Effect of Volume and Renewal of the Storage Media on the Release of Monomer from Dental Composites

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Research Article

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Abstract

Background: released monomers to the oral environment from dental composite, cause systemic or local side effects on the tissues and cells and also affect mechanical properties of the resins.

Methods: This study evaluated the effect of the volume and renewing of storage media on monomer leachability from dental composite. Samples of two dental composites (BEAUTIFIL II Gingiva (BG) and Filtek bulkfill flowable (FBF)) were stored after polymerization in 1 and 3 milt storage media (ethanol/water 75%) for seven days. Refreshing of storage media was done in half of the samples of each group. The amount of releasing monomers (UDMA, BisGMA, TEGDMA) in storage media were measured by high performance liquid chromatography. (HPLC) Data was analyzed using Two-way ANOVA and T-test. (α=0.05).

Results: Elution of TEGDMA and UDMA from both composites was significantly higher in 3ml storage media. In groups with refreshing of storage media, BisGMA had higher amounts of release. Saturation makes the storage media volume important factor in monomer elution.

Conclusion: Refreshing of storage media had significant effect on monomer release before the elution of 50% of total released monomer.

Background

Considering the ever-increasing popularity of composites, there are concerns about the release of composite compounds and their toxicity. In other words, there is a probability that resin materials would release substances such as unpolymerized monomers, additives and filler component when placed in the oral cavity [1].

In clinical conditions, with a short-time curing time (usually less than 40 seconds) and the temperature of 37°C, composites will not be fully polymerized, due to the crosslinking reactions that can considerably reduce the movements of the monomers. The degradation process occurring in the oral cavity can also increase the release of certain substances from resin materials, as mechanical, enzymal and hydraulic processes can break the polymeric chains and release the products of this breakage as monomer or polymer molecules [1, 2].

Resin matrix usually consists of two oligomers: UDMA and BisGMA [3]. Bis-GMA was synthesized from bisphenol A (BPA) and glycidyl methacrylate (GMA) [4]. The release of monomers and additives can be harmful and might have adverse local or systemic effects. Other than the allergenic properties of monomers, some of the released substances can be cytotoxic, genotoxic, and carcinogenic, and might be toxic to the reproduction system [1]. BPA is one kind of endocrine disrupting compound that can cause several health problems [3]. Recognition and evaluation of such risks require knowledge about the exact quantity of the released substances [5].

The vast heterogeneity in the evaluation methods in different studies regarding the measurement of the released compounds from dental composites has had a considerable effect on the results of such studies. While a great number of analytic studies have been carried out, not using the standard means of measurement and varieties in presenting the results, prevent an accurate analysis of the quantity of the released monomers [1, 4]. In other words, the amount of monomers released in the oral cavity cannot be precisely determined, rendering it more difficult to evaluate the possible risks of resin composites. The amount of released monomers can differ due to approximately 100,000 factors in different studies.

Although such significant variation can be explained with the fact that different composite materials have been used in each study, many factors play an important role in this matter, such as the storage media (pure ethanol, a mixture of water ethanol, artificial saliva, etc.) incubation conditions (temperature and duration) and the methods used for analysis. [1, 3, 5]. The most frequently used protocol to measure monomer elution, has been described by ISO 10993-12 2010 [6] which suggests that the ratio of the mass of the test sample to the volume of the test storage media should be at least 1gr:10ml [7]. The word “at least” and also the statement of ISO which mentions interference of degradation product with the progress of degradation process should be considered as two major factors in validity of results. However, there is a weak yet significant correlation between the amount of the storage media and the quantity of the released monomers. In other words, the greater the storage media volume is, the higher the monomer concentrations are [1, 5].

This finding can be justified with the saturation of the storage media by the released monomers. Considering the storage media reaching a state of equilibrium and a consequent diminished monomer release, it can be interpreted that the recorded values in the past in-vitro studies were speculated with less than the actual values.

