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## Valuation of Leaching of Monomers after Irradiation to Methacrylate based Nano – Composites- an Invitro Study.

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### ABSTRACT

A study was conducted to evaluate the amount of release of monomers from Methacrylate based Nano-Composites at different Electron beam radiation dosages and different storage times. Two dental Nano composite materials were used. After curing the samples for 20 seconds, they were divided into four groups (n=3) of each material for each irradiation dosage of 1kGy, 3kGy, 5kGy. After irradiating, they were immediately immersed in 2ml of absolute alcohol. The samples were stored at room temperature and the storage medium was renewed after 24 hours and again stored for 7 more days. Ethanol samples were measured using High Performance Liquid Chromatography Unit to know the amount of monomers released. The results were statistically analyzed using Kruskal Walli's test, Wilcoxon signed Rank test, Mannwhitney test. Regardless of the dose of Electron Beam Radiation, the material or storage time, a higher amount of BisGMA was released compared to TEGDMA. After 24 hours leaching of monomers was maximum in non-irradiated samples and minimum in samples irradiated at dose of 3kGy. Among irradiated samples, maximum leaching of monomers was seen in samples irradiated at 5kGy followed by 1kGy, except in case of BisGMA monomers from restorative nano-composites. After 1 week there was decrease in leaching of monomers, except in case of TEGDMA monomers from restorative nano-composites where an increase in leaching was seen.

**Keywords:** BisGMA, TEGDMA, Electron Beam Radiation, High Performance Liquid Chromatography.

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## INTRODUCTION

Composites are widely used in dentistry, owing to their esthetics which has led to an increase in their demand over the last few years. Nanocomposites are composites that have nanoparticle fillers of sizes  $\leq 100\text{nm}$ . [1] Nanocomposites have enhanced esthetics along with better wear resistance and fracture toughness. [2] They also can be easily polished and they adhere to the tooth structure. [3]

The resin matrices mainly consist of different methacrylate monomers like Bis-Phenol-A-Glycidyl-dimethacrylate (BisGMA) and Urethane Dimethacrylate (UDMA) along with other monomers of lesser viscosity, consisting of Triethyleneglycol Dimethacrylate (TEGDMA), Tetraethyleneglycol Dimethacrylate (TEGDMA), Neopen and others.

Although composites are stable materials there exists a concern regarding their effect on human health and their toxicity. Components (residual monomers, oligomers, and degradation products) may be released from the resin matrix of the composite materials in the oral environment. Elution is thought to be occurring by the diffusion of the resin matrix or by its degradation or erosion over a period of time. [4, 5] The unpolymerized monomers can be released from dental composites directly into the oral cavity, [6, 7] or into the pulp by means of dentinal microchannels. [8, 9]

Leaching of monomers raises concerns regarding its biocompatibility and causes cytotoxic reactions in the human gingival fibroblast and may even lead to cell death. [5, 10, 11] The leached monomers after dilution by the saliva can enter the intestine. [12] The monomers have shown to induce mutagenic/ carcinogenic effect in cells including those of oral cavity. [10, 13] In mammalian cells they also cause an increase in the reactive oxygen species (ROS) leading to apoptosis and oxidative DNA damages. [10, 14]

The leaching of the monomers is affected by the size of the molecules. Smaller molecules are believed to have higher mobility and thus may be eluted faster than the large molecules. [5] Studies have shown the degree of conversion of monomer-polymer conversion has been between 35-77%. [15- 20]

Incomplete polymerization of the composite resin adversely affects the biocompatibility and mechanical properties of the composites. [21-27]

Electron beam irradiation is a modern method to improve the properties of polymers. It has shown significant improvement in the mechanical the properties of composites. [43] Electron beam irradiation or e-beam irradiation is basically a form of ionizing energy. It uses a beam, a concentrated highly charged stream of electron, produced by acceleration and conversion of electricity. The beams may be either pulsed or continuous. [42]

In polymers, an electron beam leads to chain scission and cross linking. Chain scission leads to the shortening of the polymer chain, the C-C bond splits and the polymer structure may be broken. [27] Chain linkage can be initiated at several distinct points. When an electron beam hits a polymer, it will interact with its atoms and lead to formation of

backscattered electrons, secondary electrons and x-rays, visible light. Hence polymers may be arranged in a new arrangement and become cross linked. [28]

Hence we conducted this study to evaluate the amount of monomers leached from nanocomposites over different storage intervals using High Performance Liquid Chromatography (HPLC) equipment before and after irradiation by the electron beam accelerator.

