Nat. Volatiles & Essent. Oils, 2021; 8(1): pp
DOI: 10.37929/nveo

NVEO
Natural Volatiles & Essential Oils

ORIGINAL RESEARCH ARTICLE

Effect of Storage Time and Extraction Media on the Elution of Residual Monomer from Resin based Restorative Materials using HPLC

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Submitted: 00.00.2020; Accepted: 00.00.2021

Abstract

Aim: The aim of this study was to evaluate the elution of monomer UDMA from different resin based restorative materials based on the effect of different extraction media and storage time.

Materials and methods: Three materials were tested and evaluated. I: Bulk fill posterior restorative material, Filtek™ Bulk Fill Posterior Restorative material, 3M ESPE™, St. Paul, USA II: Posterior bulk fill flowable resin material, Smart Dentin Replacement, SDR™, Dentsply, Konstanz, Germany III: Alkasite restorative material – self cure mode and dual cure mode, Cention N, Ivoclar Vivadent AG, Schaan, Liechtenstein. Samples were stored in 75% ethanol solution and artificial saliva. Residual monomers eluted in the solution were analyzed using HPLC after 24 hours and 7 days. The study data was analyzed using One Way ANOVA ($p<0.05$) and post-hoc Tukey tests.

Results: Group II showed the highest amount of monomer elution, Group III had the least elution of UDMA in both extraction media over different time periods.

Conclusion: The amount of monomer UDMA eluted from the alkasite restorative material was significantly lower than that of other bulk fill resin based materials in different storage media at different time intervals which may be attributed to the presence of single monomer.

Keywords: monomer elution, resin based restorative materials, extraction media, artificial saliva, high performance liquid chromatography

Introduction

An increase in the demand for aesthetic restorative material and ease of placement for the clinician has resulted in use of resin based restorative materials which are a complex mixture, generally consisting of a polymerizable organic resin matrix containing one or more base monomers such as bisphenol A glycol dimethacrylate (BisGMA) and/or urethane dimethacrylate (UDMA), diluent co-monomers such as ethylene glycol dimethacryalte (EGDMA), diethylene glycol dimethacrylate (TEGDMA), reinforcing inorganic filler, silane coupling agents and various additives such as photoinitiators, co-initiators, polymerization inhibitors and photostabilizers. [1]

Several studies have investigated the biocompatibility and clinical safety pertaining to the elution of unreacted monomers. It has been reported that monomers can leach out of the polymerized material. These unbound monomers are known to cause significant cytotoxic and genotoxic effects. It has been

DOI: 10.37929/nveo

proven to have estrogenic, teratogenic and carcinogenic effect. The extent to which the resin based restorative materials release and are a source of unreacted monomer should be thoroughly investigated.

Various dental material analysis studies have put forth that the release of unreacted monomers depend on the type of extraction media. Usually water or artificial saliva are mainly used to represent the oral environment in studies done to evaluate monomer elution. According to USA FDA 75% solution of ethanol is considered clinically relevant food/oral simulating liquid. [1-4]

This study quantified the elution of unreacted monomer urethane dimethacrylate monomer from three materials - bulk fill posterior restorative material, posterior bulk fill flowable resin material and a contemporary alkasite restorative which is a new category of filling material essentially a subgroup of the composite resin using high performance liquid chromatography (HPLC) based on different extraction media and storage periods.

Materials and Methods

For the purpose of this study three resin based restorative materials were tested. Table 1

The materials were tested by categorizing into the following groups and subgroups based on extraction fluid. Each subgroup was analyzed for elution of monomer after 24 hours and 7 days.