Nevertheless, in in-vivo conditions, reaching the saturation state does not seem possible, as saliva is continuously being refreshed. One way to rectify this problem in in-vitro conditions is to renew the solvent medium within equal intervals, rendering it impossible to reach saturation state [6, 7]. In this study, other than the effect of the volume of storage media on the release of monomers from composites, the effect of renewal of storage media within equal intervals was evaluated. These results are essential for improving experimental protocols regarding monomer release, which will be carried out in future analytic studies with the aim of evaluating the long-term effects of released components from composites in near future. The null hypothesis is that volume and renewing of storage media do not affect monomer leachability.

Methods

Preparation of the specimens

Two types of composites (BEAUTIFUL II gingiva (BG) & Filtek bulk-fill (FBF)) were selected (Table 1).

Table 1

the properties of the used composites

Composite Brand	Manufacturer	Resin Matrix	Filler Type	Filler Amount
Filtek Bulk Fill Flowable (1)	3M ESPE GmbH, Germany	Bis-GMA, BisEMA, Procry-lat, UDMA	Zirconia or Silica, Ytterbium Trifluoride	Loading Percentage by Weight: 64.5%
BEAUTIFUL II Gingiva (2)	Shofu Inc., Japan	Bis-GMA, TEGDMA	S-PRG filler based on Fluoroboroaluminosilicate glass, polymerization initiator, pigments and others	Loading Percentage by Weight: 81%

64 Disk-shaped composite samples (height: 2mm, width: 6mm) were prepared in a Teflon mold. Before polymerization, the disks were covered with a glass plate to prevent the formation of an oxygen-inhibited layer. The specimens were cured with the 3W light curing device High power Blue light LED (guilin woodpecker, china), with the light output of 500 mw/cm^2 for 40 seconds according to the manufacturer's instruction.

Storage media preparation

The storage media was prepared using pure ethanol (99.99%, Merck, Germany) and water, with the ethanol/water ratio of 75% in two volumes of 1mlit and 3mlit. The composite disks were placed in the storage media according to table 2 (8 groups and 8 composite discs in each group).

Table 2

Test groups

Group	Composite Type	Solvent Volume	Daily Renewal
1	Filtek Bulk Fill Flowable	1 ml	No
2	BEAUTIFUL II Gingiva	1 ml	No
3	Filtek Bulk Fill Flowable	1 ml	Yes
4	BEAUTIFUL II Gingiva	1 ml	Yes
5	Filtek Bulk Fill Flowable	3 ml	No
6	BEAUTIFUL II Gingiva	3 ml	No
7	Filtek Bulk Fill Flowable	3 ml	Yes
8	BEAUTIFUL II Gingiva	3 ml	Yes

Keeping samples in storage media in different methods

The storage media in groups 1,2,5 and 6 were incubated for 7 days in 37°C temperature. The storage media in groups 3,4,7 and 8 were incubated for 24 hours in 37°C temperature, and After each 24 hours, the whole storage media was taken up for analysis following which the samples were immersed in 1mL or 3 ml of fresh ethanol/water solution. To be more precise, each composite disc was taken from the solvent, rinsed and, dried by low power air and immersed in fresh solvent. The renewed storage media was again incubated in 37°C temperature. The renewal and incubation of the storage media were repeated every 24 hours for the next 6 days. Schematic illustration of the research method used in this study is available in Figure 1.

Measurement of Monomer Release

The amount of released monomer (UDMA, BisGMA, TEGDMA) was evaluated by the HPLC 600 E waters System Controller (Waters, MA, USA) method, through The Perfect target ODS-3 column (125 mm height, 4mm width, and silica particle size of 5 µm), with a UV detector at 230 nm wave length. The mobile phase was 70% acetonitrile and

30% distilled water, at 0.8 mm flow rate, and 20µlit injection volume at room temperature. Figure 2 shows the chemical structure of these monomers.

At first, different concentrations of each monomer (0.5–50 µg/lit) were injected into the system, and a standard curve was obtained which was used to analyze the produced curve of samples. It is noticeable that in refreshing samples, the sample of each day was injected in the system in order to gain the daily releasing pattern of monomers.