**MATERIALS AND METHODS**

Two Nano - Composite materials Filtek Z350 XT Restorative (3M ESPE, USA) and Filtek Z350 XT Flowable (3M ESPE, USA) were used for the study. Their composition and manufacturers are given in Table 1.

**Table 1: Composition and Manufacturers of Filtek Z350 XT Restorative and Filtek Z350 XT Flowable Nano - Composite Materials**

MATERIALS USED	COMPOSITION	MANUFACTURER
Filtek Z350XT Restorative	<p><b>Monomers:</b> BisGMA, UDMA, TEGDMA, BisEMA.</p> <p><b>Fillers:</b> aggregated zirconia (0.6-1.4µm) and SiO<sub>2</sub>(20nm) 78.5/59.5% m/v.</p>	3M ESPE, USA
Filtek Z350 XT Flowable	<p><b>Monomers:</b>BisGMA, TEGDMA, BisEMA, dimethacrylate polymer.</p> <p><b>Fillers:</b> zirconia (5-10nm) nanofiller and Silica(75nm) 65/55% m/v.</p>	3M ESPE, USA

**Preparation of composite samples**

Samples were polymerized using composite Quartz Tungsten Halogen curing unit (QHL 75 curing light, Dentsply Caulk, Milford, CT, USA) for 20 seconds. 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.

**Standardization of Dose**

The doses of Electron Beam Irradiation to be used were standardized using an 8 MeV Microtron at Microtron Centre, Mangalore University, Mangalore, India. Since there is no literature on radiation dose for nano-composites, standardization of dose was a prerequisite for the present study. Samples were irradiated starting at 200Gy 400Gy and 600Gy initially which all gave totally negative results in handling properties. At 1kGy, 3kGy, 5kGy handling properties were similar to the normal nano-Composites which are used in the present study. Hence, 1kGy, 3kGy and 5kGy were used as standard doses for irradiation in the present study.

## Groups

After curing, the specimens were irradiated with Electron Beam Irradiation and divided into two groups-irradiated (n=18) and non-irradiated groups (n=6). The irradiated groups were further divided into three subgroups based on the radiation dose -1kGy, 3kGy and 5kGy (n=6 in each).

After irradiation samples 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. The mobile phase was acetonitrile/water (75/25% v/v) at a flow rate of 1 mL/min and detection was performed at a wavelength of 254 nm for 30 minutes. 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 and stock solutions were prepared by appropriate quantitative dilution. 20  $\mu$ l 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 KruskalWalli's test, Wilcoxon signed Rank test, Mannwhitney test.

## RESULTS AND DISCUSSION

In present study, effect of different doses of Electron Beam Radiation on leaching of BisGMA and TEGDMA monomers from Filtek Z350XT Restorative and Filtek Z350XT Flowable dental nano - composites were evaluated and compared with the non – irradiated samples of the same materials.

Electron beam irradiation (a high-energy dose irradiation) is a modern method to improve the properties of dental composites. It has shown to increase the links between polymer chains. [27]

Two types of irradiation-initiated reaction can be defined as chain linkage and chain breakage. [29, 30]

During a chemical reaction, radicals, which bring about chain linkage, are initiated from several points. It has shown that irradiation initiates the radical build-up of all

components of a polymer. The entire polymer may simultaneously be newly arranged and cross-linked when irradiated. [28]

In contrast, chain breakage can also occur. This phenomenon happens when high energy dose is used. During this phenomenon, the C–C bonds split off and the polymeric structure is broken down. [27]

In present study TEGDMA and BisGMA were identified by their retention times in HPLC. This is a standard method used for the determining the elution of monomers from dental composites.