Group I

(a) Bulkfill posterior restorative material in 75% ethanol

(b) Bulkfill posterior restorative material in artifical saliva

Group II -

(a) Posterior bulk fill flowable resin material in 75% ethanol

(b) Posterior bulk fill flowable resin material in artifical saliva

Group III

(a) Alkasite restorative material - dual cure mode in 75% ethanol

(b) Alkasite restorative material - dual cure mode in artificial saliva

Group IV

(a) Alkasite restorative material - self-cure mode in 75% ethanol

(b) Alkasite restorative material - self-cure mode in artificial saliva

Five samples for each material were fabricated using a standard teflon mould of dimension 2 x 2 x 2 mm. The mould was positioned on a transparent plastic matrix strip placed on a glass plate. After inserting the material into the mould, a transparent plastic matrix strip was placed on the top to avoid oxidation of the superficial layer. Each sample was cured as per manufacturer's instructions. The distance between the light source and the sample was standardized by using a 1cm thick glass plate. After fabrication, each sample was immediately immersed in 1.5 ml of 75% ethanol and artificial saliva used as extraction fluid and stored in amber coloured bottles. These samples were stored at room temperature for a time period of 24 hours. The storage media were renewed after 24 hours and again stored for 7 days. The samples were taken from the storage media and 1ml of solution were measured at predefined time intervals 24 hours and 7 days. These samples were analyzed by High Performance Liquid Chromatography. Optimised chromatographic conditions were formulated for the analysis. For analysis, Shimadzu UFLC with pump LC-20AD was used.

DOI: 10.37929/nveo

Column used was Phenomex luna C18, 250 x 4.6mm (5µ Particle size) with a flow rate of 1ml/min and injection volume of 5µl. Detector used was PDA detector (SPD-M20A) with a wavelength of 210nm. Mobile phase used was Water:Acetonitrile (25:75).

The data obtained was statistically analysed by One-way ANOVA and Tukey's post hoc test at a significance level of $p < 0.05$ to detect significant differences in the amount of unreacted monomer from different storage media at different time intervals.

Results and Discussion

Elution of monomer was higher in 75% ethanol extraction fluid for all the groups than in artificial saliva. A decrease in elution was observed after 7 days. The highest elution of monomer was exhibited by Group IIa followed by Group Ia and the least in Group IIIb in both time durations. (Table 2)

The mean difference in the elution of monomer UDMA values between the study groups exhibited a statistical significance of p-value <0.001. (Table 3)

In the present study, the elution of UDMA from three resin based restorative materials was evaluated at two different time intervals from two extraction media. The results exhibited that after polymerization of the tested restorative materials there was an elution of residual monomer in the extraction fluid and the amount of eluted residual monomer decreased with time.

Molecules of high molecular weight base monomers such as UDMA decompose in the gas chromatograph and only decomposition products of these are detectable. For this reason, the quality and quantity of the residual monomers eluted from dental resin materials are usually determined using HPLC, as it is a very powerful and commonly used separation method. [5,6] HPLC is preferred to gas chromatography because it provides a greater level of control over the separation process, as monomers are soluble in the mobile phase. [7] HPLC analysis was therefore used in this study to evaluate the release of monomer from the tested bulk fill resin composite materials.

According to Ferracane et al, the elution of monomers relates to the extent of the polymerization reaction, the chemistry of the solvent used, the size and the chemical nature of the released components. [8] The oral cavity presents an environment somewhere between water and more aggressive solvents (ethanol, methanol, acetonitrile). A 75% ethanol–water solution is recommended by the United States Federal Drug Administration as a clinically relevant food–oral simulating liquid, and has been used in several studies. Therefore, this solution was used in this study. Artificial saliva is a substitute to natural saliva and simulates its effects in terms of pH, enzyme activities and other factors. [8] Therefore, artificial saliva was used as the other extraction medium in this study. In this study, 75% ethanol was used to extract the unreacted monomer UDMA from the polymerized composite samples in order to identify monomer quantity. The elution pattern of unreacted monomer is higher in ethanol than in artificial saliva storage medium, because of their hydrophobic character, which can significantly reduce and rationalize examination periods. The cured composite resins are composed of polymer networks that contain some unreacted monomers trapped inside. The solvent penetrates these polymer networks and expands the spaces between the polymer chains, and unreacted monomers may be eluted out.

According to Tsitrou et al, the amounts of eluted monomers decrease with time, depending on the solvent used. [9] In this study as well, highest monomer amounts were detected within 24 hours of storage, compared to 7 days of storage in all of the bulk fill composite resins tested. In this study, Group III alkasite restorative material in dual cure mode had the least elution of UDMA in both extraction media over different time periods. This may be due to the fact that the alkasite material has only UDMA as its principle

monomer while the other resin materials tested have different and numerous types of monomers including BisGMA, HEMA, TEGDMA in their composition.

There is an inverse correlation between degree of conversion and amount of eluted monomer. The greater the extent of the polymerization reactions, the fewer residual monomers are available to be eluted. More viscous the monomer, reactivity and rate of polymerization is less and there is lower degree of conversion of monomers to polymers. UDMA has high molecular weight, high concentration of double bonds and low viscosity. Although the viscosity of UDMA is much lower than that of BisGMA, when it is mixed with the high molecular weight BisEMA or EBPADMA, it can significantly restrict the mobility of UDMA monomers and decrease their reactivity and conversion value.