Statistical Analysis

The obtained data were analyzed using the SPSS statistical software 25th version. Continuous variables were presented as mean and standard deviation. Three-way ANOVA and T-test were used. The significant level was considered at 0.05.

Results

Three tracked monomers were detected in all groups of storage media. Volume of solvent did not affect BisGMA release while TEGDMA and UDMA from had significantly higher elution in 3ml extraction solvent. To be more precise, in samples without refreshing, UDMA from both composites and TEGDMA from FBF eluted in higher amount in 3ml solvents. To define the effect of composite type in monomer elution, it should be considered that TEGDMA eluted higher from BG composite while UDMA eluted more from FBF. Refreshing of extraction solvent caused more elution of BisGMA in all samples. Besides, UDMA from FBF in 3 ml eluted more in samples with refreshing in comparison with the samples without refreshing. This result was also true in TEGDMA elution in both solvent 's volume from FBF composite. However, for TEGDMA elution from BG in 3ml solvents the reverse is true. (Table 3)

Table 3
monomers elution in tested groups. (µg)

monomer		**TEGDMA**				**UDMA**				**BisGMA**			
volume		1 mL		3 mL		1 mL		3 mL		1 mL		3 mL	
refreshing		**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
FBF	mean	**0.19**	**0.01**	**0.31**	**0.13**	**66.99**	**47.28**	**76.12**	**75.39**	**0.25**	**0.04**	**0.18**	**0.06**
	sd	**0.16**	**0.01**	**0.07**	**0.25**	**8.36**	**6.92**	**4.79**	**25.09**	**0.45**	**0.00**	**0.00**	**0.01**
BG	mean	**0.42**	**0.36**	**0.19**	**0.67**	**7.83**	**3.36**	**6.09**	**9.64**	**0.09**	**0.07**	**0.19**	**0.09**
	sd	**0.11**	**0.06**	**0.05**	**0.11**	**1.34**	**0.65**	**1.11**	**12.02**	**0.01**	**0.02**	**0.01**	**0.01**

Daily elution patterns of monomers show that except UDMA monomer eluted from BG composite in 3ml solvent, all monomers in all volume had their maximum elution in day one. While TEGDMA and UDMA released more than half of their total elution in first day. Composite BG in 1 ml storage media released 83% of its total released TEGDMA in the first day. (Tables 4 and 5)

Table 4
Daily release of monomers from FBF. (µg)

Monomer		TEGDMA		UDMA		BisGMA	
volume		1ml	3ml	1ml	3ml	1ml	3ml
Day 1	mean	**0.19**	**0.31**	**44.89**	**47.57**	**0.03**	**0.04**
	Sd	**0.16**	**0.07**	**8.73**	**4.13**	**0.00**	**0.00**
Day 2	Mean	**0.00**	**0.00**	**7.60**	**9.59**	**0.01**	**0.03**
	Sd	**0.00**	**0.00**	**0.74**	**0.18**	**0.00**	**0.00**
Day 3	Mean	**0.00**	**0.00**	**5.73**	**6.31**	**0.01**	**0.02**
	Sd	**0.00**	**0.00**	**0.36**	**0.45**	**0.00**	**0.00**
Day 4	Mean	**0.00**	**0.00**	**1.40**	**2.46**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.03**	**0.31**	**0.00**	**0.00**
Day 5	Mean	**0.00**	**0.00**	**1.29**	**1.69**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.00**	**0.51**	**0.00**	**0.00**
Day 6	Mean	**0.00**	**0.00**	**3.80**	**3.39**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.40**	**0.19**	**0.00**	**0.00**
Day 7	Mean	**0.00**	**0.00**	**2.28**	**4.59**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.82**	**0.50**	**0.00**	**0.00**

Table 5
daily release of monomers from BG. (µg)