Mean difference of BisGMA was more than TEGDMA release from both Filtek Z350XT Restorative and Filtek Z350XT Flowable nano-composite, suggesting more release of BisGMA before and after Electron Beam irradiation. 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. This could be because in present study 100% ethanol was used as a solvent for immersing the samples. To simulate clinical scenario Ferracane and Condon used ethanol as solvent to rapidly immerse the samples. Ethanol has shown maximum ability to extract unreacted monomers. Ethanol solution has the solubility parameter that is similar to BisGMA. [7, 31, 32, 35] Ethanol has the ability to penetrate and swell the polymer chains which can lead to release of residual monomers from the set composites. [17, 33, 34]

At the end of 24 hours, leaching of monomers was maximum from non-irradiated samples and minimum from samples irradiated at a dose of 3kGy, however the difference was not statistically significant. [Fig 1, 2] This could be because of the initiation of the chain linkage reaction at the dose of 3kGy. Among the irradiated samples maximum leaching of monomers was seen from the samples irradiated at 5kGy, followed by samples irradiated at 1kGy. [Fig 1, 2] We hypothesize that this could be because at 5kGy there may be chain breakage reaction occurring in the polymer of both nano-composites and the dose of 1kGy might be insufficient to initiate any of chemical reaction among the molecules of polymer. [36] Charlesby in 1953 stated that “the degree of cross-linking produced in these polymers is proportional to the radiation dose over a wide range of values, so that it is possible to prepare polymers of any required degree of crosslinking without the introduction of foreign atoms or heat treatment”. However, Schlitz et al. showed that for low dose radiation, it does not agree with the experimental results. On further research, it showed that cross-linking of polymers with radiation does not follow an easy dose–reaction relationship. [37, 38] But in case of restorative nano-composites leaching of BisGMA monomers decreased with the increase in the radiation dose, however it was not statistically significant. [Fig 2] This might be due to differences in chemical properties and reactive potentials of BisGMA and TEGDMA monomers to the electron beam radiation. [7] It is also dependent on the structure of the polymer, functional groups during the investigation and on the irradiation parameters like dose rate or acceleration of the electrons. [39]

Figure 1: Line graph showing the release of TEGDMA monomers from Restorative and Flowable Nano-composites after 24 hours

