as double bond conversion of BisGMA is lower than TEGDMA[25]. Additionally, another reason can be the solution that was used for the storage of the samples.

According to Ferracane and Condon 85-100% of monomers are eluted within 24 hours[9]. Additionally more recent studies using HPLC have shown that monomer elution continued beyond 24 hours for resin based composites and resin mixtures[26.27]. Olga Polydorou et al. in their study have used 24 h, 7 days and 28 days storage times after polymerization to test monomer release. So in present study, the release of monomers after storage times of 24h and 7 days after polymerization were tested.

In present study TEGDMA was released more within 24 hours in both Esthet X-Flow and Tetric N-Flow compared to 7 days storage period. This is because according to Nathensen et al. who has shown that TEGDMA has maximum release within the first 4 minutes and the release reduced thereafter[28]. In addition Jurgen Durner et al. has shown in his study that in beginning, higher amounts of TEGDMA were released compared to BisGMA[29]. According to other studies, as TEGDMA is a hydrophilic and smaller monomer which was identified in higher amounts into aqueous extraction media as compared to BisGMA[30,31,32].

In present study Esthet X-Flow has shown more release of BisGMA within 24 hours than 7 days[24]. It might be due to difference in chemical properties and reactive potentials of BisGMA and TEGDMA, as double bond conversion of BisGMA is lower than TEGDMA[25]. Additionally, another reason can be the solution that was used for the storage of the samples.

But Tetric N-Flow has shown more release of BisGMA after 7 days storage. This finding is an agreement with the findings of Olga Polydorou et al. who has shown in their study that elution of BisGMA continued to increase compared to TEGDMA even after 7-28 days storage[23].Yap et al. has shown that any release that occurred after 24 hours curing has been mainly due to hydrolysis process[33].

CONCLUSION

The present study was done to evaluate the amount of release of BisGMA and TEGDMA monomers from flowable dental composite materials, for different polymerization times and different storage periods.

The following conclusions were drawn:

- The findings of present study revealed that the effect of polymerization times 20 s, 30 s and 40 s on elution of monomers were not significant for both Esthet X-Flow and Tetric N-Flow.
- For the elution of TEGDMA a highly significant difference was found between 20 s, 30 s, and 40 s for 24 hours and 7 days storage periods. But for elution of BisGMA no significant difference was found between 20 s, 30 s, and 40 s for 24 hours and 7 days storage periods.
- By considering the mean values there was more release of BisGMA from both Esthet X-Flow and Tetric N-Flow compared to TEGDMA.
- Although the manufacturers' recommends 40 s polymerization time with halogen unit is enough to achieve polymerization of a 2 mm thick composite restoration. But from the present study, 40 s that are usually used as polymerization time, which is thought to have satisfying results in the mechanical properties of composite resins, compared to 20 s and 30 s polymerization time does not seem to be more effective on the release of monomers.

So, further considerations and research are needed on suitable polymerization time of composite for a satisfying polymerization.

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