Monomer		TEGDMA		UDMA		BisGMA	
volume		1ml	3ml	1ml	3ml	1ml	3ml
Day 1	mean	**0.35**	**0.15**	**4.85**	**0.21**	**0.04**	**0.05**
	Sd	**0.11**	**0.04**	**1.28**	**0.56**	**0.01**	**0.01**
Day 2	Mean	**0.04**	**0.03**	**0.63**	**0.97**	**0.01**	**0.02**
	Sd	**0.01**	**0.01**	**0.13**	**0.07**	**0.00**	**0.00**
Day 3	Mean	**0.00**	**0.00**	**0.62**	**0.80**	**0.01**	**0.02**
	Sd	**0.00**	**0.00**	**0.15**	**0.07**	**0.00**	**0.00**
Day 4	Mean	**0.00**	**0.00**	**0.00**	**1.77**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.00**	**0.56**	**0.00**	**0.00**
Day 5	Mean	**0.00**	**0.00**	**0.66**	**0.69**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.13**	**0.28**	**0.00**	**0.00**
Day 6	Mean	**0.01**	**0.01**	**0.65**	**0.69**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.08**	**0.13**	**0.00**	**0.00**
Day 7	Mean	**0.01**	**0.01**	**0.42**	**0.96**	**0.01**	**0.02**
	sd	**0.00**	**0.00**	**0.10**	**0.13**	**0.00**	**0.01**

Discussion

In this study two different composite materials were used to measure the effect of storage media volume and refreshing of it on monomer elution.

Bulkfill composites like FBF are in high demand these days because of their increased depth of cure and reduced clinical time of operation [8]. BG can provide esthetics in conservative restorations with gingival recession [9]. Despite the manufacturer's information, elution of TEGDMA from FBF- as seen in Pongprueksa P et al's [10] study, and also UDMA from BG was observed. According to Cokic SM et al [6] manufacturers do not release the exact component of composites because of trade reasons.

Ethanol was used as storage media to intensify monomer elution. In aqueous media such as artificial saliva, monomers are released in smaller amounts [11, 12]. Also, FDA declared that ethanol can simulate exposure to nutrition materials such as light drinks and chocolate [13, 14]. According to VanLanduyt et al [1] UDMA wasn't detectable during first day of elution.

According to Cokic SM et al [6] storage media volume had major effect on monomer elution. The larger the volume of the storage media, the higher the amounts of monomer elution that happens. Also, incubation time between 7 and 30 days had no significant effect in monomer release. These two observations lead to considering the limitation of monomer solubility in storage media, as a major factor in monomer elution. Polydorou O et al [14]

measured monomer elution in 1, 7 and 28 days and one year and storage media were refreshed in 28 days. Monomer elution was equal in 28 days and one year which indicates saturation of storage media. In fact, saturation of storage media prevents further elution of monomers. Higher amount of storage media reaches saturation point later, so monomer elution is higher. According to Van Landuyt KL et al's [1] meta-analysis, release of monomers is a chemical equilibrium reaction and in smaller amounts of storage media equilibrium was achieved faster and prevented more monomer release. Constant refreshing of saliva and pulpal fluid in in vivo situation prevent saturation status. High volume of storage media and refreshing of it could be used to overcome this condition.

In this study 2 volumes of storage media were used to evaluate effect of storage media volume on monomer elution. In order to evaluate the effect of storage media refreshing on monomer elution, in half of the samples, storage media was refreshed daily. Thus, daily pattern of monomer elution was obtained. Alshali et al [15] measured monomer elution after one day, one month and three months. In their study, 70% of total three month-elution of TEGDMA and 50% of the total BisGMA elution occurred on the first day. Nalcaci et al [16] declared that TEGDMA eluted faster than BisGMA in methanol as in one sample group, 92% of TEGDMA eluted in first 6 hours while at the same time BisGMA reached 57% elution. Sideridou ID et al [17] measured monomer elution in 3,6 and 24 hours and also 3,6 and 30 days. Similar to the current study, the highest amount of release of TEGDMA and UDMA was observed in first day, unlike BisGMA.