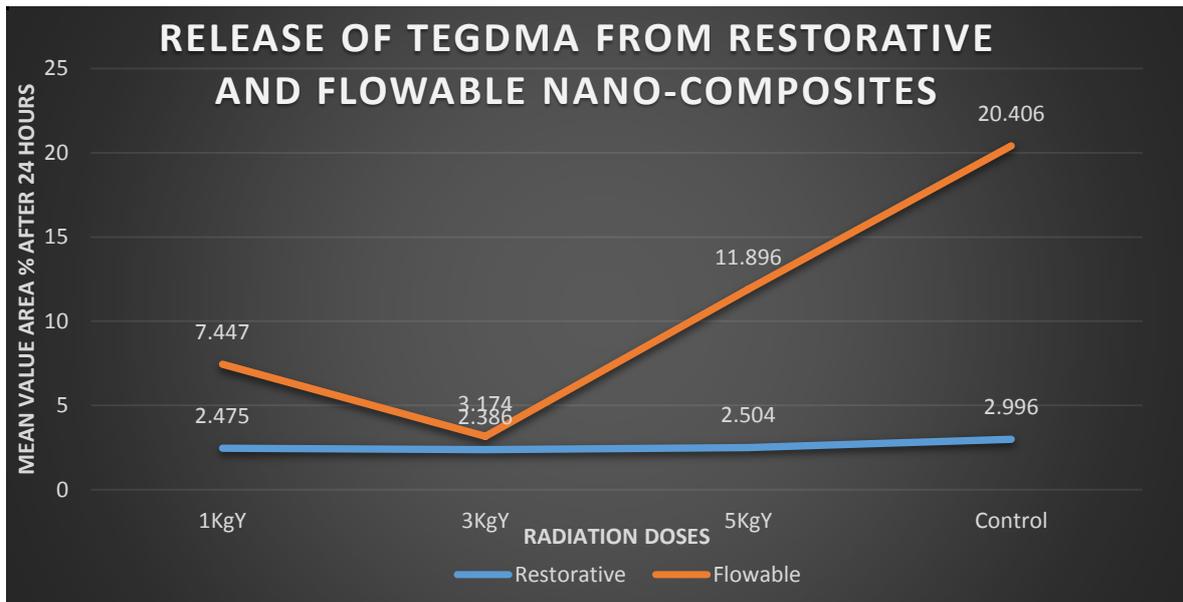
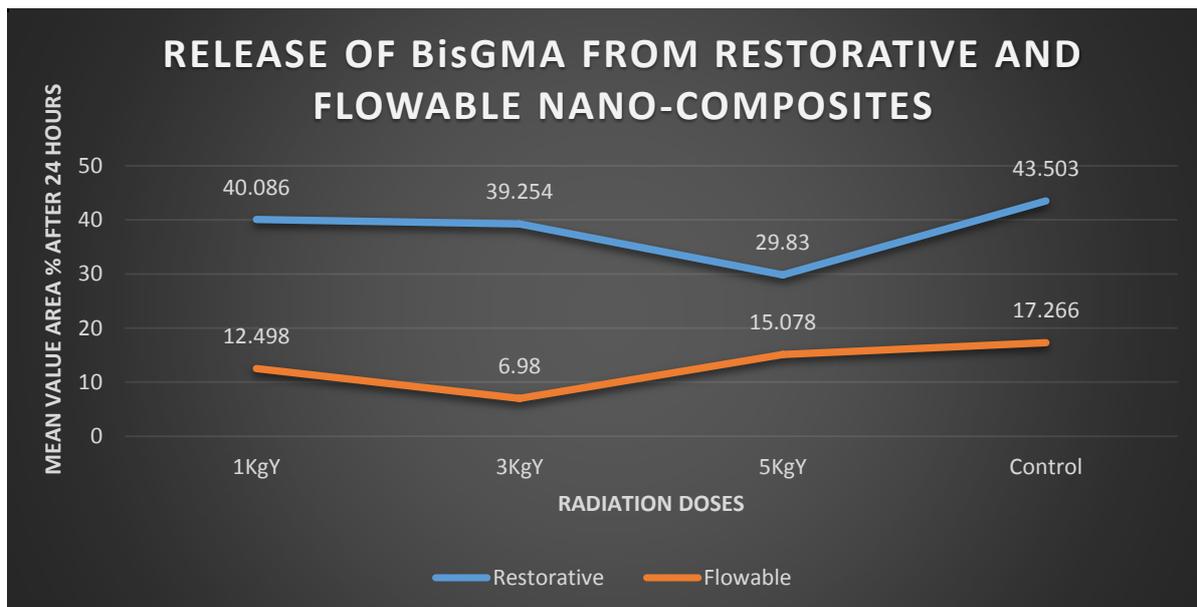


Figure 2: Line graph showing the release of BisGMA from the Restorative and Flowable Nano-composites after 24 hours.