So, the presence of UDMA as principle monomer without addition of other high viscosity monomers could be another factor that shows the alkasite restorative material as the material with least elution of monomer in this study. Monomer elution co-relates to structural stability, wear rate and biocompatibility of material.

Group II showed the highest amount of monomer elution among all the materials tested in this study. This is in accordance with the study done by Edina Lempel et al, where flowable bulk fill composite material showed 20 times higher amount of eluted UDMA than other composite resin materials tested. [10]

In this study, it was also found that alkasite restorative material - dual cure had traces or almost no elution of UDMA compared to alkasite restorative material - self cure. This may be due to the activation of photoinitiators present in the material by light curing, resulting in better degree of polymerisation and lesser elution of monomers.

Conclusion

The amount of monomer UDMA eluted from the alkasite restorative material was significantly lower than that of other bulk fill resin based materials in different storage media at different time intervals.

Tables

Table 1: Materials tested

I	Bulk fill posterior restorative material	Filtek™ Bulk Fill Posterior Restorative material, 3M ESPE™, St. Paul, USA	Fillers which comprise of a combination of non-agglomerated/non-aggregated 20 nm silica filler, a non-agglomerated/non-aggregated 4 to 11 nm zirconia filler, an aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 11 nm zirconia particles) and an ytterbium trifluoride filler consisting of agglomerate 100 nm particles for increased radio-opacity. The inorganic filler loading is about 76.5% by weight (58.4% by volume). It contains aromatic dimethacrylate (AUDMA), urethane dimethacrylate (UDMA) and 1,12–dodecanediol dimethacrylate (DDDMA)
II	Posterior bulk fill flowable resin material	Smart Dentin Replacement, SDR™, Dentsply, Konstanz, Germany	Patented urethane dimethacrylate resin (molecular weight of 849 g/mol), di-methacrylate resin, di-functional diluents, barium and strontium alumina-fluoro-silicate glasses (68% by weight, 45% by volume), photo initiating system and colourants

DOI: 10.37929/nveo

III	Alkasite restorative material – self cure mode and dual cure mode	Cention N, Ivoclar Vivadent AG, Schaan, Liechtenstein	Alkaline filler which release acid neutralizing ions – fluoride, calcium and hydroxide ions. Organic monomer comprises of urethane dimethacrylate (UDMA), tricylodecan-dimethanol dimethacrylate (DCP), tetramethyl-xlylen-diurethane dimethacrylate (aromatic aliphatic-UDMA) and polyethylene glycol 400 dimethacrylate (PEG-400 DMA) which form part of the liquid. Fillers containing barium aluminium silicate glass, ytterbium trifluoride, Isofiller, calcium barium aluminium fluorosilicate glass, calcium fluoro silicate glass are found in the powder

Table 2: One way ANOVA comparison of elution of monomer between the study groups

Time duration	Study group	N	Mean	SD	Min	Max	ANOVA	
							F	p-value
24 hours	Ia	5	2.54	0.03	2.51	2.58	25298.14	<0.001*
	IIa	5	2.67	0.02	2.64	2.69		
	IIIa	5	0.11	0.01	0.10	0.12		
	IVa	5	0.60	0.02	0.58	0.62		
7 days	Ia	5	1.91	0.01	1.90	1.93	43599.20	<0.001*
	IIa	5	2.00	0.01	1.98	2.01		
	IIIa	5	0.06	0.01	0.05	0.06		
	IVa	5	0.25	0.01	0.24	0.27		
24 hours	Ib	5	1.24	0.02	1.22	1.25	11981.65	<0.001*
	IIb	5	1.46	0.02	1.44	1.49		
	IIIb	5	0.10	0.01	0.09	0.11		
	IVb	5	0.29	0.01	0.28	0.30		
7 days	Ib	5	0.76	0.01	0.75	0.78	11785.83	<0.001*
	IIb	5	0.99	0.01	0.98	1.00		
	IIIb	5	0.04	0.01	0.03	0.05		
	IVb	5	0.18	0.01	0.17	0.19		