The first mechanism of monomer elution, is elution from the composite surface that occurs in the first 24 hours. Subsequently monomer elution continues with a slower rate, since increasing the volume of polymeric chains and release of unreacted monomers from composite, takes substantial time [11].

In the current study TEGDMA and UDMA from FBF and UDMA from BG eluted more in 3 ml storage media (when not refreshing the solvent) because of the faster saturation of 1ml storage media. In cases of refreshing storage media, two different volumes had no significant difference in reaching saturation status and thus monomer elution.

According to Cokic SM et al there are two barriers for monomer release; decreasing the concentration gradient between the sample and the storage media [6] and saturation of storage media with monomers. Thus, rather than the storage media volume and concentration of monomer in the storage media, the concentration of unreacted monomers in the composite plays an important role in reaching equilibrium. Elution of TEGDMA from BG (in cases of not refreshing the solvent) had no significant difference between two volumes of storage media. It can be observed that high concentrations of unreacted TEGDMA in BG led to the continued elution of TEGDMA in 1ml storage media. Daily pattern of TEGDMA elution also confirms this result. Since elution of this monomer from BG, despite of its high rate in elution, continued till day 7. While FBF released TEGDMA only on the first day. Elution of BisGMA from FBF and BG was in small amounts, which justifies not reaching saturation in 1ml volume and not having a significant difference in elution of this monomer in two storage media volumes.

Refreshing of storage media had significant effect on BisGMA elution. Considering that BisGMA couldn't reach saturation during 7 days because of its small amounts, refreshing of storage media should cause another phenomenon rather than preventing saturation. Refreshing storage media will reduce concentration of monomers in storage media and increase the concentration gradient. BisGMA has a heavy aromatic core, a higher molecular weight [17] and a lower elution rate than TEGDMA and UDMA. BisGMA is the only monomer which eluted lower than 50% of its total elution in day one. Storage media refreshing and increasing the concentration gradient, affect more than 50% of BisGMA 's total elution while for TEGDMA and UDMA this effect is not significant.

TEGDMA elution from FBF is significantly higher in case of refreshing storage media despite its daily elution pattern and rate of elution. Gonzalez-Bonet A et al [18] described that TEGDMA has two hydrolysable ester groups and hydrolyzing these groups creates methacrylate acid (MA), 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl methacrylate (TEGMA) and TEG. Finer Y et al [19] detected TEGDMA products such as TEGMA, triethylene glycol and methacrylicacid. They suggested that total TEGDMA elution is equal to amount of the main TEGDMA plus its products. It can be obtained that FBF releasing products such as additives and filler components could have hydrolyzed TEGDMA to its products while refreshing the storage media released TEGDMA and prevented hydrolyzing of this monomer.

3ml storage media of BG composites at the first day, eluted monomers in small amounts; Which caused more monomer elution in 1ml storage media cases with refreshing and also more elution of TEGDMA in 3ml storage media in cases without refreshing.

Conclusion

It can be suggested that in studies related to monomer elution, especially in long term evaluations, refreshing the storage media should be conducted before first 24 hours and after that due to slower rate of elution it can be done in longer periods. Higher amount of storage media volume is preferable while balance between preventing saturation and limit of detection should be considered. Monomers hydrolyzing to their products and measuring their products should be noticed.

Abbreviations

BG: Beautifil II Gingiva

FBF: Filtek Bulkfill Flowable

BisGMA: Bisphenol A Diglycidyl Ether DiMethacrylate

UDMA: Urethan DiMethacrylate

TEGDMA: Tri Ethylene Glycol DiMethacrylate

HPLC: High Performance Liquid Chromatography

BPA: Bisphenol A

GMA: Glycidyl Methacrylate

LED: Light Emitting Diode

ANOVA: Analysis of Variance

Declarations

Ethics Approval and Consent to Participate

This study was conducted under the approval of the ethical committee of Tehran University of Medical Sciences.

It is an in Vitro Study, so Consort for participant is not applicable.

Human and Animal Rights

Not Applicable

Consent for Publication

All authors declare that they agree for publishing this paper in this journal.