After 1 week of storage time, there was decrease in leaching of monomers from both nano-composites as compared to the amount of monomers leached at the end of 24 hour. This is in accordance with the study by Nathansen et al and Komurcuoglu et al who has shown that maximum release of monomers is seen within 24 hours and then it is reduced, [35, 40] but in case of restorative nano-composites there was increase in leaching of TEGDMA Monomers, however it was statistically not significant. [Table 2] This could be because time required for the release of TEGDMA monomers from the inner layers of nano-composites is more, due to high degree of cross linking at the surface of nano-composites. [41]

**Table 2: Comparison of Leaching Of Monomers from Irradiated and Non-irradiated Samples after 24 Hours and 7 Days**

		AREA % - 24 HOURS	2.475	1.240			
			3KgY	2.386	0.912	NS	
			5KgY	2.504	1.272		
			Control	2.996	0.726		
		AREA %-7 DAYS	1KgY	3.444	2.711	.863	
			3KgY	2.773	2.253	NS	
			5KgY	4.707	3.103		
	Control		5.609	4.504			
	FLOWABLE	AREA % - 24 HOURS	1KgY	7.447	5.451	.292	
				3KgY	3.174	2.980	NS
				5KgY	11.896	11.853	
				Control	20.406	12.920	
		AREA %-7 DAYS	1KgY	4.197	4.551	.826	
			3KgY	2.069	2.572	NS	
			5KgY	4.932	5.074		
Control	6.44		6.068				
<b>BisGMA</b>	RESTORATIVE	AREA % - 24 HOURS	1KgY	40.086	7.268	.589	
			3KgY	39.254	9.255	NS	
			5KgY	29.830	14.008		
			Control	43.503	3.495		
		AREA %-7 DAYS	1KgY	19.734	12.958	.972	
			3KgY	18.080	17.044	NS	
			5KgY	17.990	11.227		
	Control		20.26	15.289			
	FLOWABLE	AREA % - 24 HOURS	1KgY	12.498	6.106	.204	
				3KgY	6.980	5.527	NS
				5KgY	15.078	7.466	
				Control	17.266	5.692	
		AREA %-7 DAYS	1KgY	4.533	5.031	.287	
			3KgY	2.983	4.345	NS	
			5KgY	11.933	6.713		
Control	12.902		14.234				

### CONCLUSION

The present study was done to evaluate the amount of release of BisGMA and TEGDMA monomers from two Methacrylate based nano-composite materials, for different radiation dosages and different storage periods.

The following conclusions were drawn:

- There was significant reduction in the leaching of monomers from nano-composites after being irradiated by electron beam radiation.
- Among the irradiated samples, there is minimum leaching of monomers from samples irradiated at 3kGy.

Only few studies could be found in dentistry supporting that electron beam irradiation is able to enhance the mechanical properties. Further research should be performed with different doses of electron beam irradiation on different dental composite

materials, in order to notice reduction in the leaching of monomers from composites which in turn can be used as indirect restorations in the oral cavity.

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### REFERENCES

- [1] Musa M, Ponnuraj KT, Mohamad D, Rahman I. Nanotechnology 2013;24.
- [2] <http://www.dentsply.de/bausteine.net/file/showfile.aspx?downaid=7166&sp=D&omid=1042&fd=0>
- [3] Manhart J, Kunzelman KH, Chen HY, Hickel R. Dent Mater 2000; 16:33-40.
- [4] Jandt KD, Sigush BW. Dent Mater 2009; 25:1011-1006.
- [5] Mousavinasab SM. Dental Res J (Special Issue) 2011; 8(5):21-29.
- [6] Munksgaard EC, Peutzfeldt A, Asmussen E. European J Oral Sci 2000; 108:341-345.
- [7] Polydorou O, Trittler R, Hellwig E, Kummerer K. Dent Mater. 2007; 23:1535-1541.
- [8] Boullaguet S, Wataha JC, Hanks CT, Ciucchi B, Holz J. J Endodont 1996; 22:244-8.
- [9] Noda M, Wataha JC, Kaga M, Lockwood PE, Volkmann KR, Sano H. J Dental Res 2002; 81:265-9.
- [10] Schweikl H, Spagnuolo G, Schmalz G. J Dental Res 2006; 85:870-7.
- [11] Reichl FX, Simon S, Esters M, Seiss M, Kehe K, Kleinsasser N, et al. Arch Toxicol 2006; 80:465-72.
- [12] Reichl FX, Durner J, Hickel R, Kunzelmann KH, Jewett A, Wang MY, et al. J Dental Res 2001; 80:1412-5.
- [13] Kleinsasser NH, Wallner BC, Harreus UA, Kleinjung T, Folwaczny M, Hickel R, et al. J Dent 2004; 32:229-34.
- [14] Eckhart A, Gerstmayr N, Hiller K-A, Bolay C, Waha C, Spagnuolo G, et al. Biomater 2009; 30:2006-14.
- [15] Ferracane JL. Crit Rev Oral Biol Med 1995; 6:302-18.
- [16] Ferracane JL. J Oral Rehabil 1994; 21:441-52.
- [17] Ferracane JL, Condon JR. Dent Mater 1990; 6:282-7.
- [18] Antonucci JM, Toth EE. J Dental Res 1983; 62:121-5.
- [19] Asmussen E. Scand J Dent Res 1982; 90:490-6.
- [20] Chung K, Greener EH. J Oral Rehabil. 1988; 15:555-62.
- [21] Geurtsen W, Lehmann F, Spahl W, Leyhausen G. J Biomed Mater Res 1998; 41:474-480.
- [22] Schweikl H, Schmalz G. Mutat Res 1999; 438:71-78.