*p<0.05 statistically significant p>0.05 Non Significant, NS

DOI: 10.37929/nveo

Table 3: Pairwise comparison of elution of monomer between the study groups based on Tukey post-hoc test

Time duration	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% CI	
						Lower Bound	Upper Bound
24 Hours	Ia	IIa	-0.13	0.01	<0.001*	-0.16	-0.09
		IIIa	2.43	0.01	<0.001*	2.40	2.47
		IVa	1.94	0.01	<0.001*	1.91	1.98
	IIa	IIIa	2.56	0.01	<0.001*	2.53	2.59
		IVa	2.07	0.01	<0.001*	2.04	2.11
	IIIa	IVa	-0.49	0.01	<0.001*	-0.52	-0.45
7 Days	Ia	IIa	-0.08	0.007	<0.001*	-0.10	-0.06
		IIIa	1.86	0.007	<0.001*	1.84	1.88
		IVa	1.66	0.007	<0.001*	1.64	1.68
	IIa	IIIa	1.94	0.007	<0.001*	1.92	1.96
		IVa	1.75	0.007	<0.001*	1.73	1.77
	IIIa	IVa	-0.20	0.007	<0.001*	-0.22	-0.18
24 Hours	Ib	IIb	-0.22	0.009	<0.001*	-0.24	-0.20
		IIIb	1.14	0.009	<0.001*	1.11	1.16
		IVb	0.94	0.009	<0.001*	0.92	0.97
	IIb	IIIb	1.36	0.009	<0.001*	1.33	1.38
		IVb	1.16	0.009	<0.001*	1.14	1.19
	IIIb	IVb	-0.20	0.009	<0.001*	-0.22	-0.17
7 Days	Ib	IIb	-0.23	0.006	<0.001*	-0.24	-0.21
		IIIb	0.72	0.006	<0.001*	0.70	0.74
		IVb	0.58	0.006	<0.001*	0.57	0.60
	IIb	IIIb	0.95	0.006	<0.001*	0.93	0.96
		IVb	0.81	0.006	<0.001*	0.79	0.82
	IIIb	IVb	-0.14	0.006	<0.001*	-0.15	-0.12

*p<0.05 statistically significant p>0.05 Non Significant, NS

REFERENCES

1. Effrosyni Tsitrou, Stavros Kelogrigoris, Elisabeth Koulaouzidou, Maria Antoniades-Halvatjoglou, Eugenia Koliniotou-Koumpia, Richard van Noort Toxicol Int. 2014 Jan-Apr; 21(1): 89–95.
2. Łagocka R. et al.: Analysis of base monomer elution from 3 flowable bulk-fill composite. Med Sci Monit, 2018; 24: 4679-4690
3. Cebe MA, Cebe F, Cengiz MF, Cetin AR, Arpag OF, Ozturk B. Elution of monomer from different bulk fill dental composite resins. Dental materials. 2015 Jul 1;31(7):e141-9.
4. Schulz SD, Laquai T, Kümmerer K, Bolek R, Mersch-Sundermann V, Polydorou O. Elution of monomers from provisional composite materials. International Journal of Polymer Science. 2015;2015.
5. Altintas SH, Usumez A. Evaluation of TEGDMA leachingfrom four resin cements by HPLC. Eur J Dent 2012;6:255–62.
6. Manojlovic D, Radisic M, Vasiljevic T, Zivkovic S, Lausevic M,Miletic V. Monomer elution from nanohybrid andormocer-based composites cured with different lightsources. Dent Mater 2011;27:371–8.

7. Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. HPLC analysis of components released from dental composites with different resin compositions using different extractionmedia. J Mater Sci: Mater Med 2007;18:133–7.
8. Ferracane JL. Elution of leachable components from composites. Journal of oral rehabilitation. 1994 Jul 1;21(4):441-52.
9. Tsitrou E, Kelogrigoris S, Koulaouzidou E, Antoniades-Halvatjoglou M, Koliniotou-Koumpia E, van Noort R. Effect of extraction media and storage time on the elution of monomers from four contemporary resin composite materials. Toxicology international. 2014 Jan;21(1):89.
10. Lempel E, Czibulya Z, Kovács B, Szalma J, Tóth Á, Kunsági-Máté S, Varga Z, Böddi K. Degree of conversion and BisGMA, TEGDMA, UDMA elution from flowable bulk fill composites. International journal of molecular sciences. 2016 May 20;17(5):732.