Availability of Data and Materials

The data supporting the findings of the article is available in request.

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Competing Interest

The authors declare no conflict of interest, financial or otherwise.

Authors' contributions

SSh Study design and concept, MS and SS Experiment performing, SV Study design and concept, Writing the manuscript, HH Manuscript English editing, AS Reviewing the final manuscript. all authors have read and approved the manuscript

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Figures

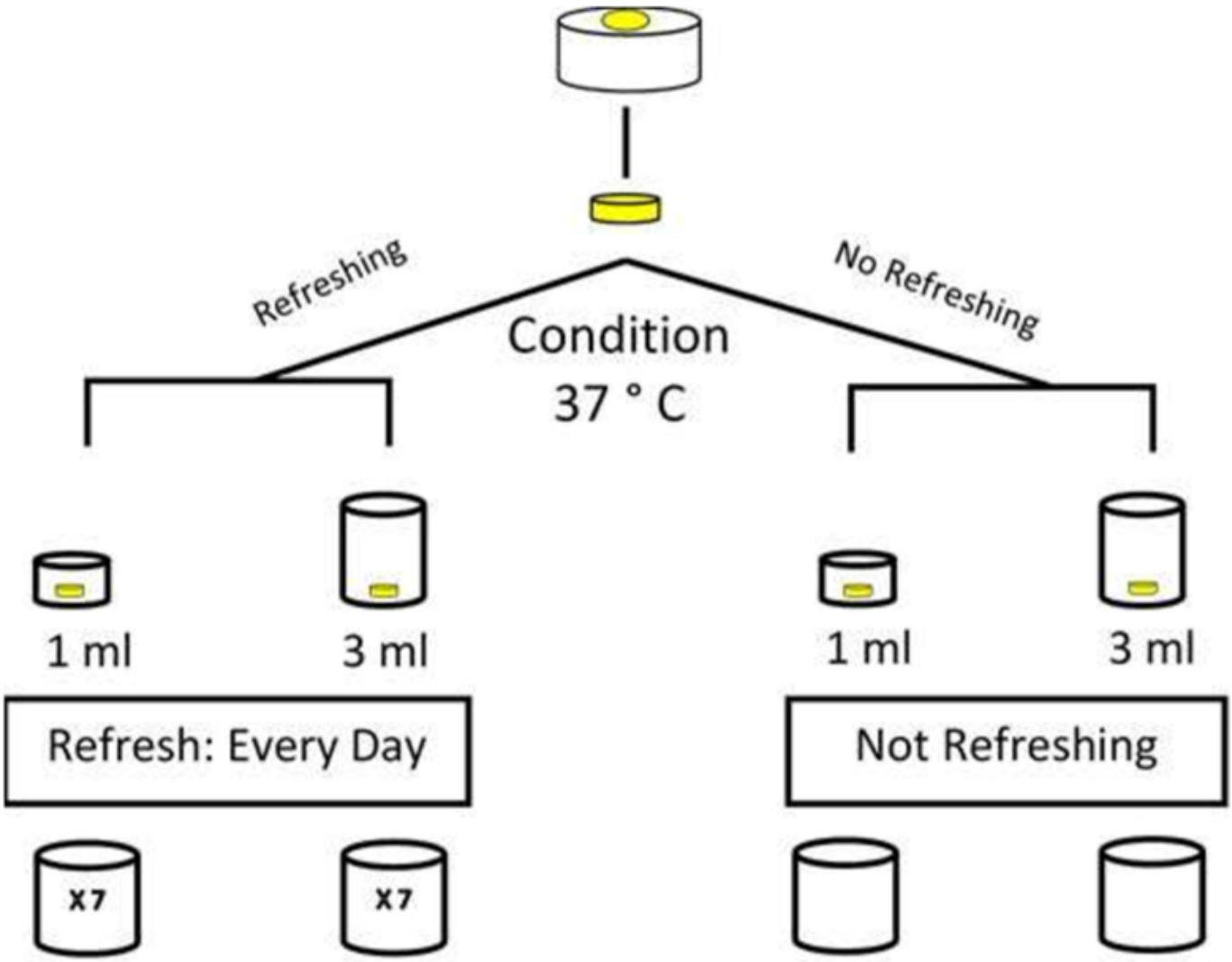

Figure 1

Schematic illustration of the research method used in this study

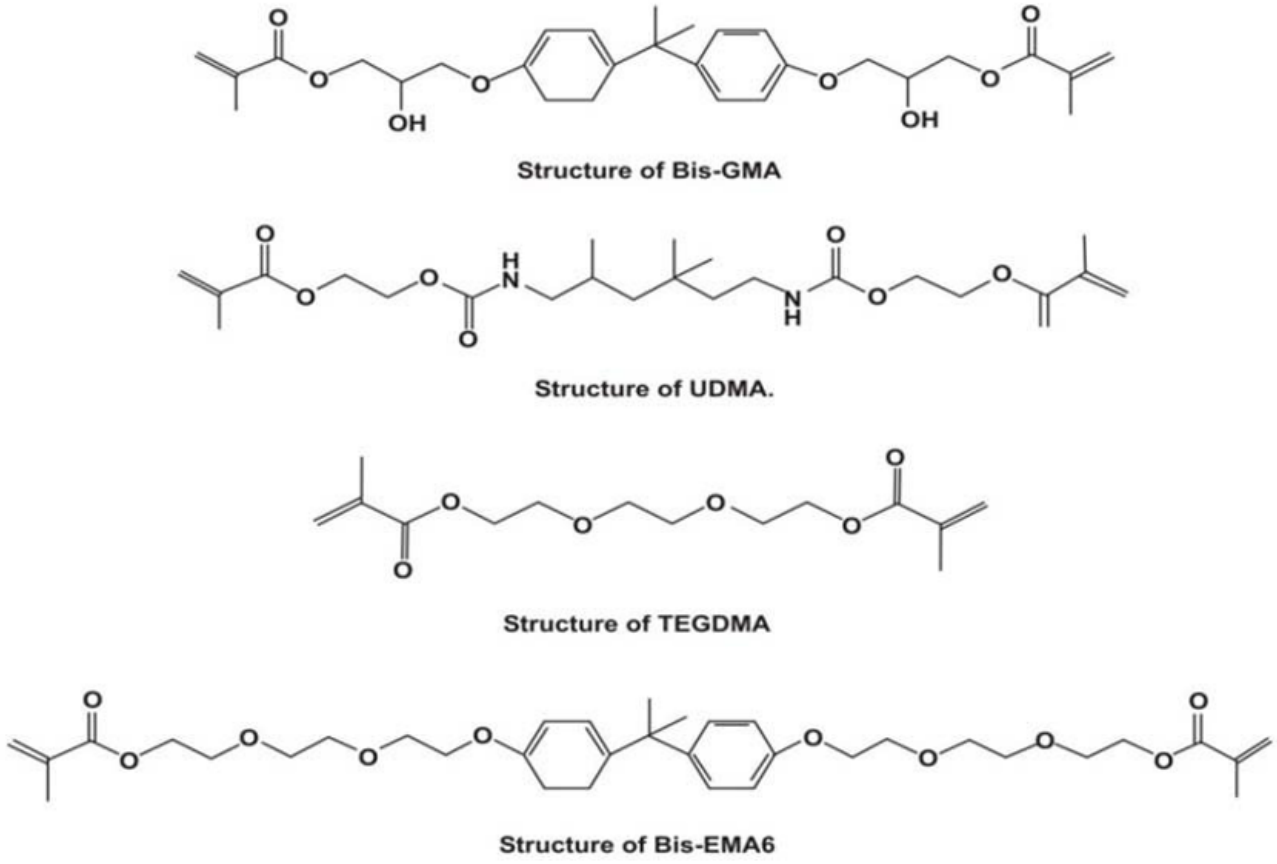

Figure 2

Monomer chemical Structure measured in this study