- [23] Munksgaard Ec, Freund M. Scand J Dent Res 1990; 98:261-267.
- [24] Wataha JC, Rueggeberg Fa, Lapp CA, Lewis JB, Lockwood PE, Ergle JW, Mettenburg DJ. Clin Oral Invest 1999; 3:144-149.
- [25] Hanks CT, Wataha JC, Sun ZL. Dent Mater 1996; 12:186-193.
- [26] Konradsson K, van Djiken JW. J Clin Periodontol 2005; 32:462-466.
- [27] Hegde M N, Puri A, Shetty S S, Hegde N D, Kumari S, Sanjeev G. Ann Res Rev Biol 2014; 4(1).
- [28] Hegde M N, S.Shabin, Hegde N D, Kumari S, Sanjeev G, Shetty S. Int J Biol Med Res 2013; 4(4): 3690-3694.
- [29] Greer RW, Wilkes GL. Polymer 1998; 39:4205–10.
- [30] Charlesby A, Ross M. Nature 1953; 171:167.
- [31] Wu W, McKinney JE. J Dent Res 1982; 61: 1180-3.
- [32] McKinney JE, Wu W. J Dent Res 1985; 64: 1326-31.
- [33] Pilliar RM, Vowles R, William DF. J Dent Res 1987; 66(3):722-726.
- [34] Thompson LR, Miller EG, Bowler WH. J Dent Res 1982; 61(8): 989-992.
- [35] Komurcuoglu E, Olmez S, Vural N. J Oral Rehab 2005; 32: 116-21.
- [36] Behr M, Rosentritt M, Faltermeier A, Handel G. J Mat Sci Mat Med 2005;16(2):175-81.
- [37] Haque S, Takinami S, Watari F, Khan MH, Nakamura M. Dent Mater J 2001;20:325–38.
- [38] Sotobayashi H, Asmussen F, Thimm K, Schnabel W, Betz H, Einfeld D. Polym Bull 1982;7:95–101.
- [39] Ratnam CT, Nasir M, Baharin A, Zaman K. Eur Polym J 2001;37:1667–76.
- [40] Dan Nathensan, Prinda Lertpitayakun, Mark S Lamkin Mahnaz Edalaptopour, Lee Choul. J Am Dent Assoc 1997; 128: 1517-23.
- [41] A. Nalçaci, N. Ulusoy and O. Atakol. Operat Dent 2006; 31(2):197-203.
- [42] [Wikipedia.org/wiki/electron\\_beam\\_processing](http://Wikipedia.org/wiki/electron_beam_processing).
- [43] S Shabin, Hegde M N, Hegde N D, Kumari S, Sanjeev G, Shetty S. J App Chem 2013; 2(6): 1589-1594.